**Pkd1** and **Nek8** mutations affect cell-cell adhesion and cilia in cysts formed in kidney organ cultures

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**Abstract**

Autosomal dominant polycystic kidney disease (ADPKD) is the most common genetic disease in humans, with an incidence of ~1:500–1:1,000 (reviewed in Refs. 21, 37, 38, and 51). It is characterized by the development of fluid-filled cysts in the kidneys, liver, and other organs in affected individuals. Cyst progression occurs through a combination of increased epithelial cell proliferation, apoptosis, and fluid secretion (5, 13, 52). Although there is no treatment available for PKD, recent advances in understanding the molecular basis of cystogenesis have led to the discovery of potential therapies targeting cell-cell adhesion and cilia in cysts formed in kidney organ cultures. Am J Physiol Renal Physiol 294: F73–F83, 2008. First published October 10, 2007; doi:10.1152/ajprenal.00362.2007.—Development of novel therapies for polycystic kidney disease (PKD) requires assays that adequately reflect disease biology and are adaptable to high-throughput screening. Here we describe an embryonic cystic kidney organ culture model and demonstrate that a new mutant allele of the *Pkd1* gene (*Pkd1*tm1Bdgz) modulates cystogenesis in this model. Cyst formation induced by cAMP is influenced by the dosage of the mutant allele: *Pkd1*tm1Bdgz−/− cultures develop a larger cystic area compared with +/+ counterparts, while *Pkd1*tm1Bdgz+/− cultures show an intermediate phenotype. A similar relationship between the degree of cystogenesis and mutant gene dosage is seen in cystic kidney organ cultures derived from mice with a mutated *Nek8* gene (*Nek8*ko). Both *Pkd1*−/− and *Nek8*−/− cultures display altered cell-cell junctions, with reduced E-cadherin expression and altered desmosomal protein expression, similar to ADPKD epithelia. Additionally, characteristic ciliary abnormalities are identified in cystic kidney cultures, with elevated ciliary polycystin 1 expression in *Nek8* homozygous cultures and elevated ciliary *Nek8* protein expression in *Pkd1* homozygotes. These data suggest that the *Nek8* and *Pkd1* genes function in a common pathway to regulate cystogenesis. Moreover, compound *Pkd1* and *Nek8* heterozygous adult mice develop a more aggressive cystic disease than animals with a mutation in either gene alone. Finally, we validate the kidney organ culture cystogenesis assay as a therapeutic testing platform using the CDK inhibitor R-roscovitine. Therefore, embryonic kidney organ culture represents a relevant model for studying molecular cystogenesis and a rapid tool for the screening of therapies that block cystic growth.

**Polycystic kidney disease; roscovitine; renal disease**

**Autosomal dominant polycystic kidney disease (ADPKD)** is the most common genetic disease in humans, with an incidence of ~1:500–1:1,000 (reviewed in Refs. 21, 37, 38, and 51). It is characterized by the development of fluid-filled cysts in the kidneys, liver, and other organs in affected individuals. Cyst progression occurs through a combination of increased epithelial cell proliferation, apoptosis, and fluid secretion (5, 13, 52). Although there is no treatment available for PKD, recent advances in understanding the molecular basis of cystogenesis have led to the discovery of potential therapies targeting cAMP-activated pathways and mammalian target of rapamycin signaling (10, 12, 45). We have therapeutically explored a link between the ciliary dysfunction seen in PKD and cell cycle dysregulation and found that the CDK inhibitor R-roscovitine was highly effective in preclinical models (10).

Effective development of therapeutic interventions requires relevant in vitro and in vivo models that can be utilized to rapidly optimize drug properties, formulation, and dosing schedule. A robust in vitro cystogenesis assay has been successfully used by us and others to identify multiple compounds that antagonize cyst development (9, 10, 24). Several PKD in vivo models with mutations in different cystogenes, including the *Pkd1* and *Pkd2* genes, are available and have been used for preclinical testing (14, 48). A model with a mutation in the *Pkd1* gene would be highly desirable because ~85% of all cases of ADPKD result from mutations in the *Pkd1* gene, encoding the polycystin 1 (PC-1) protein (19). PC-1 is a multifunctional protein with distinct roles in cell-matrix interactions, cell-cell adhesion, cell cycle regulation, and ciliary mechanotransduction (9, 25, 35, 42, 44). Cells derived from ADPKD epithelia display disruption of adherens junctions (characterized by a loss of E-cadherin and an increase of N-cadherin) and desmosomal junctions (characterized by intracellular retention of desmosomal proteins) (18, 43, 46). PC-1 has also been localized to the primary cilia, where it functions in concert with polycystin-2 (PC-2) as a mechanosensor regulating intracellular calcium in response to fluid flow (15, 35, 36, 41, 49, 54). It has been shown that mice heterozygous for targeted mutations in the *Pkd1* gene develop few kidney and liver cysts, with a high degree of variability late in life, and are not suitable as a preclinical model (27, 53). On the other hand, animals homozygous for *Pkd1* mutations represent an attractive model, because they uniformly develop kidney and pancreatic cysts by 16 days of gestation, but die during embryogenesis (22, 28, 29). Maternally administered pioglitazone was shown to inhibit cystic disease in *Pkd1* homozygous embryos (34); however, many compounds will not cross the placenta efficiently.

In an effort to develop a rapid cystic assay based on a *Pkd1*-targeted mutation, we explored the use of embryonic kidney organ culture. We therefore sought to characterize cyst formation in kidney organ culture, using embryos carrying a mutation in the *Pkd1* gene as a model for therapeutic development. Here we report the production of a novel *Pkd1* mutant mouse strain (designated the *Pkd1*tm1Bdgz allele, hereafter referred to as the *Pkd1*− allele), establishment of a kidney organ...
culture assay of cystogenesis, and show its relevance to the molecular pathology of human cystic disease. We demonstrate increased cystogenesis in kidneys derived from *Pkd1*/*−−* embryos compared with wild-type controls in a cAMP-induced embryonic organ culture assay, while heterozygous kidneys demonstrate an intermediate phenotype. Interestingly, *Nek8*<sup>−−/−−</sup> (hereafter referred to as *Nek8*<sup>−−/−</sup>) kidneys also show increased cystogenesis compared with wild-type kidneys in this assay, with heterozygotes showing an intermediate phenotype. We show that, similar to ADPKD, epithelial cells lining cysts in the *Pkd1*/*−−* or *Nek8*/*−−* cultures show abnormal cell-cell junctions, with loss of E-cadherin expression. We also show that expression patterns of PC-1 and Nek8 in primary cilia of cysts induced in organ culture resembles the in vivo expression patterns. Thus Nek8 expression is increased in the primary cilia of *Pkd1*/*−−* cystic epithelial cells, while *Pkd1* expression is enhanced in *Nek8*/*−−* cystic cells, suggesting that these proteins may function in the same molecular pathway. In support of this notion, we show that cystogenesis is more aggressive in *Pkd1*/*−−*:*Nek8*/*−−* compound heterozygous animals than cystogenesis in either individual heterozygotes alone, demonstrating a genetic interaction in vivo. Finally, we validate the cystic organ culture assay as a drug-screening tool by testing the effects of R-roscovitine, a Cdk inhibitor that has been shown to inhibit cystogenesis in *Nek8*/*−−* mice in vivo. R-roscovitine effectively inhibits cyst formation in both *Pkd1*/*−−* and *Nek8*/*−−* cultures. Taken together, our results demonstrate that the cystic epithelia in the embryonic organ culture assay reflects multiple characteristics of ADPKD epithelia. This assay may therefore be a valuable

Fig. 1. *Pkd1* gene-targeting strategy. *A*: schematic structure of the *Pkd1* gene-targeting construct. Exons are indicated by black bars; restriction sites are shown above the schematic. The expected restriction fragments recognized by the 5′- and 3′-targeting probes are shown by the lines above and below the schematic, respectively. Due to an *Sphl* restriction fragment-length polymorphism (indicated by the *Sphl*<sup>+</sup>), the 3′-probe fragment recognizes a 3-kb band from C57BL/6 DNA and a 12-kb band from 129SV DNA. The actual targeting result showed that a portion of exons 12–15 was duplicated and inverted (shown in grey with double-headed arrow above schematic), and exons 15–26 were duplicated. Note that, due to the duplication, the 3′-probe should recognize 2 bands on Southern blot analysis (15 and 5.5 kb). *B*: Southern blot analysis of the targeted allele. Results from the 5′-targeting probe are shown to the left; the 3′-probe results are shown to the right. Note the presence of 2 bands in the homozygous mutant DNA with the 3′-probe fragment. *C*: Northern blot analysis of the targeting result. Ten micrograms of adult kidney RNA from 4 independent wild-type (+/+) or heterozygous mutant (+/−) animals is shown. A single band is detected in the wild-type samples in the 12-kb size range. An additional, more slowly migrating band is detected in the heterozygous mutant samples (*).
addition to the in vitro testing regimens used to develop drugs to treat PKD.

MATERIALS AND METHODS

Knockout mouse production. A 129/Sv Lambda FixII library (Stratagene, LaJolla, CA) was screened by hybridization to 32P-labeled oligonucleotides covering intron 21 of the mouse *Pkd1* gene using standard procedures (11). Oligonucleotide sequences used as probes were 5'-GGGACTTTTGGGTAGGTGTCGTC-3' and 5'-AGTGGTTCCAGGTAGCTG-3'. A genomic clone covering exons 12–26 of the mouse *Pkd1* gene was isolated. A gene-targeting construct was generated that replaces part of exon 15 and all of exon 16 with a neomycin resistance cassette; the short arm covers intron 12 to the *NheI* site in exon 15, and the long arm extends from the *HindIII* site in intron 16 through the *SacI* site in intron 21. An external *NorI* site was used to linearize the targeting construct before electroporation. R1 embryonic stem cells were electroporated and maintained as described (32). Genomic DNA was prepared from isolated colonies, digested with *SphI*, and Southern blot analysis was performed as described (32). The following primers were used for PCR-based genotyping: primer 1, 5'-ACTGCCTTCGGTGCCAGCCTTT-3' and primer 2, 5'-AATGCCAGCCGACAGTGT-3'. The 5'- and 3'-probe fragments used for Southern blot analysis cover intron 11 and exon 26, respectively. Selected embryonic stem cells were injected into host blastocysts for chimeric animal production as described elsewhere (32).

Genotyping. DNA was isolated from tail samples as described elsewhere (23). The following primers were used for PCR-based *Pkd1* genotyping: primer 1, 5'-ACTGCCTTCGGTGCCAGCCTTT-3' and primer 2, 5'-AATGCCAGCCGACAGTGT-3'. Thirty-five cycles of PCR were performed in the presence of 1.5 mM MgCl2 using Platinum Taq DNA polymerase (Invitrogen, Carlsbad, CA). The cycling parameters were 94°C for 30 s, 55°C for 1 min, and 72°C for 1 min. Hold. PCR products were electrophoresed on 2% NuSieve agarose gels and visualized with ethidium bromide. Nekn8 genotyping was performed as described previously (47).

Northern blot analysis. Total RNA was isolated from adult kidney samples using TRizol reagent (Invitrogen) following the manufacturer’s protocol. Ten micrograms of total RNA was electrophoresed on formamide gels, transferred to Biotrans Plus membranes (ICN, Irvine, CA), and hybridized to a 32P-labeled DNA probe corresponding to the full-length mouse *Pkd1* cDNA following standard protocols (8).

Western blot analysis. Embryonic day 16 (E16) kidneys were extracted in Nonidet P-40 (NP-40) lysis buffer containing 150 mM NaCl, 1 mM EDTA, 50 mM Tris, and 1% NP-40 (LKB-Produkter, Bromma, Sweden). Kidneys were weighed and then homogenized on ice using Tissue Tearor Model 985-370 (Biospec Products, Bartlesville, OK) in seven volumes of NP-40 lysis buffer. The lysate was spun in Eppendorf tubes for 10 min at 14,000 rpm at 4°C. The supernatant was then harvested, and protein concentrations were determined with the BCA Protein Assay Kit (Pierce Biotechnology, Rockford, IL). Equal amounts were loaded on 3–8% gradient SDS-PAGE gels. Electrophoretic transfer was performed onto polyvinylidene difluoride membranes (Millipore, Billerica, MA) in SDS transfer buffer. Membranes were then probed with anti-LRR (47) or Nekn8 (26) polyclonal antibodies; GAPDH (US Biological, Swampscott, MA) immunoreactivity was used to verify equal protein loading. Primary antibodies were detected with anti-rabbit horseradish peroxidase-conjugated secondary antibodies (Promega, Madison, WI) and revealed by ECL (Amersham, Little Chalfont, Buckinghamshire, UK).

Histological analysis. Samples for histological analysis were fixed overnight in 4% paraformaldehyde in PBS before paraffin embedding following standard procedures.

Organ culture. All animals were handled in accordance with Institutional Animal Care and Use Committee guidelines. *Pkd1*+/− or Nekn8+/− males and females were mated to generate timed pregnancies; no on the day the copulation plug was observed was considered E0.5. Embryos were harvested at E13.5. Tail samples from each embryo were obtained for genotyping. Kidneys were cultured at the air-liquid interface on the surface of Transwell Filters (Corning Life Sciences, Corning, NY) in DMEM/F12 containing 10% FCS, penicillin/streptomycin, and glutamine (all from Invitrogen). For each experiment, controls were the contralateral kidney from each individ-

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Fig. 2. Phenotype of the *Pkd1* mutation. A: cystic kidneys, liver, and pancreas from heterozygous adults. Kidney cysts could be observed as early as 6 wk of age; liver cysts were not observed in animals younger than 40 wk old. Scale bars = 5 mm. B: embryonic phenotype showing gross anatomy of embryonic day 15.5 (E15.5) embryos. +/+ , *Pkd1*+/−; −/+ , *Pkd1*+/−; −/− , *Pkd1*−/−. Scale bars = 2 mm. C: histological analysis of E16.5 embryos. (K, kidney; P, pancreas; c, cysts). D: Western blot analysis of PC-1 and Nekn8 expression. Western blots from E16.5 embryos (left) or adults (right) were probed for PC-1 and Nekn8 expression. Full-length PC-1 is detectable in the wild-type embryonic kidney sample, but not the *Pkd1*−/− kidney sample. In a wild-type adult kidney samples, a weaker but detectable full-length PC-1 band is reduced compared with wild-type controls (below the limits of detection in this exposure). Nekn8 expression is elevated in both the *Pkd1*−/− embryonic kidney samples and *Pkd1*+/− adult kidney sample, relative to wild-type controls.
ul animal. Kidneys were cultured for 24 h before the additional of 100 μM 8-bromo (Br)-cAMP (Sigma, St. Louis, MO). For some experiments, R-rosocovitine (A. G. Scientific, San Diego, CA) at 10 μM was added with the 8-Br-cAMP. Media was changed daily, and cultures were harvested 5 days after initial isolation. Cultures were photographed daily using a Zeiss Axiovert 25 inverted microscope (Carl Zeiss Microimaging, Thornwood, NY) attached to a digital camera (Hamamatsu; Bridgewater, NJ) using QED imaging software (QED Imaging, Pittsburgh, PA). Cyst percentage was calculated using MetaMorph software (Molecular Devices, Downingtown, PA); cysts were identified by thresholding images and manual intervention to eliminate false positives and negatives. Cyst percentage was expressed as the total cyst area/total surface area × 100.

Antibody and lectin staining. Primary antibodies used in this study are PC-1 LRR domain (47), Nek8 (26), desmoglein (BD Biosciences, San Jose, CA), ZO1 (Invitrogen), and E-cadherin (BD Biosciences). Primary antibodies were used at a 1:100 dilution, with the exception of E-cadherin and ZO-1, which were used at a dilution of 1:200. Minimally cross-reactive secondary antibodies conjugated to Alexa 488, Alexa 546, or Alexa 647 were purchased from Invitrogen and were used at a 1:200 dilution. Organ cultures were fixed with ice-cold methanol overnight (PC-1, desmoglein, ZO1, and E-cadherin) or 10% acetic acid:50% methanol for 30 min (Nek8). Cultures fixed with methanol were rehydrated through a methanol:PBS series (75:25, 50:50, 25:75%) and washed with PBS before blocking and permeabilization. All cultures were blocked and permeabilized in blocking buffer (PBS+0.1% Tween 20 Tween 20+10% FCS) for 1 h before incubation with primary antibodies diluted in blocking buffer at 4°C overnight. Cultures fixed with PBS+0.1% Tween 20 Tween 20 before incubation with secondary antibodies at 4°C overnight. Cultures were washed at room temperature 4× with PBS+0.1% Tween 20 Tween 20 before a final overnight wash with PBS+0.1% Tween 20+10 μg/ml DAPI (Invitrogen) at 4°C. Cultures were mounted on glass slides with VectaShield+DAPI (Vector Laboratories, Burlingame, CA) and imaged using a Zeiss LSM510 META laser-scanning confocal microscope (Carl Zeiss Microimaging).

Quantitative RT-PCR. RNA extraction with RNase-free DNase I-digestion (Ambion/Applied Biosystems, Foster City, CA) was performed by homogenizing cultured kidneys in TRIzol reagent in the presence of 5 μg glycogen/sample (Invitrogen) following the manufacturer’s protocols. RT reactions were conducted with the extracted RNA from a pair of cultured kidneys in TaqMan RT reagent using random hexamers following the manufacturer’s recommendations (Ambion/Applied Biosystems). Fluorescently labeled TaqMan primer pairs and probe sets were acquired from the Ambion/Applied Biosystems TaqMan Gene Expression collection. The Pkd1 probe corresponds to sequences from exon 1 (Mm00465434_m1), and the mRPS7 probe corresponds to exon 4 (Mm00660332_m1). Reactions were performed using TaqMan Universal PCR master mix, run on an Applied Biosystems 7500 Real-Time PCR system quantitated using the ΔΔCt method as described by the manufacturer (normalized to mRPS7 expression; refer to ABI user bulletin 2).

RESULTS

Generation and analysis of a mouse model with a targeted mutation in the Pkd1 gene. Gene targeting was used to generate a mutation in the Pkd1 gene to create a mouse model of ADPKD (Pkd1tm1Bdgz). The targeting construct was designed to eliminate a portion of exons 15 and 16, which should result in a frameshift mutation and truncation of the protein before the first transmembrane domain, as shown in Fig. 1A. An embryonic stem cell clone that appeared to have been correctly targeted at the 3’-end was selected for blastocyst injection. However, subsequent Southern blot analysis revealed a complex rearrangement at the insertion site, resulting in a duplication of the region from exons 15–26, and a duplication and inversion of exons 12–15 (Fig. 1B). A combination of Southern blot analysis, long-range PCR, and inverse PCR was employed to determine the actual genomic organization (Fig. 1B). As expected, Northern blot analysis revealed a single transcript in the kidneys of wild-type animals, but an additional, larger transcript in the kidneys of heterozygous animals carrying the mutant allele (Fig. 1C).

Fig. 3. Cystogenesis in kidney organ culture. A: morphology of cultured kidneys from Pkd1 mutant embryos. Kidneys were isolated at E13.5 from wild-type, heterozygous, or homozygous mutant embryos derived from intercrossing Pkd1+/- males and females. Kidneys were cultured for 5 days in control media (control) or media+100 μM 8-Br-cAMP (8-Br-cAMP). Pkd1 genotype is indicated above the photographs. B: quantitation of cyst percentage by genotype. Kidneys from Pkd1 mutant embryos were cultured for 5 days in the presence of 100 μM 8-Br-cAMP, and cyst percentage was quantitated on day 5 using MetaMorph software. Mean percentage is indicated; error bars indicate SE. C: Morphology of cultured kidneys from Nek8 mutant embryos. Kidneys were isolated from wild-type, heterozygous, or homozygous mutant E13.5 embryos derived from intercrossing Nek8+/- males and females. Kidneys were cultured for 5 days in control media (control) or media+100 μM 8-Br-cAMP (8-Br-cAMP). Nek8 genotype is indicated above the photographs. D: quantitation of cyst percentage by genotype. Kidneys from Nek8 mutant embryos were cultured for 5 days in the presence of 100 μM 8-Br-cAMP, and cyst percentage was quantitated on day 5 using MetaMorph software. Mean percentage is indicated; error bars indicate SE. Scale bars = 1 mm.
It has previously been reported that mice carrying targeted mutations in the *Pkd1* gene develop cysts after 36 wk of age (27). To determine the effects of the disruption within the *Pkd1* gene, 80 animals heterozygous for the *Pkd1tm1Bdgz* mutation were analyzed between 6 and 78 wk of age. Gross morphology revealed surface cysts of the kidney, liver, and pancreas in ~19, 9, and 1% of animals, respectively (Fig. 2A). Liver and pancreatic cysts were only detected in *Pkd1*+/− animals from 40 wk of age. Although 90% of animals with kidney cysts were older than 24 wk, rare animals developed kidney cysts as early as 6 wk of age. Histological analysis of the kidneys revealed cyst formation or tubular dilation in ~30% of animals. In contrast to the animals with kidney cysts, 45% of animals with kidney tubular dilation were less than 24 wk of age. Pancreatic cysts occurred in only one animal that was 42 wk old (Fig. 2A).

Therefore, the *Pkd1tm1Bdgz* mutation affects multiple organs to generate an ADPKD-like phenotype in heterozygous adult mice.

*Pkd1*+/−/− embryos were analyzed at multiple stages of development. No homozygous mutant animals were identified at weaning, suggesting that the mutation is lethal, similar to previously described *Pkd1*-targeted mutations (6, 22, 28, 29). Mutant embryos developed edema by E13.5, which progressed to severe edema by E16.5 (Fig. 2B). Homozygous mutant embryos developed pancreatic cysts by E15.5, and polycystic kidneys by E16.5 (Fig. 2C). Heterozygous embryos have a normal phenotype.

We have recently demonstrated that PC-1 expression is altered in another mouse model of PKD, the *jck* mouse, which harbors a mutation in the *Nek8* gene (47). Mutant Nek8 is overexpressed and mislocalized in *jck* cystic cells, accompanied by overexpression and mislocation of PC-1, suggesting a link between these two cystoproteins. Thus we examined Nek8 expression in *Pkd1*−/− kidneys. First, we confirmed loss of PC-1 expression in *Pkd1*−/− embryos by Western blot analysis (Fig. 2D). As expected, PC-1 expression was

![Fig. 4. Cell-cell interactions in cystic epithelia. Kidneys isolated from wild-type (+/+), homozygous *Pkd1* mutant (Pkd1−/−), or homozygous mutant *Nek8* (Nek8−/−) E13.5 embryos were cultured for 5 days in the presence of 100 μM 8-Br-cAMP and stained with the antibodies indicated to the left of each row. All cultures were counterstained with DAPI (blue); in some cases, laminin staining was used to mark the basement membrane of tubules and cysts. Genotypes of the organ cultures are shown above each column. Top row: E-cadherin expression. E-cadherin (green) expression is easily detectable in the cystic epithelia in wild-type, but not *Pkd1*−/− or *Nek8*−/−, cultures. Laminin (red) was used to stain the basement membranes of tubules and cysts. Arrows indicate E-cadherin expression. Second row: N-cadherin expression. N-cadherin expression is limited to condensing mesenchyme and immature tubules in these cultures; however, N-cadherin expression is detectable in cystic epithelia in all cultures. Normal tubular regions are outlined in white. Arrows indicate N-cadherin expression. Third row: desmoglein expression. Desmoglein (red) expression is detectable at the cell-cell junctions of the normal tubular epithelia (outlined in white) of all cultures. Although desmoglein expression can be detected in cystic epithelial cells, its expression appears largely cystoplasmic regardless of genotype. Bottom row: ZO1 expression: ZO1 (green) expression is detectable in the cell-cell junctions of cystic epithelia, regardless of genotype. Laminin (red) staining indicates the basement membranes of tubules and cysts. Arrows indicate ZO1 expression. All images are optical slices obtained using confocal microscopy. C, cyst lumens; *, normal tubules. Scale bars = 20 μm.
higher in wild-type embryonic kidneys compared with wild-type adult tissue. Accordingly, PC-1 was almost undetectable in the adult Pkd1+/− heterozygous kidneys (Fig. 2D). Interestingly, the increased expression of Nek8 was detected in Pkd1−/− embryonic kidneys and even in adult Pkd1+/− heterozygote kidneys (Fig. 2D). Therefore, a mutation in the Pkd1 gene leads to altered Nek8 expression.

Development and characterization of an organ culture cystogenesis model. Although Pkd1+/−/− animals provide a relevant model for human ADPKD, disease progression in heterozygotes is too slow and too heterogeneous to provide a useful model for testing therapeutics. To overcome these limitations, we sought to develop an organ culture model of cystogenesis. Embryonic kidneys were isolated from E13.5 wild-type, Pkd1+/−, and Pkd1−/− embryos and cultured in vitro for 5 days. Cyst formation was only observed in the presence of 100 μM 8-Br-cAMP in the culture media (Fig. 3A). We noticed that cystogenesis was most prominent in the Pkd1−/− cultures. To determine whether the dosage of the Pkd1 mutant allele modulates cAMP-induced cyst formation, the percentage of cystic area in Pkd1+/-, Pkd1+-/-, and Pkd1−/- cultures was quantitated. The cyst percentage was significantly increased in Pkd1−/- kidneys compared with wild-type kidneys, while heterozygous mutant kidneys showed an intermediate phenotype (Fig. 3B), suggesting a dose-dependent relationship between mutant Pkd1 gene dosage and cystic growth.

We have previously demonstrated the utility of the Nek8−/− mice as a preclinical model of PKD (10, 47). Since mutations in the Pkd1 gene influence cystogenesis in embryonic kidney organ culture, we sought to determine whether other cysitogenic genes would have a similar effect in this system. We therefore characterized cystogenesis in similar cultures derived from kidneys of Nek8+/-, Nek8+/-, and Nek8−/- embryos. As shown in Fig. 3C, the dosage of the Nek8 mutations correlates with an increase in cystic percentage, similar to that observed for Pkd1 mutations. Quantification of these results confirmed this observation (Fig. 3D). These results suggest that a common mechanism may be involved in cystogenesis initiated by mutations in the Pkd1 gene or Nek8 gene.

Altered cell-cell adhesion in cystic cultures. We addressed whether cyst formation in kidney organ cultures parallels ADPKD cystogenesis. We and others have reported abnormal formation of cell-cell junctions in ADPKD cyst epithelial cells (18, 43, 46). To address whether similar changes occur in the organ culture cystogenesis model, we used immunofluorescent labeling to analyze the expression of proteins involved in cell-cell adhesion in organ culture. Previously, it has been shown that E-cadherin expression is reduced and mislocalized to the cytoplasm in ADPKD epithelial cells (18, 43). Therefore, we first examined E-cadherin expression in cysts induced in kidney organ cultures. In wild-type cultures, E-cadherin expression was observed at the cell-cell junctions of cystic epithelia (Fig. 4). E-cadherin expression was reduced in the cystic epithelium of Pkd1−/- cultures and Nek8−/- cultures. In cystic epithelia from the Nek8−/- cultures, some E-cadherin expression was observed to be mislocalized to the cytoplasm rather than being associated with the cell-cell junctions (Fig. 4). In all cultures, the noncystic tubular epithelial cells demonstrated normal expression of E-cadherin at the cell-cell junctions. We and others have shown previously that N-cadherin can replace E-cadherin in the cell-cell junctions of ADPKD cystic epithelial cells (18, 43). Therefore, we next examined N-cadherin expression in these cultures. N-cadherin expression was not detected in normal epithelia (Fig. 4) but was detected in condensing mesenchyme in these cultures (not shown). N-cadherin expression was detectable in cystic epithelia regardless of genotype (Fig. 4). This was true even in cystic epithelia from wild-type cultures, which express E-cadherin. Therefore, expression of N-cadherin in cystic epithelial cells occurs independently of the loss of E-cadherin.

Desmosomal junctions have also been shown to be disrupted in cystic epithelia from individuals with ADPKD (43, 46). We therefore characterized desmoglein (Fig. 4) and desmoplakin (not shown) expression in these cultures. We found no desmoglein or desmoplakin at the cell-cell junctions of cystic epithelia in any cultures. Similar to ADPKD, tight junctions (marked by ZO1) were unaffected in cysts in all cultures (Fig. 4). These data demonstrate that many of the pathological changes observed in the cytoarchitecture of cystic epithelial cells in ADPKD are recapitulated in the cystic epithelial cells in this organ culture model. Furthermore, the characteristic loss of E-cadherin expression observed in ADPKD is not observed in wild-type cysts induced in kidney organ cultures but is observed in the Pkd1−/- and Nek8−/- epithelium. This suggests that cysts formed in Pkd1−/- or Nek8−/- kidneys more accurately reproduce the changes observed in ADPKD than those formed in wild-type cultures.

Differential expression of PC-1 and Nek8 in cilia of cysts induced in organ culture models. We have shown previously that the Nek8 mutation in jck mice results in the lengthening of cilia, loss of ciliary localization for Nek8, and enhanced expression of PC-1 along the ciliary length in renal epithelia. In contrast, both proteins colocalize to cilia, with prominent basal body accentuation in wild-type kidney epithelial cells (47). We therefore sought to determine whether expression patterns of PC-1 and Nek8 are similarly affected in cysts induced in wild-type and Nek8−/- kidney organ cultures. Dual immunofluorescent staining was used to examine colocalization of...
**A**  wt  
Ac.tub  PC1  Merge

**B**  Nek8/−
Ac.tub  PC1  Merge

**C**  Pkd1/−
Ac.tub  PC1  Merge

**Diagram:**

- **A:** Wild-type (wt) kidney organ culture showing Ac.tub, PC1, and a merged image.
- **B:** Nek8/− kidney organ culture showing Ac.tub, PC1, and a merged image.
- **C:** Pkd1/− kidney organ culture showing Ac.tub, PC1, and a merged image.

**Graph:**

- **PC1 mRNA (AU):**
  - WT
  - Nek8/−

Legend:

- Ac.tub: ACTA2 tubular expression
- PC1: Proximal convoluted tubule marker
- Merge: Merged image of Ac.tub and PC1

**Note:** The images and graph illustrate the expression and localization of Ac.tub and PC1 in wild-type and mutant kidney organ cultures.
PC-1 with acetylated tubulin, a marker of cilia (Fig. 5). In cysts induced in wild-type cultures, punctate ciliary expression was detected for both the PC-1 and Nek8 proteins (Fig. 5A). In Nek8−/− cystic cultures, however, Nek8 was not expressed in cilia, while PC-1 expression was seen along the ciliary length (Fig. 5B). This was verified by quantitative RT-PCR analysis, which revealed increased Pkd1 mRNA in Nek8−/− cultures compared with wild-type cultures (Fig. 5B). Thus the expression patterns of PC-1 and Nek8 in the cystic Nek8−/− culture model parallels in vivo observations in jck mice (47). Next, we tested ciliary localization of Nek8 and PC-1 in cysts induced in Pkd1−/− cultures. Cysts induced in Pkd1−/− cultures showed no ciliary expression of PC-1, while strong punctate expression of Nek8 was found along the ciliary length (Fig. 5C). These data suggest that PC-1 and Nek8 may function in the same molecular pathway.

Cdk inhibition suppresses cystogenesis in organ cultures. Recently, we have demonstrated that the CDK inhibitor R-roscovitine effectively inhibits cystogenesis in vivo in Nek8−/− mice (10). To validate the cystic kidney organ culture model as a suitable model of cystogenesis, we first asked whether R-roscovitine would function similarly in the Nek8−/− organ culture model. R-roscovitine was added to Nek8+/, Nek8−/−, and Nek8−/− cystic cultures (Fig. 6A). R-roscovitine significantly inhibited cyst growth in Nek8−/− and Nek8+/− cultures (Fig. 6B). Therefore, a compound shown to inhibit cystogenesis in the jck mouse model in vivo can inhibit cystogenesis in the jck organ culture model. We then examined the effect of R-roscovitine in Pkd1 mutant cultures (Fig. 6C). Strong inhibition of cystic growth was detected in Pkd1−/− and Pkd1+/− cultures treated with R-roscovitine (Fig. 6D). This effect was reversible on washout of roscovitine (data not shown), as has been observed for other inhibitors of cystogenesis in organ culture (30). Our data suggest that the cystic organ culture model may be a very useful tool for rapid screening of compounds.

**Pkd1 and nek8 interact genetically to modulate cystogenesis in vivo.** The Pkd1−/− and Nek8−/− organ cultures demonstrate a number of similarities, including a similar modulation of cyst size in response to cAMP, similar abnormalities in cell-cell adhesion, and ciliary expression patterns. These data suggest that these two proteins may function in a common pathway to regulate cystogenesis. To test this possibility in vivo, cystogenesis was assessed in 16-wk-old animals carrying heterozygous mutations in the *Pkd1* or *Nek8* genes, either alone or in combination. To generate these animals, *Pkd1+/−* males were mated with *Nek8+/−* females. As indicated in Table 1, animals heterozygous for a mutation in both the *Pkd1* and *Nek8* genes showed an increased frequency of cystogenesis compared with animals heterozygous for a mutation in either gene alone. We have shown previously that *Nek8−/−* mice display gender dimorphism in PKD progression, with a more aggressive disease in males. Gender dimorphism was not observed in the cohort of 16-wk-old *Nek8+/−;Pkd1+/−* compound heterozygotes analyzed in this study. It is possible, however, that the gender effect on the severity of cystogenesis may be detected later in the course of the disease in the compound heterozygotes and, therefore needs to be further addressed in future studies. These results demonstrate a genetic interaction between the *Pkd1* and *Nek8* genes, further suggesting that these two genes may function in a common pathway.

**DISCUSSION**

Here we report the development and characterization of a kidney organ culture model with a novel *Pkd1* mutation and its application for therapeutic testing. The *Pkd1*-truncating mutation described herein leads to embryonic lethality in a
Importantly, the percentage of cystic tissue in cystic growth in all cultured kidneys, regardless of genotype. The addition of the cystogenic agent 8-Br-cAMP induced kidney cultures was higher than in similar to the previously described Table 1.

In models with mutations in other cystogenes. To address this the gene dosage and the level of cystogenesis can also be found in kidney and liver cysts late in life, similar to other reported kidney and pancreatic cysts. Heterozygous adults display homozygous state, with massive edema and development of kidney and pancreatic cysts. Heterozygous adults display kidney and liver cysts late in life, similar to other reported Pkd1 mutant alleles (22, 27–29). Development of effective therapies requires in vitro models that adequately reflect molecular cystogenesis in vivo to screen a large number of compounds that can be subsequently tested using animal models. A robust and reproducible in vitro assay of cystogenesis based on Madin-Darby canine kidney cells has been developed and utilized by us and others for testing multiple drugs (9, 33). It is important to note that the Pkd1 gene is not mutated in Madin-Darby canine kidney cells (9), although it may be possible to modulate Pkd1 expression through the use of shRNA technologies. Although this assay is highly effective as a tool for a high-throughput initial screen, secondary in vitro models carrying a mutation in the Pkd1 gene would be extremely useful in rapidly screening a group of compounds to select a small number of leads for preclinical testing in vivo.

In an effort to develop such a secondary assay, we explored the use of embryonic kidney organ culture as a potential model. Cyst formation can be induced in embryonic kidney organ culture by addition of glucocorticoids or cis-dichlorodiamminoplatinum to the culture media (1–3). Previous work on an aggressive mouse model of cystogenesis has demonstrated that preexisting cysts can regress in kidney organ cultures (4). We sought to characterize cyst formation in organ culture, using embryos carrying a mutation in the Pkd1 gene as a secondary in vitro model for therapeutic development. We found that Pkd1−/− kidneys do not form cysts spontaneously in culture, similar to the previously described cpl/cpl mouse model (4). The addition of the cystogenic agent 8-Br-cAMP induced cystic growth in all cultured kidneys, regardless of genotype. Importantly, the percentage of cystic tissue in Pkd1−/− kidney cultures was higher than in Pkd1+/+ cultures, with intermediate cystogenesis in Pkd1+/− cultures. Similar observations were also described in a different model, Pkd1m1Bei/m1Bei mice (30). Next, we asked whether such a correlation between the gene dosage and the level of cystogenesis can also be found in models with mutations in other cystogenes. To address this question, we have chosen the Nek8jck/jck mouse model of PKD.

We have previously shown that these mice develop PKD with multiple features common to human ADPKD, and they are useful in testing potential therapies (10, 47). Interestingly, the dosage of the Nek8 mutation correlated with an increase in cystogenesis, suggesting that both Pkd1 and Nek8 mutations may affect common pathogenic mechanisms. To further explore the link between the Pkd1 and Nek8 genes, we addressed the effect of mutations in these genes on intercellular junctions and cilia.

PC-1 has been shown to play a complex role in establishing and maintaining cell-cell junctions (20, 46, 50). Cells derived from ADPKD cysts display abnormal intercellular adhesion, with a loss of E-cadherin expression at the cell-cell junctions accompanied by an increase in N-cadherin expression (18, 46, 50). We also observed a loss of E-cadherin expression at the cell-cell junctions in Pkd1−/− and Nek8−/− cystic epithelium, but not in cysts induced in wild-type cultures. Other changes in cell-cell junctional protein expression, such as increased N-cadherin and loss of desmoglein (43, 46), occurred independently of genotype. The similarities between the pathological changes observed in the cystic epithelium of the mutant organ cultures and those observed in ADPKD suggest that this culture system provides an accurate model of cystogenesis.

Although mutations in the Pkd1 and Pkd2 genes are the most common cause of polycystic kidney disease, there are a number of other human and mouse genes that predispose to kidney cyst formation (14, 17, 48). It has recently become clear that the common link between these proteins is expression in the primary cilia (17, 48). In kidney epithelial cells, the primary cilia can act as a flow sensor; flow-induced bending of the cilia results in increased intracellular calcium (39, 40). Many of the cystoproteins localize to the primary cilia, and several are critical for control of function or formation of the primary cilia (17). The Nek8 protein localizes to the primary cilia, and mutation of the Nek8 gene results in lengthening of the cilia and enhanced expression of PC-1 along the cilia of jck kidney epithelia (31, 47). In embryonic kidney organ culture, an expansion of PC-1 expression was also observed in primary cilia from Nek8−/− animals. In Pkd1−/− cultures, increased expression of Nek8 was observed in the primary cilia. Together, these results support a link between Pkd1 and Nek8 function. One prediction of a functional link is that compound heterozygotes, with a mutation in one copy of each gene, would be more prone to cyst formation than animals heterozygous for either mutation individually. For example, such a link between the Pkd1 and Pkd2 genes has been observed in mice heterozygous for mutations in these genes and has been suggested for humans carrying a deletion of both the PKD1 and TSC2 genes in a contiguous gene syndrome (7, 16, 53). Animals heterozygous for the Nek8jck mutation have not been reported to develop cysts in vivo, while animals heterozygous for Pkd1 mutations are reported to form kidney cysts at low frequency later in life (27). When analyzed at 16 wk of age, 8 of 20 compound heterozygotes had multiple histologically detectable cysts, while no animals with either individual mutation had multiple cysts. Therefore, disease progression in animals with a mutation in one copy of the Pkd1 gene and one copy of the Nek8 gene develops more aggressively than in animals with either mutation alone, similar to the Pkd1/Pkd2 and Pkd1/Tsc2 situations. Further studies will be necessary to determine whether this is the result of a direct interaction between these proteins, a signaling cascade common to the two proteins, a regulatory interaction that influences expression or distribution of the proteins in parallel, or distinct signaling systems that converge to modulate cystogenesis. In support of organ culture as a useful and predictive model of cystogenesis, we demonstrate the ability of a CDK inhibitor, R-roscovitine, to block cystogenesis in the organ culture model. R-roscovitine was selected because it has recently been demonstrated to effectively inhibit cystogenesis in the Nek8−/jck mouse in vivo (10).

In conclusion, we have shown that cystic kidney organ cultures derived from mutant embryos display a number of

<table>
<thead>
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<th>Pkd1 Genotype</th>
<th>Nek8 Genotype</th>
<th>No. With Multiple Cysts</th>
<th>Total No Analyzed</th>
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</thead>
<tbody>
<tr>
<td>Pkd1+/+</td>
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</tr>
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<td>Nek8+/−</td>
<td>8 (20)</td>
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features of PKD in vivo, including characteristic changes in cell-cell junctional complexes and altered ciliary protein expression. Comparison of Pkd1−/− and Nek8−/− organ cultures revealed several common characteristics that suggest a common mechanism of cystogenesis; this was further supported by the observation of a genetic interaction between these two genes in vivo. Finally, we have validated the use of the embryonic kidney organ culture assay as a rapid tool for therapeutic development by demonstrating the inhibitory effects of R-roscovitine on cystogenesis in vitro. Taken together, our data show that cystogenesis induced in vitro in kidney organ culture is characterized by impaired intercellular adhesion and ciliary abnormalities observed in vivo and represents a useful model for molecular analysis of cystogenesis and therapeutic development.

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