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Reduced *Pax2* Gene Dosage Increases Apoptosis and Slows the Progression of Renal Cystic Disease

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The murine *cpk* mouse develops a rapid-onset polycystic kidney disease (PKD) with many similarities to human PKD. During kidney development, the transcription factor Pax2 is required for the specification and differentiation of the renal epithelium. In humans, *Pax2* is also expressed in juvenile cystic kidneys where it correlates with cell proliferation. In this report, *Pax2* expression is demonstrated in the cystic epithelium of the mouse *cpk* kidneys. To assess the role of *Pax2* during the development of polycystic kidney disease, the progression of renal cysts was examined in *cpk* mutants carrying one or two alleles of *Pax2*. Reduced *Pax2* gene dosage resulted in a significant inhibition of renal cyst growth while maintaining more normal renal structures. The inhibition of cyst growth was not due to reduced proliferation of the cystic epithelium, rather to increased cell death in the *Pax2* heterozygotes. Increased apoptosis with reduced *Pax2* gene dosage was also observed in normal developing kidneys. Thus, increased cell death is an integral part of the *Pax2* heterozygous phenotype and may be the underlying cause of Pax gene haploinsufficiency. That the cystic epithelium requires *Pax2* for continued expansion underscores the embryonic nature of the renal cystic cells and may provide new insights toward growth suppression strategies.

Key Words: **Pax2; polycystic kidney; cpk; apoptosis.**

INTRODUCTION

Polycystic kidney disease (PKD) is among the most common genetic disorders in the adult population. It has been proposed that the renal cystic epithelial cell has dedifferentiated to a more embryonic phenotype, leading to increased cellular proliferation, mislocalization of apical and basolateral cell surface markers, and increased expression of specific basement membrane components (Calvet, 1993, 1998). In humans, autosomal dominant PKD (ADPKD) has been associated with mutations in at least two genes, *ADPKD1* (The European PKD Consortium, 1994) and *ADPKD2* (Mochizuki *et al.*, 1996), that encode large transmembrane proteins which are known to associate (Qian *et al.*, 1997). In mouse, at least five loci that can generate a PKD phenotype upon disruption have been identified (Schieren *et al.*, 1996). Among the best characterized mouse PKD mutants is the *congenital polycystic kidney* (*cpk*) mouse (Gattone *et al.*, 1988, 1996). The *cpk* mutation is recessive, maps to mouse chromosome 12 (Simon *et al.*, 1994), and was originally identified in the C57BL6 strain. Homozygous *cpk* animals develop cysts from birth and die at approximately 3 weeks from acute renal failure. At the cellular level, the *cpk* mutant mouse exhibits many of the same traits found in human PKD, including mislocalization of the EGF receptor (Orellana *et al.*, 1995) and altered deposition of basement membrane proteins (Nagao *et al.*, 1994; Ojeda, 1999; Rocco *et al.*, 1992; Taub *et al.*, 1990). Mutations in PKD1 or PKD2 account for about 95% of the clinical PKD cases, leaving at least 5% of human PKD unaccounted for. Whether the mouse *cpk* gene, which has not yet been identified but does not correspond to either human PKD1 or PKD2, is associated with this minority of human PKD remains to be determined.

During embryonic development, the *Pax2* gene is essential for the differentiation and proliferation of the renal epithelium in both mouse (Keller *et al.*, 1994; Rothenpieler and Dressler, 1993; Torres *et al.*, 1995) and man (Sanyanusin *et al.*, 1995a,b; Schimmenti *et al.*, 1995). *Pax2,* like other members of the Pax gene family, is haploinsufficient such that mice and humans heterozygous for loss of func-

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tion alleles show slightly reduced renal mass, occasional unilateral renal agenesis, and eye and ear abnormalities. During the normal course of kidney development, *Pax2* is expressed in the nephric duct, the ureteric bud, and the induced renal mesenchyme which ultimately generates much of the glomerular, proximal tubular, and distal tubular epithelium (Dressler and Douglass, 1992). In the mouse, expression persists from embryonic day 11 until approximately 10 days after birth, particularly in the nephrogenic zone where new nephrons continue to from and epithelial cells are proliferating. *Pax2* expression is down-regulated in the glomerulus and in proximal and distal tubules as the epithelial cells mature (Dressler and Douglass, 1992; Ryan *et al.*, 1995). Strikingly, juvenile cystic and dysplastic kidneys show persistent *Pax2* expression that correlates with cell division and *bcl2* expression (Winyard *et al.*, 1996b). Deregulated expression of *Pax2* in transgenic mice results in glomerular and proximal tubule microcyst formation and renal failure(Dressler *et al.*, 1993). Persistent expression of *Pax2* has also been described in other proliferative abnormalities in the kidney, including Wilms' tumor (Dressler and Douglass, 1992) and renal cell carcinoma (Gnarra and Dressler, 1995).

To directly address whether *Pax2* regulates the initiation and progression of renal cystic disease or is merely a marker for proliferating, undifferentiated epithelial cells, we examined the effects of *Pax2* haploinsufficiency in mice with the *cpk* mutation. Within the *cpk* homozygous population, *Pax2* heterozygous animals exhibit more slowly progressing cystic disease, indicating an inhibitory effect of reduced gene dosage. The inhibition of cyst growth was not due to reduced cell proliferation, rather to increased apoptosis in *Pax2* heterozygotes. Furthermore, increased cell death is the underlying cause of *Pax2* haploinsufficiency in normal kidneys as well. Thus, *Pax2* expression and gene dosage in cystic epithelium can modify the progression of renal cystic disease in mice. The data support the hypothesis that the renal cystic epithelium remains in a non-terminally differentiated state and is regulated, at least in part, by developmental transcription factors.

MATERIALS AND METHODS

Animals. Male mice carrying the *cpk* mutation in a C57BL6 background were a generous gift from V. Gattone, University of Kansas Medical School. An F1 hybrid strain was generated by mating to C3H females. These F1 female offspring were tested for the *cpk* mutation by backcrossing to the founder *cpk*/+ males and checking for cystic offspring. Positive *cpk* F1 animals must carry the mutant C57BL6 allele at the *cpk* locus. The *Pax2* null mutation, originally generated in the 129 strain, was bred into the C3H strain by repeated crossing over six generations. C3H *Pax2* +/− mice were mated to the *cpk*−/+ F1 mice to generate double heterozygotes carrying the C57BL6 *cpk* allele and one copy of the *Pax2* gene. The *cpk*−/+; *Pax2*−/+ double heterozygotes were crossed to *cpk*/+ animals to generate *cpk* homozygous pups that carried either one or two copies of the *Pax2* gene.

Genotyping. Genomic DNA was extracted from tail biopsies by overnight digestion with protease K in 100 mM NaCl, 10 mM Tris, pH 8, 1% SDS. Samples were treated with RNase, extracted with phenol:chloroform, and ethanol precipitated. The microsatellite markers D12Mit10, D12Mit12, and D12Mit58 were used to distinguish the C57BL6 and C3H alleles around the *cpk* gene. PCR products were separated on 4% metaphor agarose gels (FMC). *Pax2* genotyping was done by Southern blot analysis of *Eco*RI-digested tail DNA. A 1.3-kb *Not*I–*Kpn*I fragment, extending from upstream genomic sequences into the first exon, was used to distinguish the normal from the mutant allele.

Tissue analysis. Kidneys were removed and either frozen directly in liquid nitrogen or fixed in 4% paraformaldehyde for histology and immunostaining. For immunostaining kidneys were fixed for 2 h, washed in PBS, and incubated overnight in 0.5 M sucrose, PBS before being frozen in liquid nitrogen. For histology, kidneys were fixed overnight in paraformaldehyde, dehydrated, and embedded in paraffin. Serial sections were stained with hematoxylin and eosin. Four independently derived cystic kidneys were sectioned for both *Pax2*+/+ and *Pax2*+/− genotypes at 3 days and at 10 days after birth for a total of 16 samples.

Immunostaining. Cryostat sections were cut at 8 μm, collected on gelatinized slides, and air dried for 30–60 min. Immunostaining was as described by Harlow and Lane (1988). Sections were fixed in acetone for 5 min in 3% paraformaldehyde, washed in PBS, and permeabilized in 0.05% Triton X-100 in PBS. A 10 μg/ml dilution of anti-Pax2 IgG or control, prebleed IgG was prepared in 2% goat serum in PBS, and 20 μl was applied to each section. Slides were incubated at room temperature in a humid chamber and then washed twice in PBS, 0.05% Tween. The second antibodies were diluted 1:300 for the TRITC- or FITC-conjugated anti-rabbit (Sigma) in 2% goat serum in PBS. After a 30-min incubation, slides were washed twice in PBS/Tween and covered with Gelvatol. Photomicrographs were taken with a Nikon ES800 fluorescence microscope. The E-cadherin antibody was from Sigma (U-3254); the Pax2 (Dressler and Douglass, 1992) and cadherin-6 (Cho *et al.*, 1998) antibodies have been described.

Cell proliferation and apoptosis. P3 and P21 mice were injected with BrdU (Sigma) in PBS at a concentration of 3 mg/100 g body mass. Mice were sacrificed after 2 or 4 h. Kidneys were removed, immersed in OCT, and flash frozen. Sections were cut at 8 μm and permeabilized with 2 N HCl, 0.5% Triton X-100, PBS for min. Anti-BrdU (Sigma) was diluted 1:100 in PBS, 0.5% Tween 20, 2% goat serum. After incubation for 30 min with TRITC-conjugated anti-mouse antibodies (Sigma) the sections were counterstained with 1 μg/ml DAPI. Sections were rinsed three times and mounted with Gelvatol. Micrographs were taken with a Nikon ES800 using broad band filters. The total number of cells and the number of BrdU-positive cells were counted over a given area. Four independently derived litters were injected and sections were analyzed from at least four kidneys for each genotype.

The TUNEL reaction was performed using the Apoptag kit (Boehringer Mannheim, Inc.) according to the manufacturer's protocol. The permeabilization time was increased to 20 min and the sections were counterstained with 2.5 ng/ml propidium iodide. Fore each genotype, four different kidneys were sectioned. The sections were photographed under fluorescence illumination with a 40× lens. At least 10 data points were taken for each kidney, for which the number of positive apoptotic bodies and the total number of nuclei were counted within a micrograph and the pyknotic index was expressed as the ratio of apoptotic bodies to the total number of nuclei.

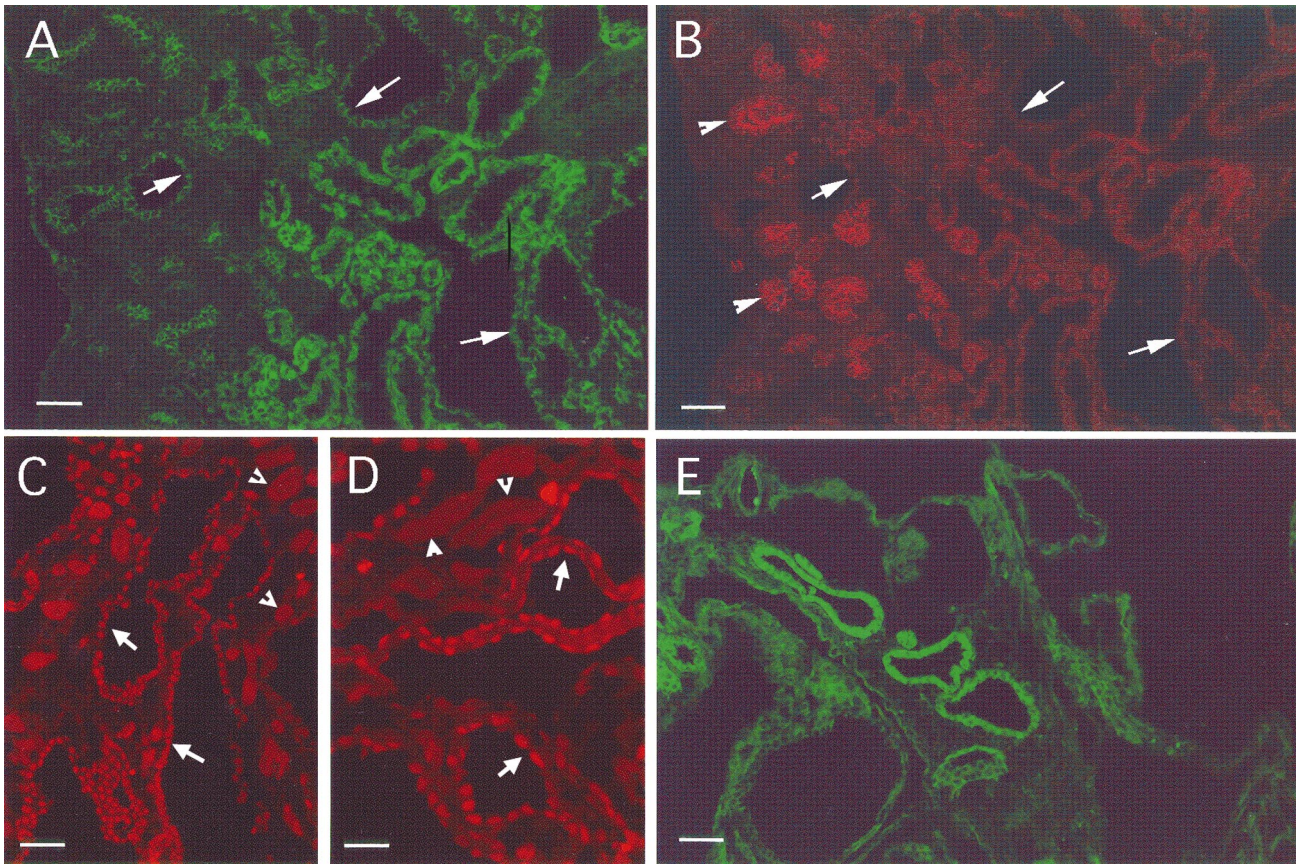

FIG. 1. Immunostaining of cystic epithelium. (A) Anti-E-cadherin staining of 3-day *cpk* kidney. Note expression in cystic epithelium (arrows). (B) Same section as A stained with anti-cadherin-6. Note expression in comma- and S-shaped bodies of the nephrogenic zone (arrowheads) but not in the cystic epithelium (arrows). (C) Expression of Pax2 in *cpk* kidney at 10 days. Note nuclear staining in cystic epithelium (arrows) but not in noncystic tissue (arrowheads). (D) Higher magnification of 10-day *cpk* kidney. (E) Anti-E-cadherin staining of 10-day *cpk* kidney. Note that large cysts have lost much of the E-cadherin staining at the basolateral sides. Bars represent 50 μm for A, B, C, and E and 25 μm for D.

RESULTS

We hypothesized that *Pax2* expression might be present in the cystic epithelium of *cpk* kidneys, given the results obtained with human juvenile cystic kidneys (Winyard *et al.*, 1996b). In the *cpk* mutant, cysts are thought to originate primarily from distal tubules and collecting ducts. This was confirmed with anti-cadherin antibodies at 3 and 10 days postpartum (P3 and P10), indicating that most cysts derived from the E-cadherin-expressing distal tubule and collecting duct epithelium (Fig. 1A) and not from the developing proximal tubules which express cadherin-6 (Fig. 1B). Immunostaining revealed distinct Pax2 nuclear expression in many cystic epithelial cells at P10, but little staining in the noncystic epithelium or interstitial cells (Figs. 1C and 1D). Only at later times were cysts positive for cadherin-6, showing that cyst growth had extended into the proximal tubule (data not shown). Strikingly, large cysts had less E-cadherin expression at the basolateral side (Fig. 1E), which may indicate a loss of epithelial cell polarity and tight junctions.

To determine whether the persistent expression of *Pax2* in the cystic epithelium was a contributing factor to cyst growth, the effect of *Pax2* gene dosage was examined by crossing the *Pax2* null allele (Torres *et al.*, 1995) into *cpk* heterozygotes. An F1 founder generation was bred by crossing *cpk* heterozygous C57BL6 males to either wild-type C3H females or *Pax2* heterozygous females within a C3H genetic background. Double-heterozygous male F1 founders that carried the *cpk* allele, derived from the C57BL6 strain, and the *Pax2* null allele were identified. These males were crossed to female F1 mice (C57BL6/C3H) which carried only the *cpk* allele to generate the F2 animals for analysis. As both parental mice were hybrid strains of 50% C3H and 50% C57BL6, the overall genetic complement of the F2 generation will still be 50:50 although individual loci can be

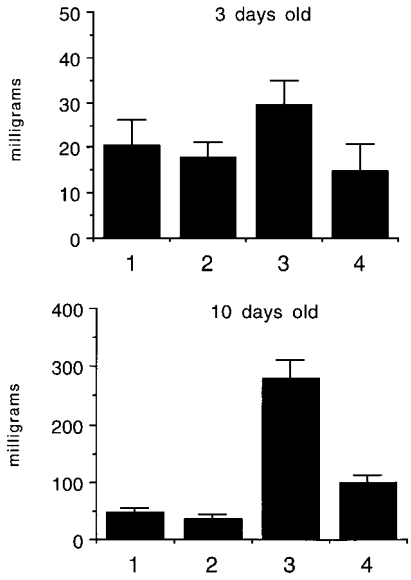

FIG. 2. Comparative wet weights of kidneys from normal and cystic mice carrying either one or two copies of the *Pax2* gene. Kidneys were taken at 3 or 10 days as indicated. Weights are averages plus 1 standard deviation from the mean. Genotypes are listed below, the number of samples (*n*) is given for 3 and 10 days respectively: 1—*Pax2*+/+:*cpk*+/+ (n = 13, 17), 2—*Pax2*+/−:*cpk*+/+ (n = 15, 12), 3—*Pax2*+/+:*cpk*−/− (n = 7, 17), 4—*Pax2*+/−:*cpk*−/− (n = 10, 9).

homozygous for either the C3H or the C57BL6 allele. The F2 pups were sacrificed 3 and 10 days postnatally and genotyped for the *cpk* allele using strain-specific polymorphisms of microsatellite markers near the *cpk* locus. At both 3 and 10 days, cyst were already evident and correlated with the presence of the C57BL6 allele near the *cpk* locus. Within a single litter, homozygous *cpk* offspring could have either one or two copies of the *Pax2* gene. Multiple litters were examined from five mating pairs over a period of 6 months.

Kidneys from P3 and P10 pups were dissected out, weighed, and prepared for immunostaining and histology. Total wet weight was used as an indication of size (Fig. 2). At P3, noncystic kidneys containing only one copy of the *Pax2* gene tended to be slightly smaller than their wild-type counterparts, 17.7 ± 3.5 mg versus 20.4 ± 5.9 mg, although this difference was not statistically significant ($P < 0.2$). This size difference was significant at P10, with *Pax2*+/− kidneys weighing 36.9 ± 7.7 mg and *Pax2*+/+ kidneys weighing 47.7 ± 7.5 mg ($P < 0.01$). Among the cystic kidneys, there was a clear effect of *Pax2* gene dosage even at 3 days (Fig. 2). Homozygous *cpk* kidneys with two copies of *Pax2*+/+ were more than twice the weight of homozygous *cpk* kidneys with only one copy of *Pax2* ($P < 0.01$). In fact, *Pax2*+/− cystic kidneys were not significantly larger than noncystic kidneys, even though histological analysis ultimately revealed small cysts present. By P10, *Pax2* gene dosage had a remarkable effect on slowing of cyst growth. Cystic kidneys wild type for *Pax2* were threefold larger than *cpk* homozygote kidneys with only one copy of *Pax2* ($P < 0.001$).

Histology revealed that *Pax2* heterozygotes had smaller cysts at both P3 and P10, compared to *Pax2*+/+:*cpk*−/− mice, and generally had more normal glomeruli and tubules remaining (Fig. 3). By P3, *cpk*−/−:*Pax2*+/+ mice had large cysts extending through the cortex and to the nephrogenic zone (Fig. 3A), with many smaller cysts in the medullary zone and cortex. In contrast, *cpk*−/−:*Pax2*+/− mice had small cysts primarily in the medullary zone (Fig. 3B). By P10, kidneys from *cpk*−/−:*Pax2*+/+ mice within this hybrid background were almost completely cystic, with large fluid-filled cysts having displaced almost all of the nephrons and extending completely to the renal capsule (Fig. 3C). In contrast, *cpk*−/−:*Pax2*+/− mice had significantly more normal renal tubules in the cortex, particularly within the periphery where the youngest nephrons are located (Fig. 3D). Since the origin of the cysts is primarily collecting duct and distal tubule (Avner *et al.*, 1987), cysts are first apparent in the medullary zone and progress into the cortex. This progression appears delayed in the *Pax2*+/− mice, as cortical cysts are rarely detected at 3 days. By 10 days, *cpk*−/−:*Pax2*+/− kidneys exhibit large cysts in the medullary zone that have not extended to the peripheral zone of the cortex, unlike the *Pax2*+/+:*cpk*−/− mice. Within this hybrid genetic background, homozygous *cpk* mice with two copies of *Pax2* always died within 3 weeks, whereas *Pax2*+/−:*cpk*−/− mice generally survived longer with many individuals lasting 4–6 weeks.

Taken together, the data demonstrate an inhibition of cyst growth in the *Pax2* heterozygous *cpk* animals. Such inhibition could be due to reduced rates of cellular proliferation or increased cell death. In order to distinguish between these two possibilities, cell proliferation was assayed by *in vivo* BrdU labeling and the number of cells undergoing apoptosis was scored by the TUNEL assay. Mice were sacrificed 2 and 4 h after BrdU injection and the kidneys sectioned for immunostaining with anti-BrdU antibodies. Cellular proliferation index was expressed as the ratio of BrdU-positive nuclei relative to the total number of nuclei per given area. Apoptosis was assayed using the TUNEL reaction and the pyknotic index was expressed as the ratio of labeled to unlabeled nuclei per given area. Representative pictures are shown in Fig. 4 and the data in Table 1. Cellular proliferation at 3 days is extensive and encompasses the nephrogenic zone, where epithelial cells are still differentiating; the cortex; and the medullary zone, where ascending and descending limbs of Henle's loop are forming in new nephrons. There was no significant difference in cellular proliferation among the genotypes tested (Table 1, Figs. 4E and 4F). *Pax2* gene dosage did not affect proliferation in either the wild-type or the *cpk* background. However, *Pax2* gene dosage had a very significant effect on cell death in both wild-type and *cpk* mice. Heterozygous

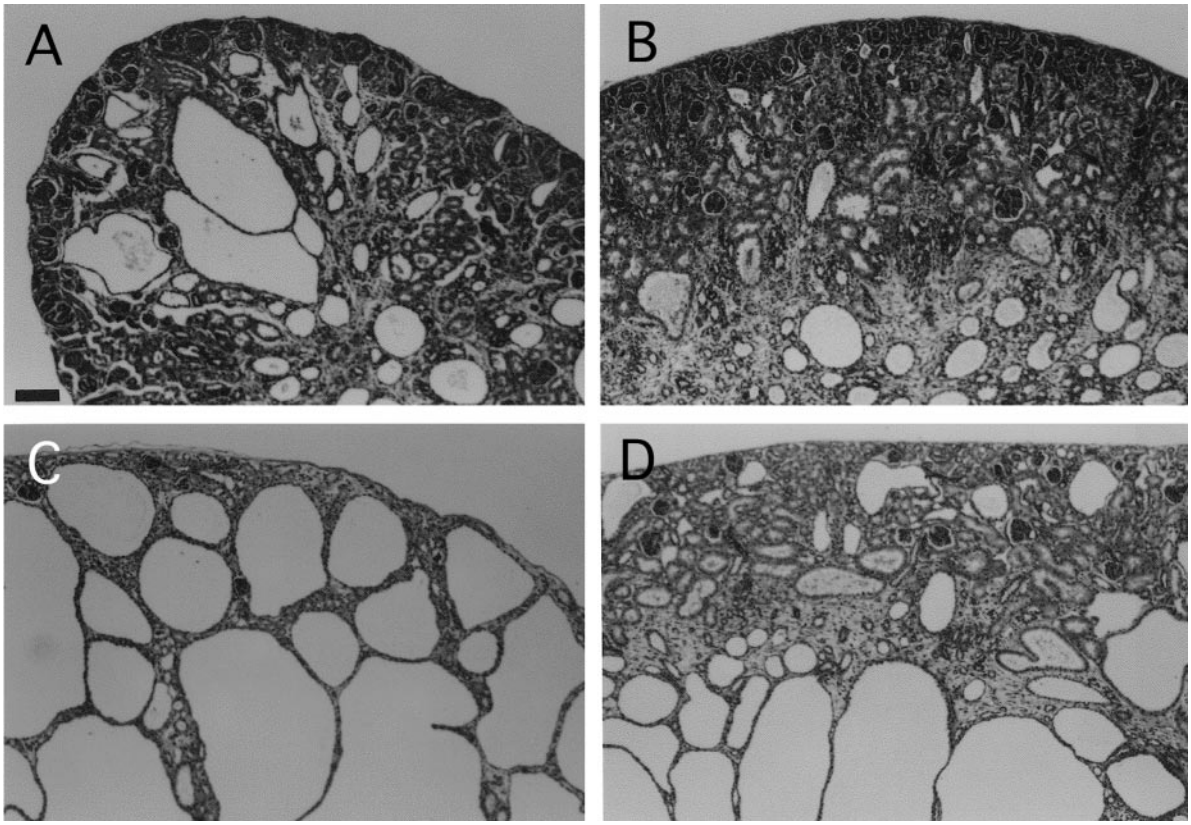

FIG. 3. Renal histology of cystic kidneys. (A) 3-day-old kidney from *Pax2*+/+:*cpk*−/− mouse. (B) 3-day-old kidney from a *Pax2*+/−:*cpk*−/− mouse. Note that smaller cysts are located more in the medullary zone and rarely extend into the cortex. (C) 10-day-old *Pax2*+/+:*cpk*−/− kidney. (D) 10-day-old *Pax2*+/−:*cpk*−/− kidney. Note the peripheral tubules are still relatively free of large cysts compared to C. The bar in A represents 100 μm. Representative sections are shown.

Pax2+/− animals exhibited a twofold increase in the pyknotic index, relative to the *Pax2*+/+ genotypes (Table 1). At 3 days, increased apoptosis was clearly evident in the nephrogenic zone of *Pax2*+/− mice (Figs. 4A and 4B), regardless of the *cpk* genotype. At 3 days, apoptosis in cystic epithelium was difficult to detect, primarily because the percentage of apoptotic bodies is low, probably due to rapid clearance, and cystic cells reflected a small percentage of the total. However, at 21 days, *Pax2*+/− mice again exhibited a twofold increase in apoptotic bodies in cystic epithelium (Figs. 4C and 4D; Table 1).

DISCUSSION

In the mouse *cpk* model, *Pax2* gene dosage can affect the progression of cystic kidney disease, which is characterized by continued proliferation and dedifferentiation of the renal epithelium. The results further underscore the embryonic nature of the cystic epithelium and point to an essential role for *Pax2* in protection from apoptosis. Persistent expression of *Pax2* has been observed in human ADPKD1 (Eng *et al.*, 1994) and in juvenile cystic kidneys (Winyard *et al.*, 1996b) and thus does not seem to be a feature unique to the *cpk* mouse. Expression of *Pax2* is also found in human Wilms tumor (Dressler and Douglass, 1992; Eccles *et al.*, 1992) and renal cell carcinoma and is necessary for the proliferation of cultured renal tumor cells (Gnarra and Dressler, 1995). *Pax2* and its close relatives *Pax5* and *Pax8* are known to suppress *p53* transcription in transfected NIH 3T3 cells by binding to *cis*-acting regulatory sequences (Stuart *et al.*, 1995). Misregulation of *Pax2* in juvenile cystic disease also correlates with *bcl2* expression (Winyard *et al.*, 1996a,b). Thus, apoptosis in *Pax2* heterozygous kidneys may be mediated in part by increased levels of *p53*, which can promote apoptosis, and decreased levels of *bcl2*, a gene that protects cells from death (Hockenbery *et al.*, 1990; Sorenson *et al.*, 1995).

In this report, we demonstrate that increased cell death is a hallmark of reduced *Pax2* gene dosage and as such can affect the rate of development and organ size. Within the nephrogenic zone, Coles *et al.* (1993) estimated the pyknotic index of a normal 3-day-old rat kidney to be approximately 0.7%, in good agreement with our results (0.6%). To

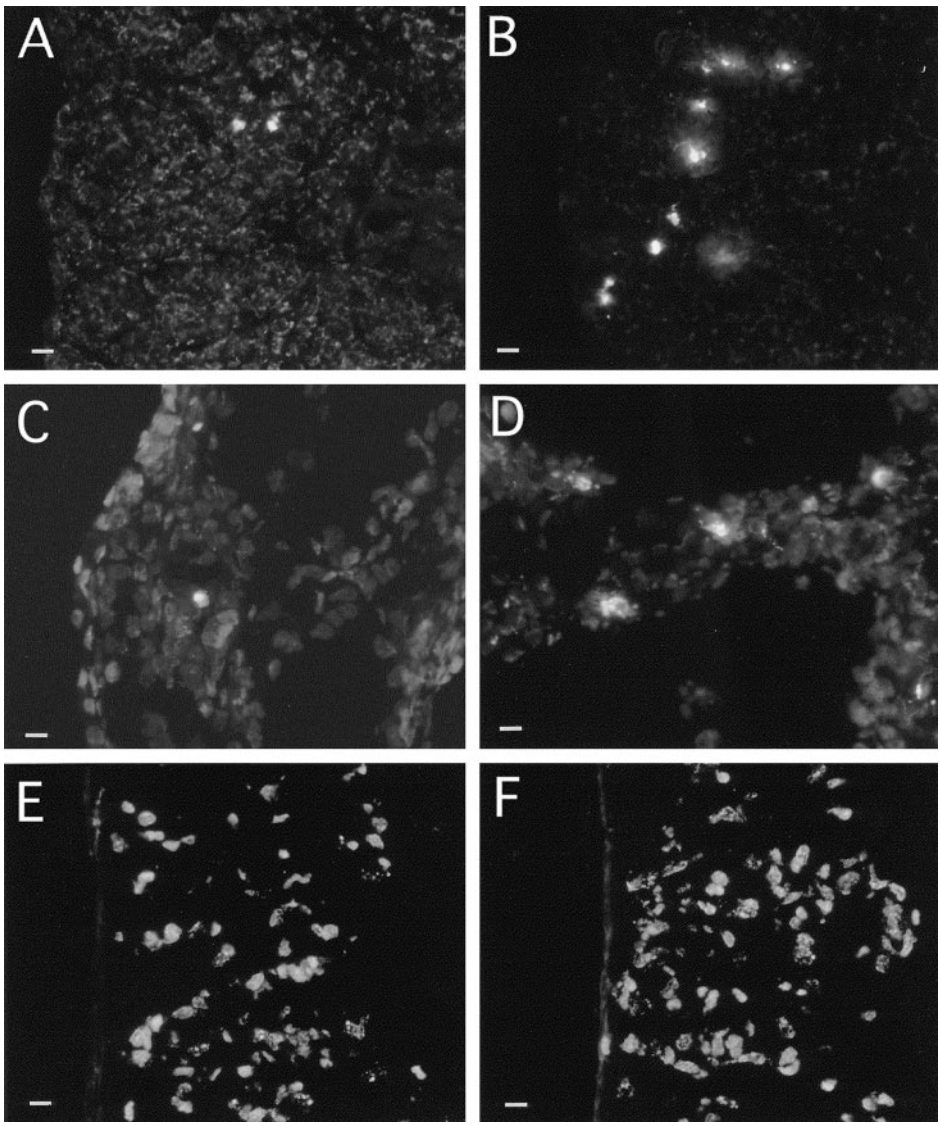

FIG. 4. Cell death and proliferation. Genotypes are *Pax2*+/+:*cpk*−/− in A, C, and E and *Pax2*+/−:*cpk*−/− in B, D, and F. (A and B) TUNEL staining in the nephrogenic zone of 3-day kidneys. (C and D) TUNEL staining in cystic epithelium of 21-day kidneys. (E and F) Anti-BrdU staining of nephrogenic zone at 3 days. The bars represent 10 μm.

calculate the actual percentage of cells fated to undergo apoptosis, the clearance rate of apoptotic bodies was estimated to be about 1.5 h in the newborn kidney, suggesting that as many as 25% of the differentiating epithelial cells may ultimately die (Coles *et al.*, 1993). The effect of reduced *Pax2* gene dosage would double this number and significantly affect the amount of tubular epithelium present at similar times. For noncystic kidneys we see a reduction in renal mass of approximately 25% at 10 days. Since *Pax2* gene dosage would not be expected to affect nonepithelial cells of the kidney, including interstitial mesenchyme, endothelial, and smooth muscle cells, the reduction in mass is consistent with the twofold increase in the pyknotic index of *Pax2*+/− mice.

It is difficult to determine precisely which cell types are undergoing apoptosis within the nephrogenic zone, because at the time of chromatin condensation and DNA fragmentation the cells have lost expression of their characteristic marker proteins. However, many apoptotic bodies are found within the lumen of the developing tubules, suggesting they were of epithelial origin. Within the cystic epithelium, apoptotic bodies are also evident. Except for their reduced size, which becomes more apparent as the animals mature, *Pax2* heterozygous kidneys are relatively normal in both

TABLE 1

Genotype		Age		BrdU staining		Apoptotic nuclei	
cpk	*Pax2*	(days)	Region analyzed	% positive	*n*	% positive	*n*
+/+	+/+	3	Nephrogenic zone	21 ± 4	9	0.6 ± 0.2	24
+/+	+/−	3	Nephrogenic zone	18 ± 5 ($P < 0.2$)	12	1.4 ± 0.5 ($P<0.001$)	23
−/−	+/+	3	Nephrogenic zone	16 ± 10	18	0.4 ± 0.3	14
−/−	+/−	3	Nephrogenic zone	21 ± 14 ($P < 0.2$)	28	0.9 ± 0.4 ($P<0.001$)	19
−/−	+/+	3	Cystic epithelium	21 ± 14	18	ND	
−/−	+/−	3	Cystic epithelium	16 ± 10 ($P < 0.2$)	28	ND	
−/−	+/+	21	Cystic epithelium	ND		1.2 ± 1.0	20
−/−	+/−	21	Cystic epithelium	ND		2.4 ± 1.8 ($P<0.02$)	20

structure and function. There is no evidence to suggest that a specific cell type is more prone to apoptosis within the heterozygous population. Given the constant rate of cell proliferation, we attribute the reduced kidney size and the reduction in cyst growth to an increase in programmed cell death due to reduced *Pax2* gene dosage.

Haploinsufficiency has been observed for *Pax1*, *2*, *3*, and *6* in both mice and humans, yet the underlying cellular effects of reduced gene dosage have not been described. In heterozygous *Pax6* mutants, the retina, lens, and pigmented epithelium of the developing eye are all reduced in size and cell number (Hanson and van Heyningen, 1995; Theiler *et al.*, 1978). In *Pax1* heterozygous mutants, segmentation of the vertebral column is affected due to reduced number of sclerotome-derived intervertebral disc and vertebral body precursors (Dietrich and Gruss, 1995). *Pax1* mutants also produce fewer thymocytes due to a reduction of the microenvironment necessary for thymocyte differentiation, the thymic epithelium and cortical stromal cells where *Pax1* is expressed (Wallin *et al.*, 1996). Although cell proliferation has not been examined in these mutants, all of the effects observed in *Pax6* and *Pax1* heterozygote animals are consistent with increased cell death, as we have observed in the kidney of *Pax2* heterozygotes.

Although genetic background can affect the rate of cystogenesis among certain inbred strains of mice (Iakoubova *et al.*, 1995, 1997; Upadhya *et al.*, 1999), we saw no evidence of a single gene enhancer or suppresser of cystogenesis between the C3H and the C57BL6 strains used for this study. Such an effect would generate a biphasic distribution of kidney size for a given genotype. Although the distribution of renal size among cystic animals, of either Pax2 genotype, showed a single peak, we cannot rule out potential modifier genes between the C3H and the C57BL6 strains that may expand the range of cystic kidney sizes. However, unless closely linked to the Pax2 locus, such modifiers would be equally distributed among *cpk*−/−:*Pax2*+/+ and *cpk*−/−:*Pax2*+/− genotypes and not affect our overall conclusions. The significant reduction of mean kidney size, the morphological observations of reduced cyst size, and the increase in apoptosis all point to a critical role for *Pax2* in the progression of renal cysts.

The results presented in this report support the hypothesis that PKD consists of dedifferentiated epithelial cells that require embryonic factors, such as *Pax2*, for continued growth and expansion. It remains to be determined just why the embryonic and cystic epithelial cells are prone to undergo apoptosis upon reduction of *Pax2*, a gene normally down-regulated upon terminal differentiation. It may be that once cells of the renal epithelial lineage exit the mitotic cycle, they no longer require high levels of *Pax2* expression to protect them from apoptosis. In any event, reducing the expression levels of such embryonic regulatory genes may thus be one potential avenue for therapeutic interventions that aim to slow cyst growth.

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