Development of Polycystic Kidney Disease in Juvenile Cystic Kidney Mice: Insights into Pathogenesis, Ciliary Abnormalities, and Common Features with Human Disease

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Significant progress in understanding the molecular mechanisms of polycystic kidney disease (PKD) has been made in recent years. Translating this understanding into effective therapeutics will require testing in animal models that closely resemble human PKD by multiple parameters. Similar to autosomal dominant PKD, juvenile cystic kidney (jck) mice develop cysts in multiple nephron segments, including cortical collecting ducts, distal tubules, and loop of Henle. The jck mice display gender dimorphism in kidney disease progression with more aggressive disease in male mice. Gonadectomy experiments show that testosterone aggravates the severity of the disease in jck male mice, while female gonadal hormones have protective effects. EGF receptor is overexpressed and mislocalized in jck cystic epithelia, a hallmark of human disease. Increased cAMP levels in jck kidneys and activation of the B-Raf/extracellular signal–regulated kinase pathway are demonstrated. The effect of jck mutation on the expression of Nek8, a NIMA-related (never in mitosis A) kinase, and polycystins in jck cilia is shown for the first time. Nek8 overexpression and loss of ciliary localization in jck epithelia are accompanied by enhanced expression of polycystins along the cilia. The primary cilia in jck kidneys are significantly more lengthened than the cilia in wild-type mice, suggesting a role for Nek8 in controlling ciliary length. Collectively, these data demonstrate that the jck mice should be useful for testing potential therapies and for studying the molecular mechanisms that link ciliary structure/function and cystogenesis.


A utosomal dominant polycystic kidney disease (ADPKD) is characterized by formation and progressive enlargement of cysts in the kidney and other organs (reviewed in references [1–5]). Mutations in either PKD1 gene (approximately 85% of cases) or PKD2 gene (approximately 15% of cases) lead to the development of cysts in multiple segments of the nephron (6,7). Cystogenesis is characterized by increased epithelial cell proliferation and apoptosis, loss of cellular polarity, and fluid secretion (2,8,9). Molecular pathways that lead to these abnormalities have been a subject of extensive research by us and others in recent years (10–15).

Animal models of PKD have been instrumental in supporting studies that aim to understand the molecular mechanisms of cystogenesis and direct assessment of potential therapies. Recently, novel therapeutic approaches have emerged (4,16–18). A number of preclinical PKD models are available with mutations in different genes, including Pkd1 and Pkd2 genes (19). No single animal model perfectly recapitulates human ADPKD in a reasonable time frame for therapeutic testing; however, each model represents a subset of human pathology. The usefulness of a particular therapy for human PKD has been determined largely by studies that were done in more than one model (20–22). Therefore, models that more closely resemble human PKD by multiple parameters are highly desirable.

Cystic disease, whether it is caused by mutations in the PKD1 gene or by mutations in a number of other genes, seems to be similar. Common features include abnormal proliferation, protein sorting, and intracellular fluid secretion. Recent data suggest that ciliary dysfunction may represent one possible common abnormality found in different cystic diseases (23–25). The gene Nek8, encoding a member of NIMA-related (never in mitosis A) kinase family protein that is responsible for the juvenile cystic kidney (jck) mouse, was mapped recently to cilia (26–29).

Unlike cpk and pcy lines, some of the first models with pathology being studied for more than two decades, the jck model is relatively new and has not been characterized fully. Although the jck mutation is transmitted in autosomal recessive mode, it resembles human ADPKD phenotypically (19).

We set out to characterize phenotypic, cellular, and molecular aspects of cystogenesis in the jck mouse to determine its utility for testing of potential therapies for PKD. Here we report for the first time that cysts in jck kidneys are formed from multiple segments of the nephron, similar to ADPKD. We show that EGF receptor (EGFR) is overexpressed and mislocalized to the apical membranes of cystic epithelia, a hallmark of human disease. In addition to the role of the EGFR pathway in jck
cystogenesis, we demonstrate increased cAMP levels in jck kidneys, which may contribute to increased proliferation and secretion of cystic epithelia, another resemblance of ADPKD. Importantly, similar to human disease and rat models of PKD and unlike many mouse models, jck mice show gender dimorphism in the progression of cystic disease, with gonadal hormones playing a role in mediating differences in disease progression in male and female mice. Finally, we demonstrate ciliary lengthening in jck kidney epithelia with abnormal ciliary expression of mutated Nek8 and polycystins. Taken together, our data suggest that the jck model should be extremely useful in further dissection of molecular mechanisms of cystogenesis and provide a foundation for testing novel therapies in this model.

Materials and Methods

C57BL/6j jck Mouse Colony and Genotyping Assay

C57BL/6j jck/+ mice were obtained from The Jackson Laboratory (Bar Harbor, ME) and were used to establish a breeding colony that is maintained at Biomedical Research Models (Worcester, MA) and Genzyme (Framingham, MA). Genzyme and Biomedical Research Models Institutional Animal Care and Use Committees approved all studies. Genotyping of jck mice was performed using a custom TaqMan SNP genotyping assay (Applied Biosystems, Foster City, CA) to monitor the mutations described for jck Nek8 gene (26) (forward primer 5′-AGC-CAGCCCCACCATGTAGA-3′, reverse primer 5′-ACAGGGCCAGCA-CATGAGAG-3′), wild-type [WT] probe 5′-VIC-CCTTGGCTGGTATG-GMBNFQ-3′, and MT probe 5′-6FAM-CCTTGGCTTTGATGAGATG-MGBNFQ-3′ (Applied Biosystems). After denaturing at 95°C for 10 min, 40 cycles of 95°C for 15 s and 60°C for 1 min were performed. PCR products were analyzed on ABI Prism 7700 sequence detector (Applied Biosystems).

Surgeries and Biochemical Studies

Mice were anesthetized with 3 to 5% isoflurane/oxygen before castor oil, ovariecctomy, or sham operation. Vehicle or dihydrotestosterone (DHT) 50 mg/kg in 90% PEG 300/10% ethanol (vol/vol; Sigma-Aldrich, St Louis, MO) was administered subcutaneously from 26 to 64 d. Mice were killed by CO2 asphyxiation, and kidneys were removed from the mice at 26, 38, 45, 50, 64, and 100 d of age. The disease was progressive with enlargement of kidneys over time, particularly in male mice, evident by initial assessment of kidney volume to a total section area.

Histological and Quantitative Analysis of Cystogenesis

Longitudinal and cross-sections were cut at 4 μm and stained with hematoxylin and eosin with a Tissue Tek 2000 processor (Sakura Finetek, Torrance, CA). Slides were digitized with an ACIS system (Molecular Devices Corp., Dowington, PA). The severity of cystogenesis was quantified from both longitudinal and transverse sections. Cystic percentage was measured as a ratio of cystic area to a total section area.

Immunostaining

Proliferating cell nuclear antigen (PCNA) staining was performed as described in the manufacturer’s protocol for the M.O.M. kit (Vector Laboratories, Burlingame, CA). Terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) staining was performed using ApopTag Apoptosis Detection Kit (Chemicon, Temecula, CA). Lectins Dolichos Biflorus Agglutinin, Lotus Tetragonolobus Lectin (Vector Laboratories), anti-calbindin (Sigma-Aldrich), and anti-Tamm-Horsfall glycoprotein (US Biological, Swampscott, MA) antibodies were used as recommended by the manufacturers. Primary kidney epithelial cells were isolated from WT or jck kidney cortex as described previously (30). All primary cultures were used between passages 2 and 5. Epithelial origin of cells was identified using mouse anti-cytokeratin pan mAb (Chemicon International). jck and WT cell cultures were stained with anti-polyxyalin-1 (PC-1; leucine rich repeats [LRP], AA 27-360) or anti–PC-2 (AA 687-968) rabbit antisera at 1:100 dilution as described previously (11,31,32). Anti-Nek8 rabbit antibody was used at 1:200 (26). Anti-acetylated tubulin (clone 6-11B-1; Sigma-Aldrich) was used at 1:400. Secondary antibodies used were goat anti-mouse Alexa fluor 488 and goat anti-rabbit Alexa fluor 546 (Molecular Probes, Eugene, OR). Cell nuclei were stained with DAPI (Vector Laboratories). Staining was visualized on an Olympus DX70 microscope (Olympus, Melville, NY). Images were captured with QED Camera Plug-In imaging system (Pittsburgh, PA).

Scanning Electron Microscopy

Kidney vibratome sections (200 μm) were fixed in 2.5% glutaraldehyde and 0.1 M cacodylate buffer, cryoprotected in 70% ethanol, and freeze-fractured in liquid nitrogen. After fixation with 1% OsO4, sections were dehydrated with ethanol and viewed with a JEOL scanning electron microscope. The analysis was performed on kidney cortex, where cortical collecting ducts can be identified by the presence of morphologically distinct intercalated cells.

SDS-PAGE and Immunoblotting

Proteins were resolved by SDS-PAGE using 3 to 12% gradient gels and blotted as described previously (33). Membranes were blocked with 3% BSA and incubated overnight with anti–extracellular signal–regulated kinase (anti-ERK), anti–phosphorylated ERK 1/2 (Thr202/Tyr204), anti–B-Raf (Cell Signaling, Danvers, MA), and anti–EGFR (Santa Cruz Biotechnology, Santa Cruz, CA) antibodies. Equal loading of protein was controlled by anti-glyceraldehyde-3-phosphate dehydrogenase staining (US Biologic). Membranes were washed in TBST and incubated with horseradish peroxidase (HRP)-labeled secondary antibodies at 1:10,000 dilution (anti-mouse IgG-HRP, anti-rabbit IgG-HRP, or anti-goat-HRP; Promega, Madison, WI). Immunoreactive proteins were detected using enhanced chemiluminescence (Amersham Pharmacia Biotech, Little Chalfont Buckinghamshire, England).

cAMP Measurement

Frozen kidneys were homogenized in 10 vol/wt 0.1 N HCl. After centrifugation at 600 × g, supernatants were collected and assayed by competitive ELISA as recommended by the manufacturer (R&D Systems, Minneapolis, MN). Protein concentrations were determined with BCA protein assay kit (Pierce, Rockford, IL).

Statistical Analyses

Data are expressed as means ± SD. Comparisons were made by two-tailed t test, and significance was accepted at P < 0.05.

Results

Sex Dimorphism in the Course of Disease Progression in C57BL/6j jck Mice

To assess the development of polycystic kidney disease in jck animals of C57BL/6j genetic background, we analyzed mutant mice at 26, 38, 45, 50, 64, and 100 d of age. The disease was progressive with enlargement of kidneys over time, particularly in male mice, evident by initial assessment of kidney weight.
weights relative to body weights (Figure 1A). To quantify the percentage of cystic tissue, we used MetaMorph analysis of hematoxylin- and eosin-stained kidney sections. This analysis confirmed that PKD was more severe in male versus female mice at a given age. Note more aggressive increase in all of the parameters in male versus female mice during the course of the disease. *P < 0.05 male versus female mice at a given age. (B) Renal pathology of jck mice. Representative scans of hematoxylin and eosin (H&E)-stained kidney sections of jck male and female mice of 26, 45, and 64 d of age are shown. (C) Kaplan-Meier survival analysis of jck male and female mice. Percentage survival of jck male (n = 80) and female mice (n = 138) is plotted against age in weeks.

Figure 1. Disease progression in C57BL/6J juvenile cystic kidney (jck) mice. (A) Quantitative analysis of disease progression. Shown are percentage of kidney to body weight ratio (K/BW ratio), percentage of cystic tissue (Cyst %), and blood urea nitrogen (BUN; mg/dl) relative to age. Note more aggressive increase in all of the parameters in male versus female mice during the course of the disease. *P < 0.05 male versus female mice at a given age. (B) Renal pathology of jck mice. Representative scans of hematoxylin and eosin (H&E)-stained kidney sections of jck male and female mice of 26, 45, and 64 d of age are shown. (C) Kaplan-Meier survival analysis of jck male and female mice. Percentage survival of jck male (n = 80) and female mice (n = 138) is plotted against age in weeks.

Microscopic examination of the jck kidney sections revealed multiple cysts in the cortex and medulla at day 26, although the majority of kidney tissue was not affected (Figure 1B). Cysts
increased in number and size over time, and disease progressed significantly by day 45. At 64 d of age, little normal tissue remained, and large cysts were seen in both cortex and medulla (Figure 1B). No extrarenal pathology was identified in the jck line as described previously (27). Kaplan-Meier analysis was used to estimate survival in jck male and female mice (Figure 1C). The median average survival for jck female mice was 19 wk compared with male mice at 16 wk of age, showing a statistical difference ($P < 0.05$) in survival between the sexes.

**Cysts Are Formed in Multiple Nephron Segments of jck Kidneys**

We examined the origin of nephron segments that were affected by cystogenesis in jck mice early and late in the course of the disease. Immunofluorescence staining of jck female kidney sections with nephron-specific markers revealed that cysts were formed in multiple segments of the nephron. Collecting ducts (CD) were stained with Dolichos biflorus agglutinin lectin (DBA); proximal tubules (PT) were stained with Lotus tetragonolobus lectin (LTL); distal tubules (DT) were stained with anti-calbindin antibodies; thick ascending limbs of Henle (LH) were stained with Tamm-Horsfall glycoprotein (THG) antibody. Note that in the early stage of disease (26 d), the majority of cortical cysts are of CD origin (top, left), cysts of DT origin are small (top, center). Later in the disease (50 d), fast-growing DT cysts are seen (bottom, center). Medullary cysts are from LH (bottom, right). PT are not affected (top, right). (B) Dynamic of cystogenesis in jck mice. Kidney sections that were stained with nephron-specific markers were processed with MetaMorph to calculate total cystic tissue (Total) and contribution of cysts of different origin. Each time point group is represented by two mice; for each mouse, two complete kidney sections were processed (longitudinal and cross-section).
disease. Immunohistochemical staining of jck kidney sections from 26-d-old (early stage) and 50-d-old (late stage) mice was performed using markers for different parts of the nephron: proximal tubule (PT), loop of Henle (LH), distal tubule (DT), and cortical collecting duct (CD) as indicated in Figure 2. At the early stage of disease, the majority of cysts were formed in cortical CD, whereas small cysts were identified in DT (Figure 2A). At the late stage of the disease (50 d), large DT and CD cysts were evident. Medullary cysts were formed in LH, but no medullary CD cysts were detected. Proximal and glomerular cysts were not detected in jck mice. Analysis of the dynamics of cystogenesis showed that CD cysts contributed to cystogenesis early in the disease, and cysts from DT and LH continued to develop during the course of the disease and contributed most to the total cystic tissue at day 64 (Figure 2B). Such detailed understanding of jck kidney cystogenesis should be useful for testing potential therapies, because it is possible that therapeutic targets may express differentially in different nephron segments.

Similarities between Pathways of Human and jck Cystogenesis

In human PKD and some but not all animal models of cystic disease, EGFR was found to be abnormally expressed at the apical membrane of cystic cells, indicating loss of epithelial polarity (34,35). We examined the status of EGFR expression in jck kidneys. Abnormal EGFR expression was detected in apical membranes of cystic epithelia both early and late in the disease by immunofluorescence and Western blot analysis (Figure 3, A and B). Therefore, similar to human PKD, EGFR was abnormally expressed in jck cystic epithelia. We also examined cAMP levels in jck kidney because recent evidence suggests that cAMP is critical in specifically promoting proliferation in cystic cells (36,37). cAMP content was measured by ELISA in jck kidneys of 26- and 50-d-old mice (Figure 3C). Significant upregulation of cAMP levels was detected in cystic samples, and the level of cAMP increased with progression of the disease. It has been shown that cAMP activates the B-Raf/ERK pathway in human ADPKD cells; therefore, we studied the possible activation of this pathway in jck mice. Western blot analysis showed that ERK 1/2 protein expression levels are increased in jck kidneys, in addition to increased levels of ERK phosphorylation (Figure 3D). We also detected increased levels of B-Raf (both 90- and 68-kD isoforms) in jck kidneys compared with WT controls (Figure 3D). Therefore, it is likely that, similar to ADPKD, increased levels of cAMP in jck kidney play a role in activating the ERK pathway.

Figure 3. EGF receptor (EGFR) axis and cAMP-activated pathways in jck model. (A) EGFR is mislocalized to the apical membranes in cystic epithelia. Shown are kidney sections from wild-type (WT) mice (left) and jck mice of 26 (center) and 50 d of age (right) stained with anti-EGFR antibody (red). (B) Upregulation of EGFR expression in jck kidney. Shown is Western blot analysis of EGFR from kidney lysates of WT and jck mice of 26 and 50 d of age (200 μg/lane). Note the increased expression of EGFR as disease progresses. (C) Activation of cAMP pathway in jck kidneys. The level of cAMP was normalized by total amount of protein. WT kidney corresponds to 64-d-old mouse. (D) Activation of extracellular signal–regulated kinase (ERK) and B-Raf pathways in jck kidneys. Note upregulation of B-Raf, ERK 1/2, and phosphorylated ERK 1/2 (p-ERK 1/2) in jck kidney extracts (50 μg/lane).
Increased Proliferation and Apoptosis of jck Cystic Epithelia

To investigate whether increased proliferation and apoptosis contribute to jck cystogenesis, as has been shown for human PKD (38), we performed PCNA immunostaining and TUNEL labeling of WT and jck kidneys. PCNA-positive nuclei and TUNEL-positive nuclei commonly were seen in cyst-lining epithelial cells, whereas very few reactive cells were found in normal mouse kidney. Likewise, apoptotic nuclei were very common in cystic epithelia, but few were seen in normal tubular epithelial cells (Figure 4). Therefore, jck cyst enlargement was accompanied by a high rate of epithelial cell proliferation and increased apoptosis.

Sex Hormones Influence Disease Progression in jck Mice

To test whether sex hormones are responsible for differences in the rate of disease progression in jck male versus jck female mice, we studied the effect of testosterone and estrogen on cystogenesis. jck male mice were castrated or underwent sham operation at 26 d of age and were treated with testosterone analogue (DHT) until day 64. Castration slowed PKD progression in jck male mice, showing statistically significant reduction in kidney to body weight ratio, percentage of cystic tissue, and blood urea nitrogen as compared with their sham-operated counterparts (Figure 5A). Administration of DHT to castrated male mice reversed this effect (Figure 5A). Kidney histology from castrated male mice showed less cystic tissue as compared with sham-operated or DHT-treated mice, as illustrated in Figure 5C. WT mice were used as control for each surgery plus treatment group (Figure 5B). Castrated WT male mice showed no significant changes in kidney to body weight ratio compared with the sham-operated cohort. Administration of DHT to jck female mice resulted in significant aggravation of PKD (Figure 6). Therefore, testosterone seems to exacerbate cystic disease in jck mice. Ovariectomy of jck female mice resulted in acceleration of cystic disease, supporting the notion that female hormones may have a protective effect (Figure 6). We also detected an increase in jck female body weights in groups that were either ovariectomized or treated with DHT (Supplementary Table 2). In WT female mice, increased kidney to body weight ratio was detected in ovariectomized and DHT-treated groups (Figure 6B).

Abnormal Expression of Nek8 and Polycystins and Lengthening of Cilia in jck Kidney Epithelia

To determine whether Nek8 mutation in jck mice affects the mRNA expression levels of Nek8 kinase, PC-1, and PC-2, we performed real-time PCR analysis in cystic versus WT kidneys (Figure 7A). A significant upregulation of mRNA levels for Nek8, PC-1, and PC-2 was detected in jck kidneys as compared with WT. The overall expression pattern of Nek8 protein in kidney sections of jck and normal mice was described previously (26). Specifically, in WT kidneys, Nek8 was found mainly in apical location of tubular epithelial cells, whereas enhanced cytoplasmic pattern of Nek8 was seen in jck epithelia. To be able to examine ciliary localization patterns of Nek8 in WT and jck epithelia, we analyzed primary kidney epithelial cells that were derived from eight WT and eight jck mice. Nek8 showed punctate pattern of expression in primary cilia of WT cells (Figure 7B, top). In contrast, Nek8 was not detected in cilia of jck cells, where only cytoplasmic/perinuclear staining was seen (Figure 7B, top). It is interesting that ciliary expression of PC-1 and PC-2 in jck epithelia also was changed (Figure 7B, middle). Whereas PC-1 and PC-2 were localized mainly to the basal body of the cilium in WT cells, enhanced accumulation along the length of the cilium was seen clearly in jck epithelia. We also noticed that jck cilia seemed longer than WT cilia (Figure 7B, acetylated tubulin staining). Therefore, we performed ultrastructural scanning electron microscopy analysis of WT and cystic kidneys (Figure 7C). We observed clear lengthening of cilia (8.5 ± 2.5 μM) in jck mutants as compared with WT controls (2.5 ± 1.5 μM). Therefore, mutation in the Nek8 gene in jck mice results in a loss of Nek8 from cilium and leads to redistribution and enhanced expression of polycystins along the lengthened cilium.

Discussion

The development of renal tubular cysts in human ADPKD and autosomal recessive PKD is characterized by several common features: increased proliferation of cyst-lining epithelial cells, alterations in cellular polarity and protein sorting, changes in extracellular matrix composition, and fluid secretion into the cyst cavity (2,39). Cysts are formed in all segments of the nephron in ADPKD, whereas collecting ducts predominantly are affected in autosomal recessive PKD. Efforts to understand the molecular pathways of cystic disease have led to identification of possible new targets for ADPKD therapeutic

Figure 4. Increased proliferation and apoptosis in jck kidney. Kidney sections from 50-d-old WT and jck mice were stained with anti–proliferating cell nuclear antigen (anti-PCNA) antibodies (green). Bars = 400 μm as indicated. An elevated level of apoptosis in jck cystic kidney is evident by terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling staining of apoptotic cells (green).
interventions (16). For elucidation of the in vivo relevance of newly identified pathways of cystogenesis, studies in animal models of PKD are crucial. Multiple murine PKD models with mutations in different genes have been used in such studies (19). Although every model characterized so far mimics a subset of human disease, no single model fully recapitulates all aspects of human PKD pathogenesis. Several spontaneous mouse models of PKD, such as cpk, bpk, and orpk, resemble human recessive disease with respect to cyst localization and rapid rate of disease progression, whereas cystic disease in pcy mice is slowly progressive with focal cyst formation similar to ADPKD. Rapidly accumulating evidence implicates defects in the primary cilium as a major cause of cystogenesis (40). Importantly, genes that are responsible for spontaneous murine models as well as genes that cause human PKD are found to be expressed at least partially in cilia, suggesting that a unifying pathogenic mechanism underlying all cystic epithelial disorders exists (41). We set out to characterize fully the jck mouse model of PKD because the gene Nek8, which is responsible for this disease, was mapped recently to the primary cilia (26–29). It is interesting that we detected overexpression of Nek8 mRNA in jck kidneys relative to WT and loss of ciliary localization of Nek8 protein in jck kidney cells. Overexpression and mislocalization of Nek8 in jck cells was accompanied by increased expression of polycystins along the jck cilia, whereas expression in normal cells was detected mainly at the base of the cillum. NIMA-related kinases were implicated recently in regulating cilia length in Tetrahymena (42). We therefore analyzed the kidneys from jck and WT mice by scanning electron microscopy. Remarkably, the cilia in jck kidneys were significantly lengthened, suggesting that Nek8 may play an important role in controlling ciliary length. It is possible that Nek8 might regulate disassembly of cilia, because previous work demonstrated that Nek8 knockdown does not affect ciliogenesis (28).

In addition, the jck mutation resembles human ADPKD phenotypically, despite its autosomal recessive mode of inheritance (19). Several features make the jck mouse an attractive and unique model for PKD. The disease progresses relatively slowly, thereby offering a convenient time frame of 3 to 5 wk for potential therapeutic testing. The jck mouse model is strikingly different from other mouse models of PKD in that it clearly displays gender dimorphism. Male gender in ADPKD is a recognized risk factor for a more aggressive course of the disease. Therefore, males with ADPKD progress faster to ESRD than females (43). Gender as a risk factor was demonstrated recently only in rat models of PKD: Han:SPRD and PCK (44,45). To define the cause of sex dimorphism that is seen in jck mice, we addressed whether gonadal hormones might be responsible. Castration of jck male mice significantly slowed the disease progression (Figure 5).
progression, whereas exogenous administration of testosterone reversed the castration effect. Therefore, testosterone is likely to be responsible for a more aggressive course of PKD in jck male mice, whereas female hormones may have a protective effect. Further studies will be necessary to determine how exactly gonadal hormones modulate cystic growth.

We also found similarities between human dominant PKD and jck cystic disease on the basis of the analysis of specific nephron segments that are affected by cystic transformation. Human ADPKD is characterized by formation of cysts in all segments of the nephron (46,47). Unlike cpk, bpk, and orpk mouse models, which develop cysts in collecting ducts, jck mice develop cysts in multiple segments of the nephron: DT, LH, and CD but not in PT and glomerulus. Moreover, we performed quantitative analysis of cystic disease dynamic and found that cysts from CD are formed early in the course of the disease, and their contribution is not significantly changed between 3 and 5 wk of age. Cysts from DT and LH, however, continue to develop during the course of the disease. Such detailed analysis of cyst progression in the jck model should be useful for dissecting pathobiology of the disease and, most important, to set out expectations for testing potential target-specific therapies because expression of some proteins may be limited to a particular segment of the nephron.

Figure 6. Effect of ovariectomy and DHT treatment on cystogenesis in jck female mice. (A) Disease progression in four groups of animals: Sham operated (sham), ovariectomized (Ox), sham-operated and treated with DHT (sham+DHT), and Ox with DHT treatment (Ox+DHT). Note that both removal of estrogen (Ox) and addition of testosterone (DHT treatment) aggravate the disease. *P < 0.05 versus Sham. (B) Ovariectomy and DHT treatment increase kidney/body weight ratio in control WT female mice. *P < 0.05 versus Sham; †P < 0.05 versus ovariectomized. (C) Representative H&E sections from Sham, Ox, and DHT-treated female mice (64 d old). Note that Ox and DHT-treated mice have larger cysts.
Evidence from multiple groups suggests an important role for EGF/TGF-α/EGFR in the progression of human PKD. EGFR has been shown to be overexpressed and mislocalized to apical membranes of cyst-lining epithelia (48,49). Great promise has been shown in modulating EGFR tyrosine kinase activity for effective PKD treatment using the bpk mouse and Han:SPRD

**Figure 7.** Ciliary abnormalities of jck renal epithelia. (A) Real-time PCR analysis of Nek8, Pkd1, and Pkd2 mRNA expression in WT and jck kidney. The expression levels were normalized to Rps12 housekeeping gene. The results are shown as the mean and SD of three different animals. (B) Differential ciliary distribution of Nek8 and polycystins in jck and WT renal epithelia. The cilia are marked with antibody to acetylated tubulin (ac. tubulin). Note punctate ciliary expression of Nek8 in WT epithelia and loss of ciliary localization in cystic epithelial cells. Polycystin-1 (PC-1) and PC-2 are expressed mainly at the base of cilia in WT cells, whereas their expression is enhanced along the cilia in jck cells. (C) Scanning electron micrographs of renal epithelial cells in WT and jck kidney. Primary cilia are significantly lengthened in cystic kidneys.
rat; however, no benefit was seen in PCK rat (21,35). In contrast with other animal models, no abnormal expression of EGFR was detected in the PCK rat cystic epithelia, which may explain the lack of therapeutic effect in this model. We analyzed expression and localization of EGFR in jck kidney and found that similar to human ADPKD, EGFR was overexpressed and mislocalized to the apical membranes of cystic epithelial cells. These data further highlight parallels between jck cystogenesis and human disease.

Because the rate of cyst proliferation is controlled by growth factors and hormones that result in activation of cAMP pathways, we also measured the levels of cAMP in jck kidneys. Indeed, we found increased levels of cAMP in jck kidneys, correlating with the severity of the disease. Recent data demonstrated that cAMP is capable of activating B-Raf and ERK in human ADPKD-derived epithelia (36), leading to increased cellular proliferation. We also detected activation of this pathway in jck kidneys, resulting in ERK activation and increased levels of B-Raf. This observation is consistent with a recent report that described increased levels of phosphorylated ERK in PCK rats (50).

Conclusion

We provide in-depth characterization of disease progression in the jck mouse model of PKD with initial characterization of cellular and molecular defects. We show for the first time abnormal expression of Nek8 and polycystins and lengthening of cilia in jck kidney tubular epithelia. The jck disease progression is more severe in male than in female mice, similar to ADPKD and rat models, but uncommon for other mouse models described. Significant similarities between human ADPKD and jck mouse were found in molecular pathways that are affected by cystogenesis, including EGFR axis and cAMP-activated pathways, leading to increased proliferation and apoptosis in jck cystic cells. Taken together, our data strongly suggest that the jck model resembles multiple facets of human ADPKD and therefore is suitable for dissecting further molecular mechanisms of cystic disease as well as for serving as a convenient model to evaluate potential therapies directly.

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