Increased Epithelial Cell Proliferation and Abnormal Extracellular Matrix in Rat Polycystic Kidney Disease

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Abstract. Proliferation of renal tubular epithelial cells is considered a major factor leading to cyst formation in human polycystic kidney disease (PKD). The Han:SPRD rat model for inherited PKD permits a close scrutiny, especially for early stages of the disease, and shows numerous similarities to human autosomal dominant PKD (ADPKD). In this study, the exact proliferation rate in Han:SPRD rat kidneys was evaluated in a cell type-specific manner, using immunohistochemistry with antibody to proliferating cell nuclear antigen (PCNA). The proliferation index (P1; percentage of PCNA-positive cell nuclei) was determined in normal and cystically altered tissue, and a relationship between proliferative activity and alterations in extracellular matrix expression was established using in situ hybridization for collagen I and IV mRNA. Heterozygously affected rats (cy/+) showed strong increases of P1 values in cystically altered nephron portions that were mostly derived from proximal tubule. Cell proliferation obviously preceded cyst formation, because early in the progression of the disease, the normal-appearing tubules from PKD kidneys had markedly increased P1 values compared with healthy controls (14.1-fold in 3-mo-old rats and 11.9-fold in 12-mo-old rats; P < 0.05), whereas later stages revealed a more generalized cyst degeneration of the nephron, with increases in P1 between 14- and 82-fold, depending on the respective category of cystic epithelia. In cysts with a distal phenotype, changes were less pronounced. No significant differences were encountered between the two age groups. Proliferation was also present in interstitial cells, whereas glomeruli were unchanged. Increases in epithelial and interstitial proliferation coincided with an overexpression of matrix compounds. For comparison, changes in homozgyously affected rats (cy/cy) showed up to several hundred-fold elevated P1 values. These results indicate that in the Han:SPRD model for ADPKD, cystic malformation of the nephron is preceded by and coincides with enhanced epithelial and interstitial cell proliferation. Altered cell–matrix interactions seem to be directly involved in the disruption of epithelial differentiation. (J Am Soc Nephrol 9: 937–945, 1998)

Autosomal dominant polycystic kidney disease (ADPKD) is a common genetic disorder characterized by progressive development and enlargement of renal cysts, leading to end-stage renal disease by late middle age in humans (1). The disease is a systemic disorder that also affects the liver, intracranial vessels, and heart valves. ADPKD is genetically heterogeneous, but the polycystic kidney disease 1 (PKD1) locus has recently been found to be implicated in the majority of cases, and expression of the PKD1 protein polycystin was shown to be overexpressed in cystic epithelia (2,3).

Despite the identification of the primary gene product involved in cystic nephron changes, the pathogenesis of cyst formation is not entirely clear. Human and experimental data suggest that increased proliferation of renal cyst cells, abnormalities in the extracellular matrix, and fluid accumulation are the basic features that are common in all of the different forms of the disease (4). An abnormal proliferation of tubular epithelial cells is considered to play a key role in cyst development, and a recent study has provided evidence for an involvement of epithelial cell hyperplasia in several forms of human PKD, using antibody against proliferating cell nuclear antigen (PCNA) (5).

The Han:SPRD rat strain with ADPKD permits a close observation of the disease, which bears similarities to human ADPKD (6,7) even though the defect in this rat model is based on the mutation of a gene locus that is not the homolog of either human PKD1 or the additionally discovered locus, PKD2 (8). As in humans, abnormalities of the tubular epithelia and concomitant changes in the extracellular matrix are a prominent feature in the rat model (6,7,9,10). In heterozygously affected Han:SPRD rats (cy/+), cystic transformation starts in the proximal tubule with loss of epithelial differentiation and overexpression of matrix compounds initially restricted to small focal areas that then become more generalized. Later, the distal tubule also is implicated in the cystic changes (7). In homozygous animals (cy/cy), all nephron epithelia are widely dilated already in early postnatal stages. Indirect evidence for a role of increased proliferation of renal cyst cells has been provided in Han:SPRD rats by the documentation of an
increased expression of the proto-oncogenes c-fos and c-myc, which are known to be associated with increased cell proliferation (6).

Antibody staining for PCNA labels the nuclei of cells engaged in the cell cycle, and by means of quantitative analysis of the staining, the proliferation index (PI; the percentage of PCNA-positive nuclei) can be evaluated. The objective of this study was to directly determine the involvement of cell proliferation in heterozygous Han:SPRD rat kidneys during the development of cystic changes. We have systematically evaluated the PI in normal nephron epithelia and in epithelia revealing loss of epithelial differentiation in the various cyst types. In addition, we have described a relationship between cell proliferation and the overexpression of extracellular matrix. For comparison, PI values were also determined in homozygously affected animals, which display a more rapid progression of the disease.

Materials and Methods

Rats from the Han:SPRD strain exhibiting PKD were bred in the local animal facility as described (Klinikum Mannheim, Germany) (7,11). Heterozygously affected male rats with ADPKD (cy/+) were checked for renal cysts by abdominal palpation. Heterozygously unaffected, age-matched littersmates (+/+) were used as controls. Homozygous males were easily checked for renal cysts by abdominal palpation due to massively enlarged kidneys. Rats had free access to tap water and standard rat chow containing 19% protein (Altromin 1934®, Lage, Germany). Clinical parameters for rats from this laboratory have been determined previously (7). For morphologic and histochemical analysis, heterozygous rats and controls were distributed into two age groups of 3 and 12 mo, respectively, and three PKD rats and three controls were chosen for each age group. For complementary information on the impact of homozygosity on cell proliferation, three homozygous male rats were investigated.

Tissue Preparation for Histochemistry

To obtain tissue for immunohistochemistry and in situ hybridization, animals were anesthetized by intraperitoneal injection of Nembutal (40 mg/kg body wt) and perfusion-fixed by cannulation of the abdominal aorta. Animals were perfused for 90 s at a pressure of 220 mmHg with 3% paraformaldehyde in phosphate-buffered saline (PBS), pH 7.4. The total osmolality of the fixative was 800 mosmol/kg H2O. After perfusion, one kidney was clamped, removed, and routinely processed for paraffin embedding. The remaining kidney was rinsed for 5 min in PBS adapted to the osmolality of the fixative by addition of RNase-free sucrose. Slices of this kidney were then snap-frozen in liquid nitrogen.

Detection of Epithelial Proliferation by Monoclonal Antibody to PCNA

Proliferation was detected using monoclonal antibody against PCNA (Dakopatts, Glostrup, Denmark). Microwave treatment was performed to improve antibody binding. Five-micrometer-thick paraffin sections were mounted on silanized glass slides. After deparaffinization, slides were placed in 10 mM citric acid buffer, pH 6.0, and heated in a commercial microwave oven at 620 W for 20 min. Slides were subsequently allowed to cool in the same buffer for 20 min at room temperature. After rinsing in PBS, slides were incubated with blocking solution (normal swine serum diluted 1:10 in PBS containing 1% bovine serum albumin) for 30 min followed by rinses in PBS and incubation with anti-PCNA antibody (1:50 dilution in PBS) for 2 h at room temperature and then overnight at 4°C. After rinsing in PBS, bound antibody was visualized with an FITC-labeled goat anti-mouse antibody (Cappel, West Chester, PA; dilution 1:200 in PBS). Cell nuclei were simultaneously stained with propidium iodide (Sigma, St. Louis, MO; dilution 1:500 in PBS). PCNA staining was validated by the comparative application of antibody against the proliferation marker Ki-67 (clone MIB5, Dianova, Hamburg, Germany); after microwave heating, consecutive sections from 3- and 12-mo-old heterozygous PKD rats were incubated with PCNA and Ki-67 antibody (dilution 1:10 in PBS), respectively. Bound antibody was detected using Cy3-labeled second antibody (dilution 1:250 in PBS). Slides were coverslipped with 50% glycerol diluted in PBS and studied by epifluorescence, using a Leica DMRB fluorescence microscope (Wetzlar, Germany).

In Situ Hybridization

mRNA expression for collagen I and the α1 chain of collagen IV, laminin, and renin was investigated by in situ hybridization, using nonisotopic riboprobes made from the respective cDNA. Five-micrometer-thick cryostat sections were mounted on poly-l-lysine-coated glass slides. Sections were post-fixed for 30 min in 4% paraformaldehyde in PBS, pH 7.4, and stored in 70% ethanol at 4°C until further use.

To generate hybridization probes for α1(IV) collagen IV, a 1139-bp fragment (kindly provided by Dr. Oberbäumer, Humboldt University, Berlin, Germany) was subcloned and transcribed, as described previously (7). To generate probes for collagen I, a 360-bp fragment of human collagen I cDNA (kindly provided by Dr. von der Mark, Max Planck Institute, Erlangen, Germany) (12) was used.

Incorporation of digoxigenin-labeled UTP was carried out by in vitro transcription using a nonradioactive nucleic acid detection kit (Boehringer Mannheim, Mannheim, Germany) to obtain sense and antisense riboprobes. To randomly shorten the α1(IV) collagen fragments of the labeled probe, time-controlled alkaline hydrolysis was performed. The hybridization procedure, including the washing steps, was essentially as described (7,13). Washing was carried out at 53°C for 3.5 h with a final high stringency wash in 0.1× SSC containing 50% formamide. After brief washes in 0.5× SSC and 0.2× SSC containing formamide, sections were incubated with alkaline phosphatase-conjugated antidigoxigenin antibody (kit from Boehringer Mannheim; antibody dilution 1:500 in blocking medium) at 4°C overnight. Subsequent color development was performed according to the manufacturer's instructions with a developing time of 24 to 48 h. Slides were coverslipped with 50% glycerol in PBS and examined by bright-field microscopy supplemented with interference contrast optics.

Quantitative Evaluation of Slides

In the heterozygotes, three nonsuccessive sections per kidney of each animal were evaluated. In each section, three independent areas of a constant size (from the cortical labyrinth, the medullary rays, and the outer stripe) were chosen at random. Within these areas, adjacent fields were manually scanned at high magnification (×400). With the aid of interference contrast optics, nephron segments were identified; two categories were established for both normal and cystic epithelia, i.e., proximal tubule segments comprising straight and convoluted parts (which accounted for the majority of cystic alterations; reference 7) and distal segments comprising thick ascending limbs, distal convoluted tubules, connecting tubules, and cortical and outer medullary collecting ducts. Cyst types were further divided according to the.
structural appearance of the lining epithelium as suggested previously (reference 7; see also Results): cysts lined by abnormal, atrophic, or hyperplastic epithelium. The number of propidium iodide- and PCNA-positive epithelial cell nuclei was counted manually in each field by two observers in a blinded manner. For each analyzed epithelial type in a given animal, a total of approximately 2000 propidium iodide-stained nuclei was collected, and mean PCNA labeling index (PI; percentage of PCNA-positive nuclei) for the respective epithelial and cyst types in each animal group was derived.

To record the rate of proliferation, specifically in early cystic events characterized by focal plaques of abnormal epithelium lying next to normal-appearing proximal tubular epithelium (7), PI was calculated over the plaques and compared with an equivalent length of adjacent, normal-appearing epithelium. Ten of these plaques were evaluated per animal in each group. Glomerular PI was calculated by counting glomerular tuft and parietal epithelial cells in 10 randomly chosen fields viewed in each animal at ×400 magnification. Interstitial cell PI was evaluated in 27 fields per animal in each group. Fields were distributed to an equal proportion in the cortical labyrinth, medullary rays, and outer stripe, respectively.

In the homozygotes, cysts were categorized as small, medium, or large, and these categories were verified according degree of epithelial dedifferentiation. PI was calculated for 10 cysts of each type in three animals. In agreement with other studies (5,14), only those nuclei that exhibited heavy and/or granular staining were evaluated and therefore were considered in the transition from G1 to S phase of the cell cycle, when PCNA expression is at its maximum intensity (15).

Statistical Analyses

Data are given as the mean ± SD for every parameter in each animal group. Statistical differences between normal and PKD rats, between the age groups, and between cyst types were evaluated using the Lord test, which is appropriate for small sample numbers (16). For comparison of the values, we used the means along with the range of the tested parameters. Levels of significance were determined, and differences were considered significant if \( P < 0.05 \).

Results

Morphology

Cystic nephron changes in heterozygously affected PKD rats were more pronounced in proximal than in distal segments. Comparing 3- and 12-mo-old rats, there was a marked increase in cyst formation in the older animals. In the 3-mo-old kidneys, proximal tubular cysts frequently showed heterogeneous phenotypes with transitions between normal-appearing and cystically altered epithelia, and numerous nephrons remained phenotypically intact. In the 12 mo-old kidneys, almost none of the proximal tubular segments had an intact structure, and distal segments were more extensively dilated than in the 3-mo-old kidneys. Three categories of cystic enlargements were established according to the varying degrees of dedifferentiation of proximal tubular epithelia:

- Tubular dilations of moderate extent that were partially or entirely lined with abnormal, cuboidal-shaped epithelial cells and revealed an increased number of cell nuclei per unit epithelial length as documented previously (7) (Figure 2, A through D). This abnormal epithelium was occasionally encountered in focal zones or "plaques" (7) surrounded by normal-appearing epithelium (Figure 2, A and B).
- Large cysts with atrophied, flattened epithelium that had mostly lost the tubulospecific characteristics (Figure 2, E and F).
- Cysts of varying size with a hypertrophic/hyperplastic-appearing epithelium composed of densely packed cells that were occasionally arranged in several layers (Figure 3, A and B).

Kidneys from homozygously affected animals displayed the aspect of widely dilated tubules with extremely thinned epithelium (Figure 4). At 3 wk of age, the epithelium of the nephron and collecting duct system revealed a simplified, dedifferentiated phenotype so that a segment-related classification of most tubular profiles was no longer possible (Figure 4). Cystic enlargement began with the onset of the proximal tubule and affected all nephron segments. Practically no nephron retained normal microanatomical appearance except for the glomeruli. As judged from the size and density of cell packing of their epithelia, small, medium, and large cysts were distinguished, provided they were adequately sectioned (Table
Figure 2. Proliferating cell nuclear antigen (PCNA) immunofluorescence staining (A, C, and E) and corresponding interference contrast microscopy (B, D, and F) in polycystic kidney disease (PKD). (A and B) Focal plaque region in proximal tubular epithelium from a 3-mo-old heterozygous PKD rat. Transition from intact-appearing epithelium to a less-differentiated plaque is consistent with a sharp increase in PCNA-positive nuclei. (C and D) Abnormal-type epithelium of a cyst derived from proximal tubular epithelium of a 12-mo-old heterozygous PKD rat. In particular, cells bulging into the lumen are positively stained. (E and F) Atrophic, proximal tubule-derived-type epithelium with less numerous positive nuclei. An abnormal-type portion of the epithelium with more numerous labeled nuclei is present between arrowheads. (G) Intraepithelial collagen IV mRNA expression by in situ hybridization of an atrophic-type cyst; underlying fibroblasts are also positive. (E through G) A 3-mo-old heterozygous PKD rat. Magnification: \( \times 440 \) in A and B; \( \times 350 \) in C and D; \( \times 160 \) in E and F; \( \times 265 \) in G.
When appropriately sectioned, smaller cysts showed a higher epithelium than larger ones.

Expression of Matrix Compounds

As shown by in situ hybridization, expression of the α1 chain of type IV collagen in the polycystic kidneys was localized in both cystically altered epithelia and underlying interstitial fibroblasts of the cortex and outer medulla, as described previously in detail (7) (Figure 2G and Figure 3, C and F). Collagen I mRNA expression was exclusively localized in the peritubular fibroblasts (Figure 3F) and was significantly enhanced in fibrotic areas of the cortex and outer stripe. Both signals were generally enhanced and more widely distributed in the 12-mo-old rats.

Proliferation Assay

Qualitative Aspects of PCNA Labeling. With PCNA immunostaining, nuclear labeling displayed a wide range of intensities. Generally, PCNA immunoreactivity is highest during the transition from G1 to S phase of the cell cycle (15). For statistical evaluation, only those nuclei that showed a strong and/or granular staining were counted; in agreement with other
work (14,17), only these staining patterns were considered to indicate the early S or G1-S transition phase.

Quantification of PCNA Labeling. In the heterozygotes, the PI of the tubular epithelia was markedly increased in both age groups when compared with the respective unaffected controls (Figure 1, Table 1). For all types of tubular epithelia from cortex and outer stripe taken together, the mean PI was 3.25 ± 1.41 in the 3-mo-old PKD kidneys and 0.24 ± 0.10 in controls (13.5-fold increase; \( P < 0.05 \)), and 3.45 ± 0.79 in the 12-mo-old PKD kidneys and 0.41 ± 0.21 in controls (8.4-fold increase; \( P < 0.01 \)); differences between the age groups were not significant. The PI values of the examined epithelia, glomeruli, and interstitial cells are indicated in Table 1.

Proximal tubules lined with normal-appearing epithelia revealed a 14.1-fold increase in PKD rats compared with controls in the 3 mo-old (\( P < 0.05 \)), and an 11.9-fold increase in the 12-mo-old (\( P < 0.01 \)), respectively.

The abnormal-type proximal tubular epithelia characteristically showed scattered clusters of three to eight PCNA-positive nuclei, which were usually located in regions with thickened basement membrane and an accompanying interstitial fibrosis (Figure 2, C and D). These sites showed enhanced signals for \( \alpha1(IV) \) collagen IV mRNA in both the epithelium and the underlying fibroblasts, and the latter were expressing collagen I mRNA as well. The PI values in these epithelia were 19.0-fold higher in the 3-mo-old (\( P < 0.05 \)) and 20.7-fold higher in the 12-mo-old (\( P < 0.01 \)) than in the respective controls.

The typical cell plaques of less-differentiated epithelium surrounded by normal-appearing proximal tubular epithelium showed the highest PI increases of all epithelia evaluated (Table 1; Figure 1 and Figure 2, A and B). Compared with adjacent, normal-appearing proximal tubule epithelium, PI values were increased 81.6-fold in the 3-mo-old (\( P < 0.01 \)) and 55.5-fold in the 12-mo-old (\( P < 0.01 \)); compared with unaffected controls, PI values in the plaques were several hundred-fold elevated. In these epithelia, \( \alpha1(IV) \) collagen IV mRNA expression was particularly strong.

In the atrophic type of cysts, some epithelia were lacking

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Table 1. PI (median ± SD) in glomeruli and epithelia lining cysts and noncystic tubules in heterozygously affected PKD rats

<table>
<thead>
<tr>
<th>Age</th>
<th>Group</th>
<th>Glomeruli</th>
<th>Proximal Segments</th>
<th>Distal Segments</th>
<th>Interstitial Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Normal</td>
<td>Plaque Abnormal</td>
<td>Atrophic</td>
<td>Hyperplastic</td>
</tr>
<tr>
<td>3 mo</td>
<td>Control</td>
<td>0.42 ± 0.29</td>
<td>0.20 ± 0.09</td>
<td>0.39 ± 0.12</td>
<td>2.02 ± 1.00</td>
</tr>
<tr>
<td></td>
<td>PKD-positive</td>
<td>0.61 ± 0.05</td>
<td>2.76 ± 1.32 54.70 ± 4.90 3.73 ± 1.53 5.66 ± 2.56 6.02 ± 1.12 3.37 ± 1.39 9.12 ± 5.16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 mo</td>
<td>Control</td>
<td>0.72 ± 0.59</td>
<td>0.27 ± 0.17</td>
<td>0.83 ± 0.30</td>
<td>2.15 ± 1.60</td>
</tr>
<tr>
<td></td>
<td>PKD-positive</td>
<td>1.80 ± 1.19</td>
<td>3.27 ± 0.39 55.50 ± 2.60 5.68 ± 1.42 3.94 ± 0.35 4.33 ± 1.28 3.41 ± 1.08 10.90 ± 1.35</td>
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<td></td>
</tr>
</tbody>
</table>

*PI, proliferation index; PKD, polycystic kidney disease.
PCNA staining, whereas others showed numerous positive nuclei. The interstitium underlying the epithelia with PCNA-positive nuclei usually was characterized by mild-to-extensive fibrosis and corresponding grades of the expression of collagen I and IV (Figure 2, E through G). The mean P1 values were elevated 28.9-fold (3 mo; \( P < 0.05 \)) and 14.4-fold (12 mo; \( P < 0.01 \)) when compared with the respective controls.

In the hyperplastic type of cysts, large numbers of PCNA-positive nuclei were seen per cross-sectioned cyst profile (Figure 3, A and B); however, relative to the number of cells, the PI was not exceptionally elevated compared with other cyst types (30.7-fold in the 3-mo-old \( P < 0.01 \) and 15.8-fold in the 12-mo-old \( P < 0.01 \)). Collagen IV expression in these cysts was maximally developed in both the epithelium and the adjacent fibroblasts (Figure 3C), whereas collagen I expression was less conspicuous.

In the distal segments, mean PI levels were elevated 8.6-fold in the 3-mo-old \( (P < 0.05) \) and 4.1-fold in the 12-mo-old \( (P < 0.05) \) compared with controls (Figure 3, D and E). However, numerous profiles showed no PCNA labeling at all in both age groups. Adjacent to distal cysts or cystically dilated tubules with high PI levels, interstitial fibrosis was strongly developed and, in particular, the expression of collagen I mRNA was high in these areas (Figure 3F).

In the cortical and outer medullary interstitium, the number of PCNA-positive nuclei per selected area was significantly increased in the heterozygous PKD animals (4.5-fold in the 3-mo-old \( P < 0.05 \) and 5.1-fold in the 12-mo-old \( P < 0.01 \)) (Table 1). PI values were particularly high in areas of interstitial fibrosis, which was encountered more frequently in the 12-mo-old. Especially in areas around the distal segments, the highest PI values were registered in parallel with enhanced matrix expression.

Glomeruli showed few PCNA-positive nuclei, with no significant differences between controls and polycystic kidneys (Table 1). Positive nuclei were more frequently located in the glomerular tuft than in Bowman's capsule in all groups.

For comparison, kidneys from homozygously affected Han:SPRD rats were evaluated as well. In these kidneys, PI values were highest in small cysts, followed by medium-sized cysts; the lowest values were found in large cysts (Figure 4, Table 2). Compared with normal epithelia from the 3-mo-old group, PI increases in epithelia from the homozygous animals were 254-fold in the small, 133-fold in the medium, and 65-fold in the large cysts. However, no significant difference was found between these cyst types due to high variability of PCNA values in the evaluated epithelia. Little fibrosis was present in

### Table 2. PI (median ± SD) of epithelium lining cysts in homozygously affected PKD rats

<table>
<thead>
<tr>
<th>Age</th>
<th>Cysts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Small</td>
</tr>
<tr>
<td>3 wk</td>
<td>61.97 ± 21.85</td>
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Discussion

We have demonstrated marked increases in PCNA-positive cell nuclei in rats with PKD. The enhanced immunoreactivity for PCNA was interpreted as an increase in cellular DNA synthesis, which can be expected to be followed by cell proliferation (14,17,18). The PI of normal proximal and distal segments of the controls was between 0.2 and 0.8, with values around 0.2 for the proximal tubule and higher values for the distal segments. The results generally agree with other sources on basal PCNA expression in rat (14) and human (5) kidney. In the proximal tubule-derived cyst epithelia of the heterozygous PKD rats, increases in PI values, depending on the different cyst categories, ranged between 14-fold and 31-fold in the 3-mo-old animals and between 12-fold and 16-fold in the 12-mo-old animals, whereas in the distal segments (i.e., from thick ascending limb to cortical and outer medullary collecting duct), relative increases were less pronounced, ranging between eightfold and fourfold in the respective groups. These increases agree with the course of cyst development in the Han:SPRD model taking its origin from the proximal tubule and then extending to distal segments (6,7).

As in human PKD, heterozygous Han:SPRD rats show a slow progression of the disease. This provides the advantage that over a broad age range, cystic stages of different degrees including the most initial stages can be observed in parallel. Most, if not all, nephrons appear to be implicated in the pathophysiologic changes, because even the microanatomically unaffected proximal tubule epithelia of polycystic kidneys show solid increases in PI. Therefore, the presumed onset of cystic transformation in genetically predestined epithelia already seems to be evident in the noncystic tubule.

The progression mode of cystic nephron changes can be interpreted to some extent from the various phenotypes of cysts. Patches or plaques of less-differentiated cells with increased nuclear-to-cytoplasmic ratio and higher incidence of mitotic figures were described earlier (6,7,19) and are regarded as initial stages of cystic dilations of the proximal tubule (7); apparently, these initial stages may also form local outpouchings of the renal tubule (20). The plaques then become more generalized in cysts with “abnormal” epithelium still showing some characteristics of the proximal tubule. The most advanced stages of cysts with large lumina showed terminal loss of epithelial differentiation and were lacking tubulospecific characteristics.

Maximal proliferation intensity was encountered in the focal plaques of proximal tubule epithelium, where almost every cell was PCNA-positive; however, even in the presumed terminal stages of cysts in the older group of heterozygotes, increased PI values were maintained. The particularly high PI values of all tubular segments in the homozygotes, which at 3 to 4 wk of age reach end-stage renal failure due to cystic enlargement of practically all tubules, support these findings and suggest that with more severe expression of PKD, more nephrons get involved in the changes more rapidly. Distal segments in the
heterozygotes with typically lower P1 values develop cysts only in very late stages, whereas in the homozygotes, there is little difference between proximal and distal cyst development. A reason for the priority of cyst formation in the proximal nephron is not evident from our data.

The hyperplastic type of cyst with prismatic and sometimes layered epithelium seems to represent a particular cyst form in the Han:SPRD rat, exhibiting less tendency to expand because these cysts are frequently found still in the older age group. On the other hand, the marked increases in P1 values in these cysts, which persist in late stages of progression, demonstrate that these epithelia are highly proliferative and therefore may be similar to polyloid hyperplasias described in human PKD (21).

Cell proliferation is thought to play a key role in cystic nephron changes (5,22-26). Pharmacologic agents such as taxol or lovastatin, which are thought to reduce the rate of cellular proliferation, were effective in slowing the progression of cystic growth in animal models for PKD (27,28). Studies in the Han:SPRD rat have suggested that an increased mRNA level for c-myc as a representative of proto-oncogenes was an indicator of cell proliferation when viewed in conjunction with the development of PKD in c-myc transgenic mice (6,29). Likewise, in an in vitro model, the significance of hyperplastic cell growth in cyst formation was illustrated by bromodeoxyuridine incorporation (25). In human ADPKD, epithelia from various histologic types of cysts were altogether showing increased P1 values as quantified from PCNA staining, although levels were generally somewhat lower than in the homozygous Han:SPRD rats (5). Similar to the homozygous rats, samples from rapidly progressive human ARPDK had a significantly higher incidence of PCNA-immunoreactive nuclei (5).

The primary cause for the observed increases of proliferation in PKD is not clear at present. This study shows the parallel occurrence of cell hyperplasia and matrix overexpression with collagen IV mRNA present in both epithelia and underlying fibroblasts and collagen I mRNA in the fibroblasts alone, thus confirming previous assumptions of such a coincidence (6,7,19). Increased matrix expression per se or compositional alterations of matrix compounds have been suggested to contribute to cyst development (30,31), possibly by inducing a loss of cellular differentiation and increased cell turnover, which may then result in an arrest of the cells in a less-differentiated state. Increase in interstitial fibrosis during progression of PKD was consistent with a five- to sixfold elevated PI value in the interstitial fibroblasts.

Epithelial hyperplasia could also be a consequence of higher cell turnover induced by increased rates of apoptosis, which was demonstrated elsewhere for PKD (32,33). In a model of renal tissue regeneration, which shares many morphologic characteristics with the pathology of renal tissue in PKD, the transient increase of apoptosis was also observed to precede a peak of regenerative proliferation (34). Disturbances in epithelial–matrix interactions have also been shown to induce apoptosis (35), so that the initial plaques of highest PI values and the concomitant matrix overexpression and loss of differentiation of the proximal tubule may go hand in hand with apoptotic events.

The intensive matrix accumulation that is a result of overexpression at the mRNA level in the heterozygotes is probably a secondary phenomenon, because in homozygotes, matrix expression is moderate and is not paralleled by interstitial fibrosis, whereas cyst development is much more pronounced than in the heterozygotes. Disturbed epithelial–matrix interactions in homozygotes may be prevalent nonetheless, but cell repair mechanisms could act differently under the condition of more rapid proliferation. In conjunction with cell proliferation, other factors such as cell stretch, possibly induced by increases in luminal pressure involving an abraction of the nephron, appear to add to cyst growth (20,25).

Expression of polycystin, the human PKD1 protein, was reported to coincide with cyst development (3), and, although the role of the gene product is still unclear, the current speculation is that there is a defective interaction of polycystin with matrix compounds. Because chromosomal mutation is not located on a homologous site in Han:SPRD rats (8), it remains to be determined whether a gene product functionally similar to PKD1 is involved.

In summary, this study provides evidence for proliferative activity in the nephron epithelia of the Han:SPRD model for ADPKD. As determined by PCNA immunoreactivity, high PI values were detected in cystic nephron epithelia, suggesting much higher proliferation in cysts than in tubular epithelia from healthy controls. Cell proliferation appears to precede cyst formation, because early during progression of the disease, the normal-appearing tubules had markedly increased PI values, whereas later stages revealed a more generalized cystic degeneration of the nephron. The association of enhanced epithelial PI levels with matrix overexpression points to altered cell–matrix interactions, which seem to be directly involved in the disruption of epithelial differentiation.

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References

18. Connolly KM, Bogdanoff MS: Evaluation of proliferating cell nuclear antigen (PCNA) as an endogenous marker of cell proliferation in rat liver: A dual-stain comparison with 5-bromo-2'deoxyuridine. *J Histochem Cytochem* 41: 1–6, 1993


