The mTOR pathway is regulated by polycystin-1, and its inhibition reverses renal cystogenesis in polycystic kidney disease


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Autosomal-dominant polycystic kidney disease (ADPKD) is a common genetic disorder that frequently leads to renal failure. Mutations in polycystin-1 (PC1) underlie most cases of ADPKD, but the function of PC1 has remained poorly understood. No preventive treatment for this disease is available. Here, we show that the cytoplasmic tail of PC1 interacts with tuberin, and the mTOR pathway is inappropriately activated in cyst-lining epithelial cells in human ADPKD patients and mouse models. Rapamycin, an inhibitor of mTOR, is highly effective in reducing renal cystogenesis in two independent mouse models of PKD. Treatment of human ADPKD transplant-recipient patients with rapamycin results in a significant reduction in native polycystic kidney size. These results indicate that PC1 has an important function in the regulation of the mTOR pathway and that this pathway provides a target for medical therapy of ADPKD.

Rapamycin | renal epithelial cells | tuberin

Autosomal-dominant polycystic kidney disease (ADPKD) is one of the most common human monogenic diseases and affects 12 million people worldwide (for recent reviews, see refs. 1–5). Excessive proliferation of renal epithelial cells leads to cysts that eventually replace most of the normal tissue. Consequently, ADPKD results in severe enlargement of the kidneys, and renal failure occurs in most cases by the age of 50. Survival depends on lifelong hemodialysis or kidney transplantation. No alternative clinical treatment is currently available.

Mutations in the PKD1 gene, which encodes PC1, account for >85% of ADPKD cases. PC1 is a multispanning membrane protein with a C-terminal cytoplasmic tail of ~226 residues. The PC1 tail has been implicated in several signaling pathways (5). Recent evidence suggests that PC1 may also play a role in cilia-mediated sensing of luminal fluid flow by renal epithelial cells (6). Whether these proposed functions are critical for renal cyst formation is unknown. Mutations in several genes unrelated to PC1 can also lead to a renal cystic phenotype in animal models and humans, but it is unclear whether all of these genotypes may converge on a common pathway that is critical for cyst formation. If such a common pathway exists, it would provide an excellent target for treatment strategies.

We have focused on the possibility that PC1 may act in a common pathway with tuberin, the product of the TSC2 gene. TSC2 mutations lead to tuberous sclerosis, a disease characterized by renal cysts and benign tumors in multiple organs (7, 8). Whereas upstream events that regulate tuberin are poorly understood, its ability to regulate the kinase activity of mTOR, via the small GTPase rheb (9), has recently been described (10, 11). mTOR has essential roles in protein translation (12, 13), cell growth, and proliferation and is up-regulated in several types of tumors (14). A possible role of mTOR in renal cystic disease is supported by two recent reports indicating that an inhibitor of mTOR slows disease progression in the Han:SPRD rat model (15, 16).

Here, we report that the C-terminal cytoplasmic tail of PC1 interacts with tuberin. The mTOR pathway is inappropriately activated in cyst-lining epithelial cells in human ADPKD and three mouse models with different affected genes, suggesting that this is a common, convergent event during renal cystogenesis. Rapamycin is a clinically approved drug and a specific mTOR inhibitor. We found that rapamycin is highly effective in reducing cystogenesis in two PKD mouse models and also leads to a significant reduction of renal size in end-stage ADPKD patients after renal transplantation. These results indicate that dysregulation of mTOR underlies changes in renal epithelial cells that cause the formation of polycystic kidneys in multiple genetic backgrounds. These results also provide a mechanistic link among PC1, tuberin, and mTOR, suggesting that rapamycin and related drugs that target the mTOR pathway are excellent candidates for a therapeutic approach to prevent or delay the onset of PKD.

Results

The Cytoplasmic Tail of PC1 Interacts with Tuberin and mTOR. To investigate signaling functions mediated by the cytoplasmic tail of PC1 in a model system relevant to renal epithelial cell biology, we generated stable clones of the Madin–Darby canine kidney (MDCK) cell line, which has been shown to express endogenous PC1 (17, 18) and is, therefore, likely to possess the components involved in downstream signaling pathways. Cells were engineered to stably express membrane-anchored fusion proteins of the entire cytoplasmic tail of PC1 (FLM-PC1) or the N-terminal (NTM-PC1) or C-terminal (CTM-PC1) half of the tail in a doxycycline (DOX)-inducible manner. Expression of NTM-PC1 containing the membrane-proximal 92 residues of the PC1 tail resulted in cell cycle arrest after 24 h and apoptosis at 48 h after induction of expression (see Fig. 6, which is published as supporting information on the PNAS web site). Expression of FLM-PC1 or CTM-PC1 had no similar effects on apoptosis (data not shown). Because increased apoptosis is a hallmark of cystic epithelial cells in ADPKD, these results suggested that expression of NTM-PC1 might affect a downstream pathway of PC1 that is relevant to renal cystogenesis.

To investigate the possibility that the effect induced by NTM-PC1 may involve tuberin, we determined the subcellular localization of...
immunofluorescence microscopy revealed that mTOR also under-
next investigated mTOR localization in NTM-PC1 cells. Confocal
isoform may be critical for PC1 binding.
been reported in human and mouse (19). This form, however, fails
tuberin coprecipitates with FLM-PC1, but not CTM-PC1, in a
of the endoplasmic reticulum and Golgi apparatus, respectively. (C)
served as controls (C and D, –DOX). Grp94 and GM130 are resident proteins
in cells that were induced to express NTM-PC1 for 16 h, a
time point before detectable effects on apoptosis or the cell cycle.
As shown in Fig. 1B, NTM-PC1 itself localizes mainly to the Golgi
apparatus. Whereas tuberin exhibits punctate cytoplasmic localization
in control cells (Fig. 1C, –DOX), expression of NTM-PC1 caused significant retargeting of tuberin to the Golgi apparatus,
where it now colocalizes with NTM-PC1 (Fig. 1C, +DOX). In
contrast, expression of CTM-PC1 did not result in Golgi localization
of tuberin (data not shown). The observed “forced” colocalization of tuberin with NTM-PC1 suggested that these two proteins
might interact.
To assess this possible interaction biochemically, FLM-PC1 was
immunoprecipitated from MDCK lysates, and binding proteins
were analyzed by Western blot. As shown in Fig. 1E, endogenous
tuberin in cells that were induced to express NTM-PC1 for 16 h, a
targeting process may be part of the multicomponent complex recruited by PC1.
E–G), exhibit intense
sibling (E–G, arrows) but not surrounding normal epithelium (E and F, arrowheads; and I). The cyst-specific cytoplasmic
immunosignal is abolished after rapamycin treatment (H, arrows). (Scale
bars, 20 μm.)

tuberin with NTM-PC1 suggested that these two proteins

The mTOR Pathway Is Inappropriately Activated in Cystic Epithelial Cells in Human ADPKD and Mouse Models. These data raised the possibility that mTOR may be regulated by PC1. To test this hypothesis, we made use of the availability of human tissue and mouse models in which PC1 is inactivated. Kidney tissue sections from ADPKD patients and normal controls were investigated by using antibodies against the phosphorylated, active forms of mTOR and its downstream effector S6 kinase. As shown in Fig. 2A, cyst-lining epithelial cells (arrows), but not surrounding normal epithelium (arrowheads) or normal tissue (Fig. 2B), exhibit intense cytoplasmic staining of phospho-mTOR (Ser 2448), indicative of elevated mTOR activity. Similarly, the same cyst-lining epithelial cells (Fig. 2C, arrows) exhibit highly elevated levels of phospho-S6 kinase (Thr 389), a surrogate marker of mTOR pathway activity, as compared with surrounding normal epithelium (Fig. 2C, arrowheads) or normal kidney (Fig. 2D). Similar staining was observed

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in five additional ADPKD cases (data not shown). These results provide evidence that dysregulation of the mTOR pathway is a biochemical feature of renal lesions in human ADPKD patients. Because available human ADPKD specimens represent advanced stage disease, we further tested whether earlier cystic lesions in a mouse model of PKD exhibit increased mTOR pathway activity. Immunostaining of kidney sections derived from 5-week-old mice that had undergone targeted recombination of the Pkd1 gene (21) (Fig. 2E) revealed that phospho-S6 ribosomal protein (Ser 235/236), a correlate of mTOR activity, is detected specifically in the cyst-lining epithelial cells (arrows) but not surrounding normal epithelium (arrowheads). To determine whether mTOR activation during cystic progression may be a general feature of PKD, we next examined the status of the mTOR pathway in polycystic mouse models independent of Pkd1 mutations. Mice overexpressing the myelin and lymphocyte (MAL) protein present with PKD (22) and, interestingly, cyst-lining renal epithelial cells exhibited specific cytoplasmic staining of phospho-S6 ribosomal protein (Fig. 2F, arrow) compared with surrounding normal epithelium (Fig. 2F, arrowhead). The orpk-rescue model, in which the cilia protein polaris is defective, results in the manifestation of a late-onset form of PKD reminiscent of human ADPKD (23). Orpk-rescue (Tg737orpk+/+;TgRsq) mutant mice exhibited intense cytoplasmic labeling of cyst-lining epithelial cells by using the phospho-S6 ribosomal protein antibody (Fig. 2G, arrows). Strikingly, rapamycin-treated orpk-rescue mutant mice (Fig. 2H, arrows) showed no evidence of cytoplasmic staining in cysts, demonstrating the effectiveness of rapamycin treatment and the specificity of the immunosignal. Renal sections derived from orpk-heterozygous-rescue (Tg737orpk+/−;TgRsq) control mice were devoid of significant cytoplasmic staining (Fig. 2G). Taken together, these results indicate that mTOR activity is significantly up-regulated in cyst-lining epithelial cells and suggest that this may be a common characteristic of renal cysts caused by defects in several genes whose functions may ultimately converge on this pathway.

**Inhibition of mTOR Alleviates the Cystic Phenotype in PKD Mouse Models.** If the abnormally high activity of mTOR in renal cystic cells contributes to cyst-formation, then compounds that inhibit mTOR should alleviate renal cystogenesis. Rapamycin is a specific inhibitor of mTOR kinase activity (24, 25). We tested the effect of rapamycin on two mouse models that exhibit late- and early-onset renal cystic phenotypes, respectively. The orpk-rescue mouse model described above served as a model of late-onset ADPKD, whereas the bpk mouse model (26) served as a model of early-onset ARPKD.

Orpk-rescue (Tg737orpk+/+;TgRsq) mutant mice and orpk-heterozygous-rescue (Tg737orpk+/−;TgRsq) control mice were treated with rapamycin (5 mg/kg of body weight per day) starting at postnatal day 150 through day 178. This treatment profoundly improved the cystic phenotype in the mutant mice (Fig. 3A–D). The histological cystic index was significantly reduced in rapamycin-treated mutant mice (Fig. 3G). To determine whether rapamycin acts mainly by preventing the formation of renal cysts or by a reversal of cystogenesis we measured the total renal volumes by MRI during the course of drug treatment. It has been shown that the degree of cystogenesis correlates with kidney volume (27). Whereas nontreated mutant animals exhibited a 13% increase in renal volume between days 150 and 178, rapamycin-treated animals exhibited a decrease of 30% (Fig. 3H; and see Fig. 7, which is published as supporting information on the PNAS web site). Rapamycin had no effect on renal volumes of control mice (Fig. 3H). This result suggests that rapamycin effects a reversal of the renal cystic phenotype, possibly in conjunction with inhibition of cystogenesis.

The bpk mouse model is characterized by an embryonic onset reminiscent of human ARPKD, and the mice generally fail to live >25 days (26). Rapamycin treatment (5 or 1.67 mg/kg of body weight per day) from day 7 postpartum for a period of 14 days resulted in strong inhibition of the renal cystic phenotype as reflected by a significant decrease (76.6% for 5 mg/kg of body weight per day treatment; 68.7% for 1.67 mg/kg of body weight per day treatment, n = 4) in kidney weight as compared with vehicle-treated bpk mutant littermates (Fig. 4D). Rapamycin-treated bpk mutant mice exhibited significantly smaller cyst sizes (Fig. 4A–C) and an improved renal cystic index (Fig. 4E) compared with controls. Furthermore, either dose of rapamycin resulted in a
normalization of plasma blood urea nitrogen levels (Fig. 4F), an indicator of renal function. Taken together, these results demonstrate that mTOR inhibition by rapamycin has a significant beneficial effect on renal cystic disease in two independent mouse models, suggesting that mTOR activity is critical for cystogenesis.

**Rapamycin Treatment Induces Apoptosis of Cystic Epithelial Cells.** Cystic epithelial cells in PKD are generally characterized by an increased rate of both apoptosis and proliferation, which indicates a high cell turnover (1–4). Because rapamycin treatment appeared to not only prevent the onset of cyst formation but also affected a reduction in renal volume, we investigated whether apoptosis may be a mechanism underlying this reduction. As shown in Fig. 5A, rapamycin-treated orpk-rescue (Tg737^orpkTgRsq) mutant mice exhibited increased numbers of TUNEL-positive cyst-lining epithelial cells and the presence of luminal TUNEL-positive cells (Fig. 5A Lower) as compared with nontreated orpk-rescue mutant mice (Fig. 5A Upper). Quantification (Fig. 5B; and see Materials and Methods) revealed a significant (P < 0.001, n = 4) increase in cyst-lining and luminal TUNEL-positive cells per cyst in rapamycin-treated orpk-rescue mutant mice when compared with vehicle-treated orpk-rescue mutant mice. These results suggest that rapamycin reduces renal cysts, at least partially, through the selective induction of apoptosis and luminal shedding of cyst-lining epithelial cells in the orpk-rescue mutant mouse model.

**Rapamycin Treatment After Renal Transplantation Reduces the Size of Affected Kidneys in ADPKD Patients.** To determine the possible clinical relevance of our findings, we performed a retrospective study. Advanced-stage ADPKD patients frequently receive a renal transplant without removal of the affected cystic kidneys. Rapamycin treatment is used in some of these patients as an immunosuppressant to prevent transplant rejection (28). We hypothesized that the drug may also have a beneficial effect on the remaining polycystic kidneys. The criteria used to select these patients were that (i) they retained one or both of their native polycystic kidneys, (ii) they had an initial CT scan no more than 12 months before or 5 months after kidney transplantation, and (iii) they had a follow-up CT scan at least 11 months or later after transplantation. The control group, which satisfied these same criteria, consisted of patients receiving adjunct immunosuppression using compounds other than rapamycin. Total kidney volumes were determined by quantification of CT scans.

As shown in Table 1 (which is published as supporting information on the PNAS web site), the rapamycin group showed a statistically significant (P < 0.001) decrease in kidney volumes by 24.8 ± 7.7% over an average period of 24 months. In contrast, the control group exhibited a decrease of only 8.6 ± 11.2% in renal volume over an average period of 40 months, which was not statistically significant. There was a statistically significant difference between the rapamycin and the nonrapamycin groups (P = 0.03). Considering that the dose of rapamycin administered to transplant patients is significantly lower than what was used in the above animal experiments, these results suggest that rapamycin may have a similar beneficial effect in human ADPKD as we find in the mouse models.

**Discussion**

In this study, we show that the membrane-proximal half of the cytoplasmic tail of PC1 interacts with a component of the tuberous sclerosis complex, tuberin, and with the kinase mTOR, which is regulated by tuberin. We show that the mTOR pathway is inappropriately activated in cyst-lining epithelial cells in ADPKD patients, most of whom will have mutations in the PKD1 gene. Together, these results are consistent with a model in which PC1,
Epithelial cells were observed in sections of nontreated Tg737orpk proteins: (activity may be a common process underlying renal cyst formation. Earlier-onset PKD than patients with for the presence of apoptotic cells by TUNEL. (mycin or vehicle as indicated. Renal tissue sections were processed and analyzed of cyst-luminal epithelial cells were observed in sections of rapamycin-treated Tg737orpk mutant mice, whereas a significant increase in TUNEL-positive, cyst-lining and renal sections derived from vehicle or rapamycin-treated orpk-rescue mutant animals expressed as an average of TUNEL-positive cells per cyst (n = 4 kidneys). *** P < 0.001.

Fig. 5. Rapamycin treatment induces increased apoptosis and luminal shedding of cystic epithelial cells. Orpk-rescue (Tg737orpk;TgRsq) mutant and orpk-heterozygous-rescue (Tg737orpk;TgRsq) control mice were treated with rapamycin or vehicle as indicated. Renal tissue sections were processed and analyzed for the presence of apoptotic cells by TUNEL. (A) TUNEL-positive, cyst-lining epithelial cells were observed in sections of nontreated Tg737orpk;TgRsq mutant mice, whereas a significant increase in TUNEL-positive, cyst-lining and cyst-luminal epithelial cells were observed in sections of rapamycin-treated Tg737orpk;TgRsq mutant mice. (B) Quantification of TUNEL-positive cells in renal sections derived from vehicle or rapamycin-treated orpk-rescue mutant animals expressed as an average of TUNEL-positive cells per cyst (n = 4 kidneys).

Our results suggest that inappropriate stimulation of mTOR activity may be a common process underlying renal cyst formation. We observed this effect in systems with defects in very diverse proteins: (i) ADPKD patients and a mouse model with defective PC1; (ii) a mouse model with a defect in polaris, a protein implicated in cilia transport; and (iii) a mouse model that overexpresses MAL, a protein implicated in apical membrane targeting. Rapidmycin was also highly effective in the bpk mouse model with a defect in bicaudal C, the homologue of a protein implicated in translational regulation in Drosophila (34). Furthermore, defects in the tuberin gene are directly associated with renal cystic disease in humans (7), and increased mTOR activity has been reported in tumor cells derived from a rat model with a defect in tuberin (35). We therefore suggest that mTOR is a downstream effector at a converging point of signaling pathways that involve all of the above gene products. Because rapamycin alone was able to reverse the cystic phenotype in the PKD mouse models, we also conclude that inappropriate activation of the mTOR pathway is likely the sufficient and predominant defect that causes renal cyst formation. It was recently reported that rapamycin improves the cyst phenotype of another model of PKD, the Han:SPRD rat (15, 16). Although the underlying gene defect in this model is still unknown, this finding is consistent with and supports our model that mTOR is a converging point of mechanisms that lead to renal cyst formation.

Even though our retrospective studies on rapamycin-treated ADPKD transplant patients involved only small numbers, the observed significant reduction in renal volumes is highly encouraging and suggestive of a potential for clinical intervention. The impact of ADPKD on patients and the health care system is enormous. Chronic pain, palliative surgery, renal failure, dialysis, and transplantation as well as death are all outcomes of this genetic disease that still has no medical therapy to slow or reverse its progression. The dramatic shortage of donor organs for transplantation makes the search for medical therapy for this disease even more important. This study implicates PC1 as a regulator of mTOR function, shows that mTOR is inappropriately active in ADPKD, and provides evidence that this morbid path may be averted with medical treatment by rapamycin, a drug that is already approved for human use.

Materials and Methods

Reagents. Antibodies were anti-tuberin (C-20) and anti-mTOR (N-19) (Santa Cruz Biotechnology); anti-phospho-mTOR (Ser-2448), anti-phospho-p70 S6 kinase (Thr-389), and anti-phospho-S6 ribosomal protein (Ser-235/236) (Cell Signaling Technology); anti-CD16 (Chemicon); anti-Grp94 (StressGen); anti-GM130 (Transduction Laboratories); anti-acetylated tubulin (Sigma); and anti-Golgin 97 (Molecular Probes). For Western blotting, an ECL kit was used (SuperWest Pico; Pierce).

PC1 Constructs and Generation of Stable MDCK Cell Lines. The cDNAs of membrane-anchored PC1 constructs have been described in ref. 36 and were cloned into pcDNA4/TTO (Invitrogen). All fusion proteins contain the extracytoplasmic domain and signal peptide of CD16, the transmembrane domain of CD7 and the full-length cytoplasmic tail of PC1 (FLM-PC1, residues 4077–4302 of human PC1), the N-terminal half of the tail (NTM-PC1, residues 4077–4168), or the C-terminal half of the tail (CTM-PC1, residues 4191–4302). Constructs were transfected into MDCK cells that had been stably transfected for expression of the TET-repressor (37), and stable clones were selected.

Cell-Cycle Analysis. Cells were cultured in the presence or absence of DOX for 24 or 48 h. Cells were harvested, fixed, stained with propidium iodide, and analyzed on a fluorescence-activated cell sorter (Becton Dickinson). The percentages of cells in Gi/G0, S, and G2/M were determined by using the program MODFIT (Verity, Sunnyvale, CA).

Cell Proliferation Assay. Cells were cultured in 96-well plates at 10^4 cells per well in the presence or absence of DOX. Cell numbers were evaluated by using the program CYQUANT (Molecular Probes).

Immunohistochemistry and Immunofluorescence Microscopy. Paraffin-embedded tissues were processed and stained as described in ref. 38. Immunofluorescence microscopy on cultured cells was carried out as described in ref. 38. Apoptotic cells in tissue sections were detected by using a TUNEL-based assay (ApopTag; Chemicon).

Commmunoprecipitation. FLM- and CTM-PC1 MDCK cells were incubated for 16 h in the presence or absence of DOX. Cells were lysed in buffer (50 mM Hepes, 50 mM potassium acetate, 1% 3-[3-cholamidopropyl]dimethylammonio]-1-propanesulfonate (CHAPS), and protease and phosphatase inhibitors) for 30 min at 4°C. Precleared lysates were incubated with anti-CD16 antibody for
3 h, followed by overnight incubation with protein A-Sepharose at 4°C. Binding proteins were analyzed by Western blot using the indicated antibodies.

**Animals.** The *orpk*-rescue and the *bpk* mouse models of PKD have been described in refs. 23 and 26. *Bpk* mice were kindly provided by Calvin Cotton (Case Western Reserve University). Tissue sections of MAL-transgenic mice were kindly provided by Ueli Suter (Eidgenössische Technische Hochschule, Zurich) and have been described in ref. 22. The mouse model with a floxed *Pkd1* allele (*Pkd1*cond/cond) has been described in (21), and tissue sections from *Pkd1*cond/cond mice coexpressing the balance Cre, TgN(balancer1)2Cgn, were used in this study. For rapamycin experiments, mice received no injections or daily i.p. injections of 1.67 or 5 mg/kg rapamycin or vehicle (21.4% DMSO, 21.4% ethanol, and 57.2% saline). For the *orpk*-rescue model, rapamycin was administered starting at postnatal day 150 for a period of 28 days. For the *bpk* model, rapamycin was administered starting at postnatal day 7 for a period of 14 days.

**MRI Analysis of Mice.** Mice were anesthetized under isoflurane and analyzed in a clinical 1.5T MRI scanner (Sonata; Siemens) using a T2 and T1 fat-suppressed sequence. Kidneys were scanned with a high-resolution 3D True fast-induction steady-state potential (FISP) acquisition [resolution = 260 × 260 × 500 μm, time of repetition (TR)/time of echo (TE) = 10.4/5.2 ms, number of signal averages (NSA) = 4, 24 slices per slab]. Renal volumes were quantified by using the program OsiriX by measuring slice-by-slice renal areas and summing the individual slice volumes.

**Analysis of Mouse Tissues and Blood Samples.** After treatment, mice were weighed and anesthetized, and blood was drawn via retroorbital puncture. Blood was separated by centrifugation in a lithium–heparin tube (Becton Dickinson), and plasma blood urea nitrogen levels were determined by using a clinical laboratory service. After cervical dislocation, kidneys were removed, weighed, and fixed in 10% neutral-buffered formalin at 4°C. Binding proteins were analyzed by Western blot using the indicated antibodies.

**Cystic Index Calculations.** Representative images of H&E-stained kidneys were acquired. A grid was placed over the images, and the cystic index was calculated as the percentage of grid intersection points that bisected cystic or noncystic areas.
Supporting Information

Files in this Data Supplement:

Supporting Figure 6
Supporting Figure 7
Supporting Table 1

Fig. 6. The N-terminal cytoplasmic domain of PC1 induces cell cycle arrest and apoptosis. (A and B) Identical numbers of MDCK cells stably transfected for the membrane-anchored N-terminal cytoplasmic domain of PC1 (NTM-PC1) were cultured in the presence or absence of DOX for 48 h. (A) Numbers of cells attached after this time were quantified and are expressed in arbitrary units. Note that induction of expression of NTM-PC1 resulted in a significant decrease of cell numbers. (B) DNA of detached cells was stained with DAPI to reveal typical condensed and fragmented apoptotic nuclei in NTM-PC1-expressing cells (+DOX). (C) The DNA of attached cells was isolated and analyzed by agarose-gel electrophoresis. Note that induction of NTM-PC1 expression results in DNA-laddering, indicative of apoptosis. (D and E) Cell cycle analysis of attached cells was carried out by FACS in induced and noninduced NTM-PC1 cells. (D) Note that at 24 h, cells exhibited a significant increase in G1/G0 cell cycle and a significant reduction of cells in S-phase. (E) The fraction of apoptotic cells is significantly increased after induction of expression of NTM-PC1 for 24 and 48 h. *, P < 0.05; **, P < 0.01; ***, P < 0.001.
**Fig. 7.** Analysis of renal volume changes by MRI scanning. Vehicle- and rapamycin-treated orpk-rescue (Tg737orpk/orpk;TgRsq) mutant mice were subjected to MRI scanning at day 150, day 164, and day 178. The maximum slice volume for each kidney was determined by using the analysis program OSIRIX. The outline of each kidney at each time point was traced as shown and is represented as a merged image. Note the visual increase in maximal slice volume in vehicle-treated orpk-rescue mutant mice vs. the decrease in maximal slice volume in rapamycin-treated orpk-rescue mutant mice over the time course of the study. Cystic structures, evident as black areas within the kidney parenchyma, are significantly fewer in rapamycin-treated orpk-rescue mutant mice vs. vehicle-treated orpk-rescue mutant mice. (Scale bar, 3 mm.)
Table 1. Calculated CT renal volume of native polycystic kidneys in kidney transplant patients receiving rapamycin vs. nonrapamycin immunosuppression

<table>
<thead>
<tr>
<th>Patient</th>
<th>Kidney</th>
<th>Initial renal volume, ml</th>
<th>Final renal volume, ml</th>
<th>Volume change, ml</th>
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<th>Follow-up, months</th>
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<td>−230</td>
<td>−12.8%</td>
<td>14</td>
</tr>
<tr>
<td>Averages</td>
<td></td>
<td>1,232</td>
<td>931</td>
<td>−301</td>
<td>−24.8%</td>
<td>24</td>
</tr>
</tbody>
</table>

Analysis

<table>
<thead>
<tr>
<th>Total % Volume Change:</th>
<th>−24.8% ± 9.7% (P = .001)</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Volume Change per Month:</td>
<td>−1.4% ± 0.8% (P = .005)</td>
</tr>
</tbody>
</table>

Nonrapamycin treatment

<table>
<thead>
<tr>
<th>Patient</th>
<th>Kidney</th>
<th>Initial renal volume, ml</th>
<th>Final renal volume, ml</th>
<th>Volume change, ml</th>
<th>Volume % change</th>
<th>Follow-up, months</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>M</td>
<td>1,621</td>
<td>1,406</td>
<td>−215</td>
<td>−13.3%</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>1,174</td>
<td>1,251</td>
<td>77</td>
<td>6.6%</td>
<td>22</td>
</tr>
<tr>
<td>6</td>
<td>O</td>
<td>464</td>
<td>459</td>
<td>−5</td>
<td>−1.1%</td>
<td>69</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>735</td>
<td>575</td>
<td>−160</td>
<td>−21.8%</td>
<td>69</td>
</tr>
<tr>
<td>7</td>
<td>Q</td>
<td>1,949</td>
<td>1,689</td>
<td>−260</td>
<td>−13.3%</td>
<td>20</td>
</tr>
<tr>
<td>Averages</td>
<td></td>
<td>1,189</td>
<td>1,076</td>
<td>−113</td>
<td>−8.6%</td>
<td>40</td>
</tr>
</tbody>
</table>

Analysis

<table>
<thead>
<tr>
<th>Total % volume change</th>
<th>−8.6% ± 11.2% (P = 0.16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Volume change per month:</td>
<td>−0.3% ± 0.4% (P = 0.22)</td>
</tr>
</tbody>
</table>

Volumes of native polycystic kidneys in human transplant patients were calculated by using an accepted ellipsoid volume formula from dimensions measured retrospectively on patient CT scans. Initial volumes were calculated from CT scans performed no more than 12 months prior to 5 months after receiving a kidney transplant and initiating immunosuppression therapy. Final volumes of these same native kidneys were also measured retrospectively from a CT scan performed at least 11 months after transplantation. The follow-up period between the two scans was measured and is shown as follow-up in months. Patients who received rapamycin as part of their immune suppression were placed in the rapamycin treatment group and those that did not were placed in the nonrapamycin treatment group. Volume changes for the kidneys were then analyzed statistically within groups by using a paired t-test and between the two groups by using an unpaired t-test.