Cyclin-Dependent Kinase 1 Activity Is a Driver of Cyst Growth in Polycystic Kidney Disease

Chao Zhang,1 Bruno Balbo,1 Ming Ma,1 Jun Zhao,2,3 Xin Tian,1 Yuval Kluger,2,3,4 and Stefan Somlo1,5

1Department of Internal Medicine, Yale University, New Haven, Connecticut
2Department of Pathology, Yale University, New Haven, Connecticut
3Interdepartmental Program in Computational Biology and Bioinformatics, Yale University, New Haven, Connecticut
4Program in Applied Mathematics, Yale University, New Haven, Connecticut
5Department of Genetics, Yale University, New Haven, Connecticut

ABSTRACT

Background Mutations in PKD1 and PKD2, which encode the transmembrane proteins polycystin-1 and polycystin-2, respectively, cause autosomal dominant polycystic kidney disease (ADPKD). Polycystins are expressed in the primary cilium, and disrupting cilia structure significantly slows ADPKD progression following inactivation of polycystins. The cellular mechanisms of polycystin- and cilia-dependent cyst progression in ADPKD remain incompletely understood.

Methods Unbiased transcriptional profiling in an adult-onset Pkd2 mouse model before cysts formed revealed significant differentially expressed genes (DEGs) in Pkd2 single-knockout kidneys, which were used to identify candidate pathways dysregulated in kidneys destined to form cysts. In vivo studies validated the role of the candidate pathway in the progression of ADPKD. Wild-type and Pkd2/Ift88 double-knockout mice that are protected from cyst growth served as controls.

Results The RNASeq data identified cell proliferation as the most dysregulated pathway, with 15 of 241 DEGs related to cell cycle functions. Cdk1 appeared as a central component in this analysis. Cdk1 expression was similarly dysregulated in Pkd1 models of ADPKD, and conditional inactivation of Cdk1 with Pkd1 markedly improved the cystic phenotype and kidney function compared with inactivation of Pkd1 alone. The Pkd1/Cdk1 double knockout blocked cyst cell proliferation that otherwise accompanied Pkd1 inactivation alone.

Conclusions Dysregulation of Cdk1 is an early driver of cyst cell proliferation in ADPKD due to Pkd1 inactivation. Selective targeting of cyst cell proliferation is an effective means of slowing ADPKD progression caused by inactivation of Pkd1.

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Autosomal dominant polycystic kidney disease (ADPKD) is the most common monogenic cause of ESKD and accounts for approximately 4% of patients receiving RRT in the United States.1 Mutations in PKD1 encoding polycystin-1 (PC1) or PKD2 encoding PC2 account for most affected families. PC1 is a large polytopic integral membrane protein that undergoes extensive post-translational modification, including autoproteolytic cleavage, and has been hypothesized to function as a receptor for either ligands or mechanical stimuli.1–3 PC2 is a nonselective cation channel member of the transient receptor potential family.

C.Z. and B.B. contributed equally to this work.

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Present addresses: Dr. Bruno Balbo, Division of Anesthesiology at Hospital das Clinicas, University of Sao Paulo School of Medicine, Sao Paulo, Brazil. Dr. Ming Ma, School of Life Sciences, Southwest University, Beibei, Chongqing, China.

Correspondence: Dr. Stefan Somlo, Section of Nephrology, Department of Internal Medicine, Yale University School of Medicine, PO Box 208029, 333 Cedar Street, New Haven, CT 06520-8029. Email: stefan.somlo@yale.edu

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ADPKD is inherited as a germline autosomal dominant heterozygous trait, but cyst formation in ADPKD is focal and typically manifests in adulthood following the second decade of life. Multiple lines of evidence show that cyst initiation requires second-hit somatic mutations inactivating the normal allele of the respective PKD gene in cells along the nephron. Following initiation, cyst progression manifests increased proliferation in cyst-lining epithelia. Kidney tissues from patients with ADPKD as well as kidneys from mouse models on the basis of orthologous genes consistently show higher rates of nuclei staining positive for the proliferating cell nuclear antigen (PCNA) and mKi67 mitotic markers as well as enhanced incorporation of bromodeoxyuridine. Multiple signaling pathways, including mTOR, MAPK/ERK, and cAMP-dependent pathways, have been suggested to regulate cyst growth at least in part by altering cell proliferation. The molecular components of CDCA are unknown, although some ciliary pathways, such as Hedgehog, have been excluded.

In this study, we used transcriptional profiling in adult mouse kidneys at a stage before cyst formation to identify the earliest in vivo transcriptional changes that follow inactivation of polycystins. To identify CDCA-related transcriptional responses specific to cyst-forming cells, we compared three groups of kidney-specific knockout mice: Pkd2 single mutants destined to form cysts and Ifit88/Pkd2 double mutants and nonknockout controls, which are protected from cyst progression. This analysis identified 241 differentially expressed genes (DEGs) in the Pkd2-only knockouts relative to both control groups. Upregulation of genes involved in cell cycle progression was the strongest signal in this gene set. One candidate gene in proliferation-related group was cyclin-dependent kinase 1 (Cdk1), a member of the serine/threonine protein kinase family essential for cell cycle progression. Cdk1 showed a similar “CDCA pattern” of differential expression in Pkd1 models. Inactivation of Cdk1 in both early-onset and adult-onset Pkd1 models of ADPKD markedly decreased cyst cell proliferation and improved polycystic kidney disease progression. The protective effect of Cdk1 inactivation on cyst growth occurred through inhibition of the cell cycle possibly affecting the G1-S phase. These studies identify Cdk1 as a critical early driver of cyst cell proliferation in ADPKD and show that targeting polycystin-dependent cyst cell proliferation may be an effective means of slowing polycystic kidney disease progression in ADPKD.
performed with DESeq2. The RNA-sequencing data reported in this paper are available on the Gene Expression Omnibus (accession no. GSE149739).

**Functional Enrichment Analysis and Gene Interaction Network Analysis**

The 241 DEGs were applied to KEGG pathway analysis in DAVID 6.8. The Benjamini–Hochberg procedure was used for multiple test adjustment, and FDR<0.05 was selected as the statistical significance threshold. Gene interaction network analysis was performed using 15 genes from 241 DEGs, which were included in KEGG cell cycle pathway using Ingenuity Pathway Analysis (Qiagen).

**Quantitative PCR**

Total RNA was extracted from cold PBS-perfused kidney tissue using the RNeasy Mini Kit (Qiagen), and cDNA synthesis was performed using SuperScript II Reverse Transcription (Invitrogen). Quantitative RT-PCR was performed with SYBR Green fluorescence reagent (BioRAD) and analyzed by the CFX96 Touch Real-Time PCR Detection System (BioRAD). The following primers were used:

- **Cdk1**: forward 5'-TACACACACGGGTAGTGCAG-3', reverse 5'-TCAACCGGAGTGGAAGTAACG-3'.
- **Cdt1**: forward 5'-GCAACCATGTGCTGAGAAGC-3', reverse 5'-TGCTGCGGAACATCTCAACT-3'.
- **Rrm2**: forward 5'-AGCTACGTATGGAGAACGC-3', reverse 5'-ATCAGCCCCGTTTTCTTGAG-3'.
- **Top2a**: forward 5'-GACAGCAACAAAAAGGCAGTC-3', reverse 5'-CAGGAGCACTCAAGGCTGA-3'.
- **Mcm3**: forward 5'-AGCAAAACTCGTTCCAAGGATG-3', reverse 5'-AGCAAGCAGAGGATTGCCTT-3'.
- **Mcm5**: forward 5'-TGTTGGGAGACCCTGGTACA-3', reverse 5'-AGGCTGGGCAATTCTTGTGT-3'.
- **Mcm7**: forward 5'-AGGAGATGTGTACACCCCAAT-3', reverse 5'-AGGCTGGGCAATTCTTGTGT-3'.
- **Gapdh**: forward 5'-GTCCCGTAGACAAAAATGTG-3', reverse 5'-ATTCTCGGCCTTGACTGTG-3'.

**Immunoblotting**

Frozen PBS-perfused kidneys were homogenized using a 1-ml Dounce homogenizer (Wheaton) in ice-cold RIPA buffer (140 mM NaCl, 1% Triton X-100, 0.1% sodium deoxycholate, 0.1%, 10 mM Tris-HCl, pH 8.0, 1 mM EDTA, 0.5 mM EGTA) containing Complete EDTA-free Protease Inhibitor cocktail tablets (Roche) and PhosSTOP phosphatase inhibitor cocktail tablets (Roche). An equal amount of protein was loaded to 4%–20% Mini-PROTEm TGX Precast Protein Gel (Bio-RAD) and transferred to PVDF membranes. Membranes were incubated with 5% powdered milk or 3% BSA at room temperature for 1 hour and then incubated with primary antibodies at 4°C overnight.

The following antibodies were used: anti-Cdk1 (#28439, 1:1000), anti-Pcna (#13110, 1:1000), anti-Cyclin E1 (#20808, 1:2000), anti-phospho H3 (Thr3; #13576, 1:3000), and anti-Hsp90 (#4877, 1:3000) from Cell Signaling Technology and anti-Cyclin D1 (ab134175, 1:2000) from Abcam. Secondary anti-rabbit HRP-conjugated antibody (1:5000; Jackson ImmunoResearch Laboratories) was then incubated with the membrane for 1 hour at room temperature. Pierce ECL Western Blotting Substrate or Supersignal West Femto Maximum Sensitivity Substrate (Thermo Scientific) was used for chemiluminescence detection. The images were acquired by LI-COR Odyssey Fc Imaging System.

**Cell Proliferation and Apoptosis Analyses**

Proliferation was measured by ethynyl-2'-deoxyuridine (EdU) incorporation. Mice received 50 mg/kg EdU (Invitrogen) by intraperitoneal injection 4 hours before euthanasia. Kidneys were fixed with 4% PFA overnight, embedded in OCT after 30% sucrose infiltration, and processed for immunofluorescence. EdU staining was performed with Click-it EdU Alexa Fluor 594 and Alexa Fluor 488 Imaging Kit (Invitrogen). Apoptosis was determined using terminal deoxynucleotidyl transferase–mediated digoxigenin-deoxyuridine nick-end labeling (TUNEL) with the In Situ Cell Death Detection Fluorescein Kit (Roche) POD or TMR red directed by the manufacturer's instructions. Sections were costained with segment markers (*Lotus tetragonolobus agglutinin* [LTA]-FITC 1:200, *Dolichos biflorus agglutinin* [DBA]-Rhodamine 1:50; Vector Laboratories). The numbers of EdU-positive and TUNEL-positive nuclei were counted in at least 1000 DBA- or LTA-positive nuclei per animal.

**Statistics Analyses**

Data were analyzed by one-way ANOVA followed by Tukey multiple-comparison test with GraphPad Prism 7 software. *P*=0.05 was used as the threshold for statistical significance. All data are presented as mean±SEM.

**RESULTS**

**Transcriptional Profiling Identifies Cdk1 as Upregulated in the Early-Stage Cyst Cell Proliferative Response in ADPKD**

To investigate the transcriptional response following cyst initiation in ADPKD, RNA sequencing was performed in models...
on the basis of the biallelic Pax8ERTA; TetO-cre kidney-selective, whole-nephron, doxycycline-inducible, adult-onset model. We conditionally inactivated Pkd2 for the ADPKD model and intraflagellar transport 88 (Iftr88) for the cilia inactivation step. Three genotypes with three biologic replicates of each were compared: (1) poly cystic kidney disease forming single-mutant Pkd2fl/fl; Pax8ERTA; TetO-cre mice, (2) Iftr88/Pkd2 double-mutant Iftr88fl/fl; Pkd2fl/fl; Pax8ERTA; TetO-cre mice that are protected from cyst formation despite inactivation of Pkd2, and (3) noncystic controls without gene knockou t.9 Mice in all three groups were administered oral doxycycline from P28 to P42, and kidney tissues were obtained at P70 when kidneys of Pkd2 single-mutant Pkd2fl/fl; Pax8ERTA; TetO-cre mice had mild tubule dilation but no overt cyst formation (data not shown). This precystic stage was selected to limit potential confounding by secondary changes, such as inflammation and fibrosis, that accompany advancing cyst growth. Pairwise comparison between Pkd2 single mutant and the wild type identified 1047 (805 + 241 + 1) DEGs, whereas Pkd2 single mutant compared with Iftr88/Pkd2 double mutant identified 439 (197 + 241 + 1) DEGs (Figure 1A, Supplemental Table 1). Because the Iftr88/Pkd2 double mutants are strongly protected from cyst formation, we hypothesized that genes differentially expressed with the same direction of change in Pkd2 single mutants when compared with both the wild type and Iftr88/ Pkd2 double mutants are most likely to include the crucial drivers for cyst progression in ADPKD. A group of 241 DEGs met this criterion (Figure 1A). One DEG was excluded because it had lower expression in Pkd2 single mutants compared with the wild type but higher expression compared with Iftr88/Pkd2 double knockouts, therefore not meeting our criterion for the same direction change in expression. KEGG pathway enrichment analysis applied to the 241 DEGs indicated that cell cycle was the major pathway dysregulated at the early stage of ADPKD (Figure 1B). We refer to these 241 DEGs as the genes dysregulated in the “CDCA pattern.” Fourteen of the 15 CDCA pattern DEGs, which are included in KEGG cell cycle pathway, showed increased expression in Pkd2 single mutants (Figure 1C, Supplemental Table 1).

To identify a critical regulator of abnormal cell proliferation in ADPKD, the subgroup of 15 DEGs included in the KEGG cell cycle pathway was applied to the gene interaction analysis in Ingenuity Pathway Analysis. All 15 genes showed potential interactions on the basis of this in silico analysis (Figure 1D). Cdk1, a member of the serine/threonine protein kinase family that interacts with multiple interphase cyclins and is essential for cell cycle progression, was central to the interaction network (Figure 1, C and D). This suggested the hypothesis that Cdk1 played a pivotal role in the cell cycle regulation of ADPKD. To verify that transcriptional change of Cdk1 is broadly applicable for both Pkd1 and Pkd2 models, Cdk1 expression was measured in wild-type, Pkd1 single-mutant Pkd1fl/fl; Pax8ERTA; TetO-cre, and Kif3α/Pkd1 double-mutant Kif3αfl/fl; Pkd1fl/fl; Pax8ERTA; TetO-cre mice. Three biologic repeats for each genotype underwent the same doxycycline induction regimen as above, and kidney mRNA was extracted at 10 weeks age when there was minimal tubule dilation present in the Pkd1 single knockout (Supplemental Figure 1). Kif3α was used for cilia inactivation in place of Iftr88 to further ensure that the results are independent of the mechanism of cilia removal. The result showed increased expression in the Pkd1 single-inactivation model compared with both wild-type and Kif3α/Pkd1 noncyst-prone models, confirming the CDCA pattern of expression changes for Cdk1 across ADPKD models (Figure 1E, left panel). Immunoblotting confirmed the increased expression of Cdk1 protein in Pkd1 single-mutant kidneys (Figure 1E, right panel). Immunocytochemical tissue staining from kidney sections showed that Cdk1 was present in cyst-lining cells from both proximal tubule and collecting duct origin in the Pkd1 single-knockout kidneys at 18 weeks of age (Supplemental Figure 2).

Loss of Cdk1 Slows Cyst Progression in an Early-Onset ADPKD Model
To examine the functional significance of Cdk1 in ADPKD, the conditional allele Cdk1fl/fl was crossed to an early-onset Pkd1 mouse model Pkd1fl/fl; Pkh1d1-cre.18 Pkh1d1-cre gives complete inactivation in the principal cells of the collecting duct by P7.9 All of the mice were examined at P24. Perinatal deletion of Cdk1 alone in collecting duct did not induce structural or functional effects on the kidney as determined by kidney-body wt ratio, cystic index, or serum urea nitrogen (Figure 2). All histologic sections used in the analysis are shown in Supplemental Figure 3. Deletion of Pkd1 alone resulted in a severe polycystic phenotype as expected. Double mutants with both Cdk1 and Pkd1 deleted by Pkh1d1-cre showed significantly reduced cyst growth, which was reflected by a 60% reduction in kidney-body wt ratio and a 34% reduction in cystic index measured by the cystic area in sagittal sections (Figure 2). Cdk1 and Pkd1 double mutant had a 54% improvement on serum urea nitrogen, indicating improved renal function compared with Pkd1 single mutants (Figure 2). These findings suggest that concomitant Cdk1 inactivation is protective against cyst growth resulting from Pkd1 loss in an early-onset rapidly progressive ADPKD.

Reduced Expression of Cdk1 Improves Cyst Phenotype in an Adult-Onset Pkd1 Mouse Model
Cyst growth in adult-onset ADPKD models is much slower than early-onset models36,37 and may more accurately represent the course of ADPKD in the majority of human patients. To determine whether the effect of Cdk1 inactivation on cyst growth is also applicable to the more indolent adult models, we introduced the conditional allele Cdk1fl/fl into the Pkd1 single-mutant Pkd1fl/fl; Pax8ERTA; TetO-cre adult model. All of the mice were administered doxycycline from P28 to P42 and examined at 18 weeks age. Similarly to the early-onset model, inactivation of Cdk1 alone along the kidney nephrons did not result in any discernible kidney phenotype, whereas Pkd1fl/fl; Pax8ERTA; TetO-cre mice showed marked polycystic
kidney disease with elevated serum urea nitrogen indicative of kidney functional impairment (Figure 3). Cdk1/Pkd1 double mutants showed a much milder phenotype than Pkd1 single mutants when evaluated by kidney-body wt ratio, cystic index, and serum urea nitrogen level (Figure 3). The selective inactivation of Cdk1 in kidney tubule cells resulted in complete absence of Cdk1 immunostaining in kidney tissue sections, confirming the role of Cdk1 in cyst

Figure 1. Increased cell cycle signatures in ADPKD models shown by RNA sequencing analysis. (A) Venn diagram of DEGs. The center circle shows the 241 DEGs with the same direction change in Pkd2 single knockouts relative to both the wild type and Ift88/Pkd2 double-knockout controls. Ten-week-old mice were used (Supplemental Figure 1); n = 3 for each genotype. (B) KEGG pathway analysis using 241 DEGs. Only pathways that pass FDR < 0.05 are shown. (C) Volcano plots of statistical significance against fold change for the indicated genotype pairs. Above the horizontal dashed lines, FDR is < 0.05; the vertical dashed lines mark the two-fold change threshold. The final group of 241 DEGs identified that are significantly dysregulated in the same direction of change between Pkd2 single knockouts and both the wild type and Ift88/Pkd2 knockouts is labeled with blue dots. Representative DEGs in the KEGG cell cycle pathway analysis are marked with the gene names and red dots. (D) Gene interaction analysis using 15 of 241 DEGs included in the KEGG cell cycle pathway. Cdk1 is one of the central nodes (red box); the labeled representative DEGs in (C) are shown in blue boxes. (E, left panel) Quantitative real-time PCR validation of Cdk1 expression in Pkd1 mouse models: wild type (WT), Pkd1fl/fl;Pax8rtTA;TetO-cre (Pkd1), and Kif3afl/fl;Pkd1fl/fl;Pax8rtTA;TetO-cre (Kif3a/Pkd1); n = 3 for each group. Gene expression was normalized to Gapdh. One-way ANOVA was followed by Tukey multiple comparison adjustment; mean ± SEM. *P < 0.05; **P < 0.01. (E, right panel) Immunoblot showing increased Cdk1 protein in 10-week-old Pkd1fl/fl;Pax8rtTA;TetO-cre mice compared with the wild-type (WT) control. The upper band of the Cdk1 doublet is the phosphorylated form. Hsp90 was used as the loading control.
progression and the role of cyst progression in the increased expression of Cdk1 (Supplemental Figure 2). All histologic sections used are shown in Supplemental Figure 4. Interestingly, even loss of one-copy $Cdk1$ in $Cdk1^{fl/fl};Pkd1^{fl/fl};Pax8^{rtTA};TetO-cre$ mice significantly improved cystic burden in $Pkd1$ mutant (Figure 3). The cystic index suggested that inactivation of a single copy of $Cdk1$ resulted in an intermediate phenotype between the severe $Pkd1$-only knockout and the $Cdk1/Pkd1$ homozygous double knockout. The results in the adult-onset ADPKD model further demonstrate the essential role of $Cdk1$ in progression of cyst growth and renal dysfunction in ADPKD.

**Cdk1 Inactivation Reduces Cell Proliferation in Cystic Kidneys**

To determine the mechanism by which inactivation of $Cdk1$ slows cyst growth, we examined proliferation in cyst-lining cells by measuring EdU incorporation during 4 hours after intraperitoneal injection. We quantified the rate of nephron segment–specific cyst cell DNA synthesis by determining the percentage of nuclei from cells marked by segment-specific markers that were positive for EdU in both the early-onset $Pkd1$-only knockout and adult-onset $Pax8^{rtTA};TetO-cre$ models. Collecting duct cysts marked by lectin DBA in $Pkhd1-cre$ model at P24 showed approximately 0.2% EdU-positive nuclei in wild-type kidneys and approximately 2.5% EdU-positive nuclei in $Pkd1$ single mutants (Figure 4A). $Cdk1/Pkd1$ double mutants had significantly reduced EdU incorporation rates (approximately 1%) when compared with the $Pkd1$ single mutants. In the adult-onset $Pax8^{rtTA};TetO-cre$ model at 18 weeks of age, collecting ducts had approximately 1.4% nuclei with EdU incorporation in the $Pkd1$-only cystic models. Concomitant inactivation of $Cdk1$ significantly reduced the EdU incorporation to approximately 0.4% (Figure 4B). Proximal tubules marked by LTA showed similarly increased proliferation in the $Pkd1$-only knockout and significant reduction when $Cdk1$ was also inactivated.
inactivated (Figure 4C). We excluded effects of Cdk1 inactivation on cyst cell apoptosis using similar quantitative analyses with TUNEL. The late-stage polycystic kidneys in both early and late models show a modest elevation in apoptotic rates in cyst cells, but these rates did not further significantly increase when Cdk1 was inactivated (Supplemental Figure 5). Cdk1 inactivation slows ADPKD progression by reducing cyst cell proliferation.

The increased proliferation in the Pkd1-only mutants was associated with increased expression of Cdk1 protein as well as of Pcna and phosphohistone 3 in whole-kidney lysates from the adult Pax8rtTA;TetO-cre models at 18 weeks (Figure 4D). Pcna and phosphohistone H3 expression returned to baseline levels in the Cdk1/Pkd1 double knockouts. Although Cdk1 is known to be responsible for G2-M–phase regulation, reduction of EdU incorporation in the Cdk1/Pkd1 double mutants implicated loss of Cdk1 in inhibition of DNA replication, which occurs in S phase of cell cycle. To determine whether Cdk1 loss affects the earlier G1-S phase as well, the expression of G1-S–phase regulatory factors Cyclin D1 and Cyclin E1 was determined in the adult-onset model. Both cyclins showed increased expression in Pkd1 single mutant, whereas the expression in Cdk1/Pkd1 double mutant was indistinguishable from the wild type (Figure 4E). Finally, quantitative real-time RT-PCR of several genes involved in DNA replication and expressed in S phase consistently showed a reduced expression in the Cdk1/Pkd1 double mutants compared with the Pkd1-only mutant kidneys from the 18-week-old adult-onset model. These results show that deletion of Cdk1 slows cyst cell proliferation, possibly by inhibiting the G1-S phase of the cell cycle. Dysregulation of Cdk1 is a critical early driver of proliferation in ADPKD cyst cells. Selective targeting of cyst cell proliferation is an effective means of significantly slowing ADPKD progression.

DISCUSSION

There remain persistent gaps in understanding of in vivo polycystin function underlying ADPKD. To address this in this study, we began with an unbiased discovery approach in mouse kidney models on the basis of orthologous ADPKD genes and followed this by validation using in vivo systems. Our studies incorporated several unique features not previously applied to pathway discovery in ADPKD. First, our study was on the basis of a mouse model that uses the conditional adult inactivation of Pkd1 or Pkd2 along much of the nephron by the Pax8rtTA; TetO-Cre digenic system. This offers two
Figure 4. Loss of Cdk1 reduces cell proliferation of cyst-lining cells by inhibiting G1-S phase. (A) The aggregate quantitative data at P24 along with the representative images of the percentage of EdU-positive nuclei (red) in DBA-positive collecting duct cells (green) in the early-onset model with the indicated color-coded genotypes. (B and C) The aggregate quantitative data along with the representative images at 18 weeks for the percentage of EdU-positive nuclei (red) in (B) DBA-positive collecting duct cells (green) and (C) LTA-positive proximal tubule cells (green) with the indicated color-coded genotypes. Quantification in (A–C) was done by counting the number of EdU-positive nuclei among at least 1000 DBA-positive or LTA-positive cells from each mouse. The biologic replicate numbers are noted in each panel. (D) Immunoblotting for Cdk1, PCNA, and phosphohistone 3 (p-H3) in the adult Pax8rtTA;TetO-cre models at 18 weeks. The upper band of the doublet in the Cdk1 blot is the phosphorylated form of Cdk1. The lower band in the doublet on the p-H3 blot is the nonphosphorylated form of histone 3. Hsp90 was used as loading control. (E) G1-S–phase markers Cyclin D1 and Cyclin E1 in the adult Pax8rtTA;TetO-cre models at 18 weeks. Hsp90 was used as loading control. (F) Quantitative real-time RT-PCR of multiple S-phase genes in 18-week-old adult model kidneys. All of the gene expressions were normalized to Gapdh. One-way ANOVA followed by Tukey multiple comparison adjustment was used in (A–C and F); mean ± SEM. Cdk1 + Pkd1, Cdk1fl/fl;Pkd1fl/fl;Pax8rtTA;TetO-cre; Pkd1, Pkd1fl/fl;Pax8rtTA;TetO-cre; WT, wild type. *P<0.05; **P<0.01; ***P<0.001; ****P<0.001. Scale bar, 40 μm.
potential advantages. By virtue of its broad Cre activity along much of the nephron, it offered the potential for a robust Pkd-specific signal, even though using whole-kidney bulk RNA sequencing. More importantly, it allowed us to interrogate an early stage following doxycycline-induced gene inactivation when polycystin proteins disappear from the nephron but there is only mild tubule dilation without frank cysts. Access to this early stage is a unique benefit of the mouse model that cannot be achieved in human tissue studies, which are most often accessible at end stage when a multitude of secondary processes, including infection, inflammation, and fibrosis, masks the earliest polycystin-dependent functional alterations. Second, we used a tripartite rather than binary comparison system to define DEGs. We took advantage of our previous work showing that concomitant inactivation of Pkd and cilia markedly reduces cyst formation.9 We focused on transcriptional changes observed in the Pkd-only knockout that are significantly different in the same direction of change from both the wild type and the cilia and Pkd double knockout.

We defined a list of 241 genes that are likely to be a resource describing the early transcriptional changes specifically correlated with the propensity to form cysts with time. Several features indicate that this gene set has validity and value. For example, one of the upregulated genes is Mki67, with a more than two-fold increased expression only in kidneys destined to become polycystic. Ki67 has been extensively shown to be a marker for cyst cell proliferation in ADPKD.18,20,39 Notably, the most strongly upregulated KEGG pathway in this gene set is the cell cycle, which is in keeping with earlier studies. Aberrant cell proliferation of cyst-lining cells is a hallmark of ADPKD in both human and mouse.9,19–21 Nonetheless, there is still a lack of understanding specific molecular components that affect cell proliferation in ADPKD. We defined at least 15 genes involved in cell cycle regulation (14 of 15 were upregulated) whose expression in precystic stage of ADPKD fits a pattern consistent with CDCA.

Cdk1, which encodes an essential Ser/Thr protein kinase for cell cycle progression, is one of the most significantly upregulated of the cell cycle genes in Pkd2 mutants and is also significantly upregulated in Pkd1 models. Inactivation of Cdk1 in cyst cells significantly protects against cyst growth in both early- and late-onset ADPKD models, and in the case of the adult models, it suggests a gene dosage relationship with cyst growth. These studies provide in vivo genetic validation that the unbiased discovery paradigm we used can identify transcriptional alterations that interact functionally with cyst growth in ADPKD. Although we selected one gene for further investigation in this study, we anticipate that evaluation of other DEGs from the gene set defined in this study may yield additional insights into the mechanisms of ADPKD and perhaps even identify specific components of CDCA.

The discovery of Cdk1 as an essential regulator of cyst-lining cell proliferation is in line with previous studies on cell proliferation inhibition in ADPKD. The nonselective, broad-spectrum CDK inhibitors roscovitine and S-CR8, which target at least Cdk1, Cdk2, Cdk5, and Cdk7,40 were reported to be beneficial in slowing cyst growth when applied to both nonorthologous and orthologous ADPKD mouse models.41,42 Our finding of the strong protective effect of Cdk1 inactivation on cyst growth suggests that action of roscovitine and other broad-spectrum CDK inhibitors on Cdk1 may be sufficient to explain the beneficial effects in ADPKD. This conclusion is further supported by a recent study on the role of Cdk2 in cystic kidney disease; it showed that Cdk2 inactivation, which also caused reduced expression of Cdk5, was not able to suppress cyst formation.43 Neither Cdk2 nor Cdk5 were found dysregulated in our study.

The MAPK/ERK pathway has been suggested to be involved in cyst growth by affecting cell proliferation.22–24 The activation of receptors, including receptor tyrosine kinases, GPCRs, and integrins, could lead to the phosphorylation of MAPK/ERK. The phosphorylated ERK would then activate various transcriptional factors, including c-Myc, c-Fos, and Foxm1.44,45 Foxm1 is an indispensable transcriptional factor for cell cycle progression and has been shown to be the upstream regulator of Cdk1 expression.46 Thus, it is conceivable that MAPK/ERK signaling regulates the cell proliferation of cyst-lining cells in ADPKD partially by changing Cdk1 expression level.

Cell cycle transition in higher eukaryotes is regulated by different CDKs and their activating cyclin partners. Cdk1, known as a catalytic subunit of the highly conserved protein kinase complex M phase-promoting factor, is indispensable for G2-M–phase transition,38 but it has a broader role as it is able to substitute for most of the other CDKs.47 Genetic studies showed that following deletion of Cdk1, mouse embryos fail to develop to the morula and blastocyst stages, whereas the simultaneous inactivation of Cdk2, Cdk3, Cdk4, and Cdk6 still supports mouse embryo organogenesis until midgestation.47 Cdk1 alone is sufficient to drive cell proliferation, and Cdk1 has the ability to regulate other phases other than G2-M phase,48 including the G1-S–phase transition.49 Our in vivo mechanistic studies in cystic kidneys show that in cystic cells, the role of Cdk1 is not confined to G2-M–phase regulation and likely participates in the G1-S phase as well. EdU incorporation and the expression of DNA replication–related genes were found to be significantly elevated in Pkd1-only knockouts and reduced to wild-type levels in Cdk1/Pkd1 double mutants, indicating that S phase was disrupted due to loss of Cdk1. The reduction of G1-S–phase regulation factors Cyclin D1 and Cyclin E1 provided further evidence of dysregulated G1-S phase after inactivation of Cdk1 in cyst epithelial cells. Therefore, it can be suggested that Cdk1 is a master regulator of cell proliferation in cyst-lining cells of ADPKD. Discovery of upstream regulators of Cdk1 expression following loss of polycystins may help identify the links to the CDCA pathway, to polycystin function, and to the molecular determinants of proliferative signals that accompany cyst growth in ADPKD.
DISCLOSURES
S. Somlo is a founder, shareholder, consultant, and scientific advisory board member for Goldfinch Bio. He also receives honoraria and consulting fees from Otsuka Pharmaceuticals. All remaining authors have nothing to disclose.

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S. Somlo and C. Zhang designed the experiments and cowrote the manuscript; B. Balbo, M. Ma, X. Tian, and C. Zhang performed experiments; B. Balbo and C. Zhang produced the figures; and Y. Kluger and J. Zhao performed bioinformatics analyses.

SUPPLEMENTAL MATERIAL
This article contains the following supplemental material online at http://jasn.asnjournals.org/lookup/suppl/doi:10.1681/ASN.2020040511/-/DCSupplemental.

Supplemental Figure 1. Kidney histology in the Pax8rtTA;TetO-cre models at 10 weeks.

Supplemental Figure 2. Cdk1 immunocytochemistry in the Pax8rtTA;TetO-cre models at 18 weeks.

Supplemental Figure 3. Images of all kidney histologic sections used in Figure 2.

Supplemental Figure 4. Images of all kidney histologic sections used in Figure 5.

Supplemental Figure 5. Apoptosis rates in Pkhdl-Cre models at P24 and Pax8rtTA;TetO-cre models at 18 weeks.

Supplemental Table 1. Differentially expressed genes from RNA sequencing.

REFERENCES
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