Epithelial-to-mesenchymal transition in cyst lining epithelial cells in an orthologous PCK rat model of autosomal-recessive polycystic kidney disease

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PKHD1 (polycystic kidney and hepatic disease 1), is a large gene located on chromosome 6p21.1-p12 (27, 39). It is predicted to yield a novel 4,074-amino acid, multidomain, integral membrane protein called fibrocystin (43), or polyductin (27), of unknown function. Despite impressive advances in the genetic and molecular characterization of PKD gene products, the normal cellular function of these proteins and their role in cyst formation and growth are unknown.

PKD cystic epithelia share common phenotypic abnormalities despite the different genetic mutations that underlie the disease (37). Cystic epithelial cells are characterized by altered proliferative activity, a secretory rather than absorptive function, and an abnormal matrix microenvironment (11, 20). The cyst lining epithelial cell pathophysiology in PKD is characterized by dedifferentiation and perturbations of the polarized phenotype. Some of these features resemble an early developmental epithelial cell phenotype. From a developmental perspective, cystogenesis associated with PKD can be considered a state of abnormal tubulogenesis in which proliferative influences predominate over differentiation factors (2, 10).

In relation to an abnormal matrix microenvironment, renal interstitial fibrosis is an important characteristic of PKD (10, 37). One of the key features of fibrosis is epithelial-to-mesenchymal transition (EMT) (17). Epithelial cells undergoing EMT acquire mesenchymal features, which leads to excessive production and deposition of extracellular matrix components (14, 17). Recently, a role of EMT has been implicated in the pathogenesis of PKD. Schieren et al. (34) studied the expression profiles of 12 human ADPKD kidneys using a 7,000 cDNA microarray and concluded that ADPKD was associated with increased EMT markers, suggesting that EMT contributed to the progressive loss of renal function in ADPKD. Song et al. (35) performed global gene profiling on renal cysts from five PKD1 human polycystic kidneys and reported that epithelial dedifferentiation accompanied by EMT may be required for PKD1 cyst growth and disease progression. Chea and Lee (9) reported that the end stages of ADPKD kidneys from five patients were associated with markers of EMT, suggesting that EMT has a role in progression of ADPKD. Okada et al. (25) examined fibroblast-specific protein 1 (FSP1), α-smooth muscle actin (α-SMA), vimentin, and heat shock protein 47 (HSP47) in the recessive pcy mouse model of late-onset cystic kidney disease (an ortholog of human adolescent nephronophthisis, NPHP3; MIM 604387) and described that some epithelia among remnant tubules trapped within fibrotic septa around adjacent cysts also acquired the phenotype of FSP1, HSP47 collagen-producing fibroblasts, suggesting a possible role for

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EMT in this model. Many of the proteins implicated in PKD and nephronophthisis are linked to ciliary function and localize to the primary cilia, basal body, and/or centrosome. There are several indications of a relationship between the primary cilium and renal cystic diseases, which belong to a group of disorders with abnormalities in the primary cilium, referred to as ciliopathies (30). The mechanisms by which ciliopathy gene product deficiencies lead to EMT of tubular epithelia are not yet fully understood. It has been shown that EMT is active in a human nephronophthisis (NPHP7; MIM 611498) orthologous mouse model (1). The proposed pathogenesis of EMT in this model is loss of function of downstream effectors of hedgehog signaling, which is active normally in mesenchymal-to-epithelial transition in renal tubulogenesis (1, 45). The similar pathophysiology may also contribute to PKD.

Polarization is essential for epithelial cell function, and apicobasal cell polarity is lost during EMT, a program of events characterized by loss of cell polarity (40). E-cadherin is a calcium-dependent cell adhesion molecule present in most types of renal tubule cells. Assembly of the E-cadherin/β-catenin complex is the first step in formation of a polarized epithelium and plays an important role in maintenance of an epithelial phenotype (29). Recent studies (8, 31) have suggested that aberrant expression of E-cadherin and/or β-catenin may be associated with PKD. Loss of E-cadherin expression is a hallmark of EMT. Recently, it was clarified that Snail1 (known previously as Snail), a transcriptional repressor of epithelial phenotype (29), was connected using an image-joining software package.

Materials and Methods

PCK model. PCK rats were originally derived from a spontaneous mutation in a strain of Sprague-Dawley rats (15, 16). The animals used in this study were from a colony established at the Education and Research Center of Animal Models for Human Diseases of Fujita Health University. The PCK rats are homozygous for the gene and maintained as such for ease of breeding. In 2002, Ward et al. (43) demonstrated that the mutation in the PCK rat was an exon deletion in the rat homolog of human PKHD1. As controls, Sprague-Dawley rats were obtained from Charles River Japan (Kanagawa, Japan). The protocol for the use of rats was approved by the Animal Care and Use Committee at Wakayama Medical University.

Immunohistochemistry. From male PCK (n = 5) and control rats (n = 5) each at day 0, 1, 3, 10, and 14 wk, and 4 mo of age, both kidneys were removed rapidly. One kidney was fixed in 6% formaldehyde, and the other was frozen in liquid nitrogen for frozen sections, Western blotting, and real-time PCR. Kidney samples were embedded in paraffin and cut into sections 4 μm thick. Serial sections were incubated for 1 h at room temperature with tubule segment-specific markers (Supplemental Table 1; supplemental data for this article is available online at the American Journal of Physiology-Renal Physiology website) (24), and primary antibodies against E-cadherin (1:1,000; BD Transduction Laboratories, San Jose, CA), Snail1 (1:400, ab17732; Abcam, Tokyo, Japan), N-cadherin (1:500; BD Transduction Laboratories), β-catenin (1:100; Santa Cruz Biotechnology, Santa Cruz, CA), Na⁺/K⁺-ATPase α1-subunit (1:500, clone C464.6; Upstate, Temecula, CA), vimentin (1:200; Dako Cytoplasm, Glostrup, Denmark), fibronectin (1:400; Dako), α-SMA (1:1,000; Progen Biotechnik, Heidelberg, Germany), and fibrocytin (1:50; Santa Cruz Biotechnology). After washing, they were incubated with the secondary antibody conjugated to a peroxydase-labeled polymer supplied with the Envision plus system (Dako), except for fibrocytin. Immunostaining for segment-specific markers, except for Phaseolus vulgaris erythroagglutinin (PHA-E), was performed using the labeled streptavidin-biotin complex (LSAB) method employing a LSAB kit (Dako). For PHA-E staining, avidin-binding horseradish peroxidase (HRP) (ExtrAvidin; Sigma, St. Louis, MO) was used. For fibrocytin staining, anti-goat HRP-labeled immunoglobulin (Dako) was used as a secondary antibody. Target retrieval solution (Dako) for E-cadherin, Snail1, β-catenin, N-cadherin, vimentin, and fibrocytin, and proteinase K (Sigma) for α-SMA and Na⁺/K⁺-ATPase α1-subunit, were used for antigen retrieval. The samples were visualized with 3,3’-diaminobenzidine tetrahydrochloride and counterstained with hematoxylin. Masson-trichrome stain was used to assess fibrosis. The samples were photographed using an all-in-one light field and fluorescence microscope, BIORÉVO (BZ-9000, Keyence; original magnification, ×40), were connected using an image-joining software package.

Table 1. Tubular segment-specific cyst count in PCK rats

<table>
<thead>
<tr>
<th>Segment</th>
<th>0 days</th>
<th>1 wk</th>
<th>3 wk</th>
<th>10 wk</th>
<th>14 wk</th>
<th>4 mo</th>
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<tbody>
<tr>
<td>PT</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>2+</td>
<td>3+</td>
</tr>
<tr>
<td>LH</td>
<td>+</td>
<td>-</td>
<td>2+</td>
<td>3+</td>
<td>5+</td>
<td>5+</td>
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<tr>
<td>DT</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>2+</td>
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<tr>
<td>CD</td>
<td>+</td>
<td>-</td>
<td>2+</td>
<td>2+</td>
<td>4+</td>
<td>5+</td>
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Cyst numbers were counted in a plane showing the maximum area of each kidney sample. Number of cysts was graded semiquantitatively on a scale from − to 5+: −, 0; +, ≤25; 2+, ≤50; 3+, ≤100; 4+, ≤200; and 5+, >200. PT, proximal tubule; LH, loop of Henle; DT, distal tubule; CD, collecting duct.
and blotted with an antibody against E-cadherin (1:50,000; BD Transduction Laboratories) and β-catenin (1:5,000; Santa Cruz Biotechnology). An ECL detection system was used for detection (Amersham Biosciences, Little Chalfont, UK). The blots were then reprobed with an antibody against β-actin (1:5,000, Sigma) as a loading control. Images of the blots were captured, and densities of bands were analyzed using a CS Analyzer 3.0 with a charge-coupled device (CCD) camera (ATTO, Tokyo, Japan).

Quantitative real-time PCR. RNA was harvested from kidney lysates using an RNeasy kit (Qiagen, Hilden, Germany). RNA samples were then tested with primers specific for E-cadherin and Snail1 mRNAs, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control. The primers used for real-time PCR are shown in Supplemental Table 2. First-strand cDNA was produced from total RNA using a PrimeScript RT reagent kit (Takara Bio, Otsu, Japan). A SYBR Premix Ex Taq kit (Takara Bio) was used to perform quantitative PCR. Data were collected and analyzed with the Thermal Cycler Dice real-time PCR system and software (Takara Bio). The expression of E-cadherin or Snail1 mRNA was calculated relative to that in the control. Each RNA sample was analyzed in triplicate, and each experiment was performed independently at least three times. The ΔΔCt method was used to analyze the data.

Statistical analysis. Data were analyzed using the Mann-Whitney U-test at a significance level of P < 0.05. The results were analyzed with JMP 7.0.1J software (SAS Institute Japan, Tokyo, Japan).

RESULTS

Profile analysis of cyst formation using segment-specific markers. Detection of tubule segments was performed using segment-specific markers (Supplemental Figs. 1 and 2). Renal cysts were evident from 3 wk of age in PCK rats (Fig. 1). Most of these cysts had developed in the loop of Henle (LH) and collecting duct (CD) in the corticomedullary region and outer medulla. There was also cyst formation in distal tubule (DT), in which the number was relatively small but the size was large. However, although small in number, proximal tubule (PT)-derived cysts were present at birth and from 10 wk of age (Table 1). The number and size of the cysts increased markedly from 14 wk of age (Table 1 and Figs. 1 and 2). Interstitial fibrosis and inflammation were also evident from 10 wk of age, in accordance with disease progression (Supplemental Fig. 3).

E-cadherin. Immunohistochemistry showed that E-cadherin was abundant in LH, DT, and CD but was present at only a very low level in PT in control rats at all ages (Supplemental Fig. 1, Fig. 3, and Table 2). This expression pattern was correlated with the pathology of the disease in that the tubule

![Cyst number and size in PCK rats.](http://ajprenal.physiology.org/)

![E-cadherin/β-catenin expression in tubule epithelial cells in control and PCK rats.](http://ajprenal.physiology.org/)
segments showing the highest expression level in control rats had much severer cyst formation in PCK rats. In control rats, E-cadherin was localized primarily in areas of cell-cell contact in a basolateral pattern, and in LH of control rats it was also distributed in a basolateral and cytoplasmic pattern. From 3 wk of age, the level of E-cadherin expression in cystic tubules was attenuated and localized to areas of lateral cell-cell contact, in accordance with cyst enlargement in PCK rats, whereas E-cadherin expression in noncystic tubular epithelia remained high (Fig. 8). In PCK rats, the level of β-catenin expression demonstrated by immunohistochemistry in cystic tubules was attenuated and localized to areas of lateral cell-cell contact, in accordance with cyst enlargement, whereas β-catenin expression in noncystic tubule epithelia in PCK rats remained high (Table 2 and Fig. 3). These expression patterns were similar to those of E-cadherin. Western blot analysis showed decreased β-catenin expression in PCK kidneys relative to controls, in accordance with disease progression (Fig. 7).

**Snail1.** Snail1 was expressed predominantly in the nuclei of tubule epithelial cells forming large cysts, especially in adjacent fibrotic areas (Fig. 6). Snail1 expression was maximal in enlarging cysts, i.e., the largest cysts showed slightly lower expression of Snail1. There was no significant immunostaining for Snail1 in the controls. Expression of Snail1 mRNA was also upregulated in an age-dependent manner, although expression of Snail1. There was no significant immunostaining in cystic tubules was attenuated and localized to areas of lateral cell-cell contact, in accordance with cyst enlargement in PCK rats, whereas E-cadherin expression in noncystic tubular epithelia in PCK rats remained at a high level (Table 2 and Fig. 3). Western blot analysis and real-time PCR analysis showed decreased E-cadherin expression in PCK kidneys relative to controls, in accordance with disease progression (Figs. 4 and 5A).

**β-Catenin.** Like E-cadherin, β-catenin was strongly expressed in LH, DT, and CD in control rats. β-Catenin was localized primarily in areas of cell-cell contact and was also distributed in the cytoplasm in control rats (Fig. 3). In PCK rats, the level of β-catenin expression demonstrated by immunohistochemistry in cystic tubules was attenuated and localized to areas of lateral cell-cell contact, in accordance with cyst enlargement, whereas β-catenin expression in noncystic tubule epithelia in PCK rats remained high (Table 2 and Fig. 3). These expression patterns were similar to those of E-cadherin. Western blot analysis showed decreased β-catenin expression in PCK kidneys relative to controls, in accordance with disease progression (Fig. 7).

**Na⁺-K⁺-ATPase α1-subunit.** To examine the polarization of epithelial cells in PCK rats, we examined the subcellular distribution of Na⁺-K⁺-ATPase α1-subunit during cyst formation. In control rats, basolateral expression of Na⁺-K⁺-ATPase α1-subunit was evident in LH and DT, where cyst formation was severe in PCK rats (Fig. 8). In PCK rats, expression of Na⁺-K⁺-ATPase α1-subunit in cystic tubule epithelia was decreased in accordance with disease progression, whereas basolateral expression of Na⁺-K⁺-ATPase α1-subunit in noncystic tubule epithelia remained high (Fig. 8).

**N-cadherin and mesenchymal markers.** In control rats, N-cadherin expression was detected only in PT and was absent in other segments (Table 3). The subcellular distribution of N-cadherin differed between segments S1/S2 and segment S3 of PT. Segments S1/S2 showed mainly apical expression, whereas segment S3 showed mainly basolateral expression. N-cadherin expression began to increase from 10 wk of age in

Table 2. *Epithelial expression of E-cadherin and β-catenin in control and PCK rats*

<table>
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<th></th>
<th>Control</th>
<th>PCK (3 wk–4 mo)</th>
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<tr>
<td></td>
<td>0 days</td>
<td>1 wk</td>
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<td><strong>E-cadherin</strong></td>
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<td>PT</td>
<td>±</td>
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<td>DT-CD</td>
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<tr>
<td>Outer medulla</td>
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<tr>
<td>LH</td>
<td>b</td>
<td>b</td>
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<td>CD</td>
<td>b</td>
<td>b</td>
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<tr>
<td>Inner medulla</td>
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<tr>
<td>CD</td>
<td>±</td>
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<tr>
<td><strong>β-Catenin</strong></td>
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<td>Cortex</td>
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<td>DT-CD</td>
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<td>Outer medulla</td>
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<td>Inner medulla</td>
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<td>CD</td>
<td>±</td>
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The intensity of staining was graded semiquantitatively on a scale from – to 3+: -, none; ±, trace; +, slight; 2+, moderate; and 3+, intense. A large cyst was defined as a tubule >200 μm in diameter. Dominant pattern of staining: b, basolateral only; b/c, basolateral and cytoplasmic; l, lateral only. No cyst, no cyst formation.
the controls (Table 3). There was no remarkable difference in N-cadherin expression between control and PCK rats until 3 wk of age. N-cadherin expression was evident in cyst epithelial cells in PCK rats from 10 wk of age as cyst enlargement progressed. In PCK rats, all cysts derived from PT showed mainly basolateral expression of N-cadherin, whereas noncystic PT epithelial cells showed apical or basolateral expression of N-cadherin, in accordance with the segmental distribution in control rats (Table 3 and Fig. 9A). Cytoplasmic expression of N-cadherin was observed in cyst lining cells derived from LH and CD (Fig. 9B and C), indicating de novo synthesis of N-cadherin.

Some tubule epithelial cells forming large cysts, especially in adjacent fibrotic areas around large cysts. Weak α-SMA expression in cyst lining cells was also observed in PCK rats (Fig. 10). Cells with α-SMA positivity were also distributed in the interstitium. There was no significant staining for mesenchymal markers in the controls.

**Fibrocystin/polyductin.** Although fibrocystin/polyductin was strongly expressed in CD in normal adult kidney, it was widely expressed in epithelial cells in other segments in control rats. In PCK rats, fibrocystin/polyductin expression was reduced in large cysts but not completely absent.

### DISCUSSION

The current study provided further evidence of EMT in PKD. The novelty of our study is systematic evaluation of EMT markers in a nephron segment-specific and time-course manner in an orthologous ARPKD model. Clarification of the segment-specific cyst formation profile related to EMT in the PCK rat allows us new insight into PKD pathophysiology as discussed in detail below.

Our studies demonstrate that EMT may play a role in PCK rats, although investigation of mechanistic insights into pathological processes of EMT is needed for further confirmation of EMT in PKD. Recent evidence suggests that fibrocystin/polyductin, polycystin (PC)-1, and PC-2 are all localized at plasma membrane and primary cilium (42). Therefore, analysis of cyst formation mechanism in PCK rats would likely provide valuable information about not only ARPKD but also ADPKD in humans. Because our study was performed by focusing on specific segments in rats at different ages, it clarified the process of disease progression in vivo. Sato et al. (33) reported that cholangiocytes of the PCK rat may acquire mesenchymal features and participate in progressive hepatic fibrosis by producing extracellular matrix molecules, which seems to be a different event from EMT. The reason of this difference is unknown. There is the possibility of organ-specific features in the model.

The data presented in the present study reveal that tubule segments with the highest level of E-cadherin expression in control rats showed much severer cyst formation in PCK rats. The expression level and pattern of cadherins differed among the tubule segments. In control rats, N-cadherin was predominant in PT and absent in other segments, whereas in control rats E-cadherin was highly expressed in LH, DT, and CD but...
present at a very low level in PT. Moreover, E-cadherin expression was strongest in the thick ascending limb (THAL) of LH in the outer medullary segment. These localization patterns of cadherins were in accord with results reported previously (29). On the other hand, Lager et al. (16) described that PCK rats develop renal cysts in LH, DT, and CD. In our study, the segments most affected in PCK rats were the medullary THAL and CD in the outer medulla, where the level of E-cadherin expression is highest in control rats. In addition, the expression of E-cadherin decreased gradually in accordance with cyst enlargement in PCK rats. These findings raise the possibility that, in the PCK model, differences in cadherin expression may be a marker of susceptibility to cystogenesis.

Nephron segment-specific cyst formation is a key feature of PKD. Our observations may therefore provide an important clue to the mechanism of segment-specific cyst formation.

In the present study, E-cadherin expression decreased gradually and was limited to the lateral-lateral cell membrane, in accordance with cyst enlargement. Loss of E-cadherin expression is closely linked to plasma membrane destabilization, which is also observed in fetal development and certain pathological processes including carcinogenesis, metastasis of cancer cells, and EMT. Aberrant E-cadherin expression has been reported in PKD (7, 28). Huan and van Adelsberg (13) reported that the PKD1 gene product PC-1 formed a complex containing E-cadherin and β-catenin. Roitbak et al. (32) reported that the PC-1/PC-2/E-cadherin/β-catenin complex was disrupted in cells from ADPKD patients and that loss of E-cadherin expression was compensated by N-cadherin expression, which apparently stabilized β-catenin in place of E-cadherin. In our present study, segments of epithelial cells in areas of severe cyst formation showed de novo expression of N-cadherin, whereas it was negative in control rats. Expression of N-cadherin might therefore represent transdifferentiation from an epithelial to a mesenchymal phenotype, as well as loss of E-cadherin. These findings suggest that cadherin-related functions may play a key role in PKD.

In the present study, expression of the Na+/K+-ATPase α1-subunit in cystic tubular epithelia decreased in parallel with disease progression in PCK rats, suggesting a loss of apico-basal cell polarity. Avner et al. (3) reported that the apical membrane Na+/K+-ATPase α1-subunit persisted in cystic kidneys of the PCK mouse, suggesting that its expression might be a manifestation of the relatively undifferentiated phenotype of the epithelial cells lining CD cysts. Although there is a difference in the abnormal expression pattern of the Na+/K+-ATPase α1-subunit in cystic epithelium between the PCK rat and CPK mouse models, these findings may suggest diversity of the peripheral phenotype of epithelial cells from the common pathophysiology of PKD.

Renal fibrosis, like that in PKD, is considered to be a common key pathway leading to renal failure, irrespective of the underlying disease (17). Although EMT is known to play a significant role in renal fibrosis, the exact mechanism involved is unclear. In the present study, some tubule epithelial cells in large cysts in PCK rats showed phenotypic and morphological change, including loss of E-cadherin expression, and de novo expression of N-cadherin, vimentin, and fibronectin. Loss of E-cadherin expression is thought to be an early event that precedes EMT (17). In PCK rats, even at 3 wk of age, E-cadherin expression was evidently decreased in cystic epithelial cells. On the other hand, mesenchymal markers were expressed after 10 wk of age. At the same time, in interstitial fibrotic areas adjacent to large cysts, expression of vimentin...
and fibronectin was upregulated, suggesting an accumulation of fibroblasts. α-SMA is a molecular marker of myofibroblasts. The presence of activated myofibroblasts is thought to be one of the most distinctive features of interstitial fibrosis. It has been reported that the expression of α-SMA is increased in rat models of unilateral ureter obstruction (UUO) (28). In our present study of PCK rats, weak α-SMA expression in cyst lining cells was also observed, in addition to a few α-SMA-positive cells scattered in the fibrotic area. As discussed in previous reports, such fibroblasts may have a different origin. Iwano et al. (14) showed that 36% of new fibroblasts originated from local EMT and that 15% were from bone marrow, the rest being local proliferation. Together, the data suggest that EMT of tubule epithelial cells may contribute to renal fibrosis in the PCK rat. These observations raise the question of whether EMT is a PKD-specific pathophysiology or merely a common key pathway leading to renal failure, regardless of the underlying disease. However, the early change of E-cadherin expression before evident fibrosis suggests the former possibility. Further investigations are needed to clarify this issue.

Although the mechanism of EMT appears complex, its consequences are likely mediated in part through the effects of β-catenin. Disruption of the E-cadherin/β-catenin complex induces loss of β-catenin in the cell membrane. Cytoplasmic free β-catenin works as an essential signal mediator in the Wnt signal pathway. The Wnt/β-catenin signal pathway mediates many inductive events such as kidney development and cancer. Cytoplasmic β-catenin translocates to the nucleus and binds

Table 3. **Epithelial expression of N-cadherin in control and PCK rats**

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<tr>
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<th>Control (≤3 wk)</th>
<th>Control (≥10 wk)</th>
<th>PCK Rat (≥10 wk)</th>
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<tr>
<td></td>
<td>Cortex</td>
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<td>PT</td>
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The intensity of staining was graded semiquantitatively on a scale from − to 3+: −, none; ±, slight; +, moderate; and 3+, intense. A large cyst was defined as a tubule >200 μm in diameter. Dominant pattern of staining: a, apical; b, basolateral; c, cytoplasmic. S1–S3, PT segments.
with T-cell factor/lymphoid enhancing factor (TCF/LEF) in the nucleus, thus activating various target genes (5). Aberrant β-catenin signaling is reported to play a role in cyst formation. Nuclear catenin staining has been found in the bcl-2 null mouse model of PKD (36). Muto et al. (21) showed that the expression of E-cadherin and β-catenin was attenuated in transgenic mice with targeted deletion of exon 2–6 of Pkd1. We speculate the alterations of β-catenin expression are associated with cyst enlargement.

We observed a clear increase in the nuclear expression of Snail1 in PCK rats, and Snail1 mRNA was also upregulated. These findings may support a role of EMT in the PCK rat model. One possible mechanism to account for the loss of E-cadherin expression is a reduction of gene expression. The transcription factor Snail1 is activated and binds to specific DNA sequences known as E-boxes in the promoter of the E-cadherin gene. Subsequently, transcriptional repression of E-cadherin occurs during EMT and cancer (6, 38). It has been reported that Madin-Darby canine kidney (MDCK) cells transfected with Snail1 show downregulation of E-cadherin expression and increased expression of mesenchymal markers (7). In our study, Snail1 mRNA upregulation was slightly late for the start of E-cadherin mRNA decrease. The reason of this gap between the E-cadherin expression and its inhibitor Snail1 level is unknown. Several possibilities are conceivable. Because of the nature of our study, the change of expression levels on time course in an individual rat is not available. Therefore, if expression levels vary widely among individuals, the mean of the data may also vary. Another possibility is that the initiation of decrease in E-cadherin may be caused by other factor(s) than Snail1. Snail1 may work for the continuity of suppression of E-cadherin.

The PKHD1 gene product, fibrocystin/polyductin, is a membrane-bound protein widely expressed in epithelial cells (46). The cellular functions of fibrocystin/polyductin are not fully understood but likely include roles in epithelial cell proliferation. Fibrocystin/polyductin seems to participate in tubule morphogenesis. Ward et al. (43) showed that fibrocystin/polyductin was strongly expressed in CD of fetal and adult human kidney. Fibrocystin/polyductin also has been detected in LH in fetal kidney (19, 43). The PCK rat has a mutation in the ortholog of human PKHD1. Immunohistochemical analysis has shown that fibrocystin/polyductin expression is reduced in the kidney of the PCK rat but not completely absent (46). Similar findings also have been obtained by Western blot analysis (18). Our observations in fibrocystin/polyductin in the PCK rat are similar to those reported previously. Loss of E-cadherin and β-catenin might disrupt the PC1/E-cadherin/β-catenin complex and induce loss of function of PC1 as a mechanosensor, as has been reported in ADPKD (32). It is known PC-1 and PC-2, which are mutated in ADPKD, interact through their COOH-terminal cytoplasmic tail (41). Moreover, recent evidence suggests that fibrocystin/polyductin and poly-cystins are colocalized at the primary cilia (23, 42, 44). Fibrocystin/polyductin is reportedly related to the flow-stimulated intracellular calcium signaling pathway (12, 22). These findings suggest that the pathophysiology of the PCK rat model may involve a common pathway that alters ciliary functions, thus leading to cyst formation.

In conclusion, we have shown that nephron segments exhibiting severe cyst formation correspond to the segments with high expression of E-cadherin and fibrocystin/polyductin in PCK rats. These distribution patterns are probably associated with cyst formation in PKD. E-cadherin expression was shown to decrease in parallel with cyst enlargement, suggesting that adhesion molecules play a key role in cyst formation and enlargement in PCK rats. In addition, epithelial cells in cysts were shown to acquire mesenchymal features in response to cyst enlargement and probably participate in the progression of renal fibrosis. EMT may play an important role in the pathophysiology of PKD, and we speculate that it could become a potential therapeutic target for human PKD.

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