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Targeting of the *ras*T24 Oncogene to the Proximal Convoluted Tubules in Transgenic Mice Results in Hyperplasia and Polycystic Kidneys

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Five families of transgenic mice were derived from one-cell-stage embryos injected with γGT-rasT24, a fusion gene consisting of the γ-glutamyl transpeptidase (γGT) 5′ flanking region containing promoter I linked to a mutated (codon 12) human H-ras oncogene. The transgene was expressed selectively in the kidneys, eyes, and brains of all families as determined by reverse transcription-polymerase chain reaction, nuclease protection assays, and in situ hybridization. In two of five families, kidney lesions consisting of proximal tubular hyperplasia, renal cysts, and microadenomas developed in male animals; males also expressed higher levels of γGT/rasT24 RNA. Early lesions consisted of proximal tubular hyperplasia as defined by alkaline phosphatase histochemistry, γGT immunohistochemistry, and electron microscopy and could be correlated with the presence of rasT24 RNA within the cystic proximal tubular epithelium by in situ hybridization. Advanced lesions also involved other segments of the nephron and consisted of cysts lined by a flattened unicellular layer of attenuated epithelium. No rasT24 could be identified within cystic lesions of the distal nephron and collecting tubules by in situ hybridization, and they most likely arise by external compression. Animals from the two transgenic strains exhibiting cystic lesions die of renal failure beginning at 8 months of age. No difference in cell-cycle parameters or DNA ploidy between transgenic and control kidneys was identified by flow cytometric analysis. No renal carcinomas developed. The primary renal effects of the H-rasT24 oncogene in this model system consist of proximal tubular hyperplasia and polycystic kidneys. This model appears to provide a useful in vivo system for the study of ras oncogene function and control of renal cell proliferation. (Am J Pathol 1993, 142:1051–1060)

The members of the ras gene family (c-Ha-*ras*, c-Ki-*ras*, and N-*ras*) encode 21-kd, membrane-associated, guanosine triphosphate–binding proteins thought to function in signal transduction and regulation of cell proliferation. Mutations involving codons 12, 13, or 61, identified in both experimental and human cancers, are associated with loss of intrinsic GTPase activity by the p21 protein and are strongly implicated in the pathogenesis of malignant disease. Such mutant *ras* oncogenes are sufficient by themselves to produce transformation in transfection experiments with NIH3T3 cells,[1–3] whereas transformation in other *in vitro* systems requires the cooperation of the *ras* oncogene with other activated oncogenes.[4] Analysis of human tumors has revealed genetic alterations in *ras* genes in a significant number of pancreatic, thyroid, gastrointestinal, lung, and acute myeloid malignancies, but little or no involvement in cancers of other organ systems.[5] *In vivo* experiments with transgenic mice carrying activated *ras* oncogenes have also yielded variable results, ranging from cell hyperplasia without malignant transfor-

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mation, to early carcinomas, to the development of tumors only after long periods of latency.[6–14] However, in spite of extensive experimentation, the role of activated *ras* genes in the pathogenesis of neoplasia remains poorly understood.

The possible contribution of *ras* oncogenes to the genesis of renal cell carcinoma also remains unclear. Ohgaki et al[15] have recently shown a high incidence of Ki-*ras* codon 12 mutations in epithelial and mesenchymal renal tumors induced by intraperitoneal injection of chemical carcinogens in rats. Although cultured human kidney proximal tubular cells have been shown to be capable of transformation by retroviruses carrying either v-Ki-*ras* or v-Ha-*ras*,[16] studies of human renal cell carcinomas have identified only the infrequent presence of activated *ras* oncogenes.[17,18]

To study the possible role of *ras* oncogenes in kidney neoplasia, it would be desirable to have transgenic animals in which the *ras*T24 oncogene was directed to the proximal convoluted tubular epithelium, the site of origin of most human renal cell carcinomas. Recently, three species of rat γ-glutamyl transpeptidase (γGT) messenger (m)RNA (types I, II, and III) have been identified that differ in their 5′ untranslated sequences but give rise to identical γGT proteins.[19–21] The type I RNA has been shown to be expressed almost exclusively in the proximal convoluted tubules of the fetal and adult kidney.[19,21] Thus we have made transgenic mice carrying *ras*T24 (a *ras* oncogene mutated in codon 12) driven by the γGT promoter I.[22] In the present communication, we analyze the biological effects of expression of *ras*T24 in the mouse kidney.

Materials and Methods

Transgenic Mice

The details of the transgene construction and microinjection procedures have been reported previously.[22] Briefly, a γGT/*ras*T24 construct was created by placing a 2.0-kb fragment containing the rat γGT promoter I[23] 5′ of the c-Ha-*ras*T24 oncogene. A 4.9-kb *Eco*RI–*Acc*I fragment was isolated and used for microinjection into the inbred albino mouse strain FVB/N using established microinjection techniques.[24] The construct was coinjected with a 4.1-kb TyBS tyrosinase minigene[25] that would cointegrate with the γGT/*ras*T24 construct, allowing transgenic animals to be identified on the basis of coat color. The transgenic families are maintained in the hemizygous state by mating transgenic males with FVB/N females.

Histology and Blood Chemistry Analysis

Animals were sacrificed and autopsied at ages ranging from day 11 of gestation to about 18 months. Serum was obtained from 23 of these animals and assayed for sodium, blood urea nitrogen and creatinine concentrations with a Kodak Ektachem 700 chemistry analyzer. Portions of most organs were fixed in 10% neutral-buffered formalin, dehydrated through graded organic solvents, and embedded in paraffin. Six-μ sections were cut and stained for routine histology with hematoxylin and eosin (H & E). Fragments of tissue from representative kidneys were fixed for ultrastructural studies in 3% glutaraldehyde, postfixed in osmium tetroxide, and embedded in Spurr (Ted Pella, Costa Mesa, CA). Sections were cut at 0.005 μ and examined on a JEOL 100C electron microscope. Portions of representative kidneys were also embedded in optimal cutting temperature compound (Miles Scientific, Naperville, IL) and rapidly frozen in methylbutane cooled by liquid nitrogen. Four-μ sections were cut for histochemistry and *in situ* hybridization.

Alkaline Phosphatase Histochemistry and γGT Immunohistochemistry

Alkaline phosphatase and γGT were localized in frozen sections of transgenic and control kidneys using alkaline phosphatase histochemistry and γGT immunohistochemistry. For demonstration of alkaline phosphatase activity, frozen sections were fixed in acetone for 30 seconds and incubated for 15 minutes in a solution of 1 ml sodium nitrite and 1 ml naphthol AS-B1 (Sigma Chemical Co., St. Louis, MO). The slides were rinsed in phosphate-buffered saline and counterstained in hematoxylin Gill Num. 3 (Sigma).

For demonstration of γGT, frozen sections were fixed for 3 minutes in acetone. Endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide in methanol, and nonspecific staining was eliminated by incubation with a 1:20 dilution of normal goat serum. The sections were incubated 1 hour at room temperature with a 1:50 dilution of polyclonal rabbit anti-γGT.[26] The washed slides were sequentially incubated with a biotinylated goat anti-rabbit antibody and an avidin-biotin-peroxidase complex. The color was developed with 3,3′diaminobenzidine, and the sections were counterstained with Harris hematoxylin.

Measurement of RNA by Reverse Transcription-Polymerase Chain Reaction

RNA from skin, lung, liver, spleen, kidney, eyes, and brain were surveyed for γGT/*ras*T24 transgene expression by reverse transcription followed by polymerase chain reaction (RT-PCR). RNA was extracted by the acid guanidinium thiocyanate-phenol-chloroform method,[27] and RT-PCR was performed on RNA from all five families of transgenic mice and FVB/N control mice. PCR of γGT/*ras*T24-containing genomic DNA served as a positive control. The reverse transcription reaction was performed using 1 μg of total RNA, 8 units AMV reverse transcriptase (Promega Corp., Madison, WI), 0.5 mmol/L deoxynucleotide triphosphates, 20 units RNAsin (Promega), and primed with 100 pmole random hexamers as previously described.[28] The resulting complementary DNA was amplified for 35 or 40 cycles, denaturing 1 minute at 95 C, primer annealing 1 minute at 60 C, and extension 1 minute at 72 C. The PCR primers consisted of 5′-GCCTCTTTGACTCCAGAGTTC-3′ located within the γGT promoter sequence and 5′-CATCAATG-ACCACCTGCTTCC-3′ located within the second coding exon of *ras*. Parallel PCR was performed on all samples using hypoxanthine-guanine phosphoribosyl transferase primers as positive controls (data not shown). The PCR products were electrophoresed through 2.5% agarose gels (1.25% NuSieve and 1.25% SeaKem) with a *Hae*III digest of Phi X174 as a size marker. A 359-bp band was expected for the RT-PCR product, and amplification of intron-containing γGT/*ras* genomic DNA results in a 626-bp band.

Generation of Riboprobes

A 593-bp *Not*I/*Sac*I fragment representing the 3′ untranslated region of *ras*T24 extending from nt 3382 to 3975[29] was cloned into a Bluescript vector (Stratagene, Heidelberg, Germany). The plasmid was linearized with *Not*I before transcription, and ^{32}P-labeled anti-sense riboprobes for nuclease protection assays were generated using T7 RNA polymerase. Digoxigenin-labeled riboprobes for *in situ* hybridization were similarly generated using digoxigenin-labeled UTP (Boehringer Mannheim, Mannheim, Germany). Sense riboprobes were also generated for use as negative controls with *in situ* hybridization using T3 RNA polymerase.

Nuclease Protection Assays

Relative abundance of γGT/*ras*T24 RNA in the kidneys of each of the five transgenic families was determined using the Ambion ribonuclease protection assay kit (Ambion, Austin, TX). The assays were performed according to the standard kit protocol. Briefly, 15 μg of total RNA was hybridized with ^{32}P-labeled *ras* riboprobe for 16 hours at 45 C. RNA from cultured C5 cells known to produce a large amount of *ras*T24 RNA[30] was used as a positive control, and RNA from nontransformed cultured liver 228 cells and organs of nontransgenic albino animals were used as negative controls. Digestion was performed using a combination of ribonuclease A and ribonuclease T1 at 37 C for 30 minutes. The RNA was electrophoresed through a 6% denaturing polyacrylamide gel, and an autoradiograph was produced using Kodak X-OMAT film. A protected fragment of 375 bp was expected.

In Situ *Hybridization*

In situ hybridization for transgenic mRNA was performed on kidney cryostat sections fixed in 4% paraformaldehyde in phosphate-buffered saline for 3 minutes, rinsed in phosphate-buffered saline, and incubated in 0.001% proteinase K for 20 minutes at 37 C.[31] After acetylation with acetic anhydride (to block positive charges on tissue induced by protease digestion), the sections were dehydrated and prehybridized for 1 hour at room temperature in a hybridization buffer consisting of 50% deionized formamide, 2× standard saline citrate (SSC), 20 mmol/L tris[hydroxymethyl]aminomethane, pH 8.0, 1× Denhardt's solution, 1 mmol/L ethylenediaminetetraacetic acid, 100 mmol/L dithiothreitol, and 0.5 mg/ml yeast transfer RNA. The sections were incubated with a digoxigenin-labeled *ras* riboprobe at 45 C overnight. Some sections were hybridized with digoxigenin-labeled sense RNA probe made by transcribing with T3 polymerase. The sections were rinsed in 2× SSC for 1 hour at room temperature, 1× SSC for 30 minutes at room temperature, 0.5× SCC with RNAse A at 65 C for 30 minutes, and 0.5× SSC for 1 hour at room temperature. The tissues were then incubated with a polyclonal sheep anti-digoxigenin antibody labeled with alkaline phosphatase. The alkaline phosphatase was finally demonstrated with Nitroblue Tetrazolium and X phosphate (Boehringer Mannheim) to which 1 mol/L levamisole was added to inhibit endogenous alkaline phosphatase activity.

Flow Cytometry

Transgenic and control kidneys from 10 animals ranging in age from 1 to 11 months were processed for flow cytometric analysis.[32,33] Cells were dispersed by mincing the tissues with scalpel blades and digesting with collagenase at 37 C for 10 minutes. Washed monodispersed cells were fixed in 95% ethanol, treated with RNAse and stained with propidium iodide for at least 30 minutes. The stained cells were analyzed in a FACScan flow cytometer. Fixed chick erythrocyte nuclei were used as an external standard. The cell cycle statistics were estimated using the second order polynomial (SFIT) model.

Results

Five families of transgenic animals were obtained. The families were named according to the founder animals 16497, 16498, 16499, 16500, and 16502, and correspond to OVE153 through OVE157 reported previously.[22] At autopsy, kidney lesions were present in mice of two of the five families, 16499 and 16500. No cystic, hyperplastic, or neoplastic kidney lesions were observed in albino control animals. Eye lesions were present in all five families. The details of the eye lesions will be reported elsewhere (Chevez et al, in preparation). Other organ systems, including brains, were normal in all five families.

Kidney Lesion Morphology

The kidneys in both 16499 and 16500 families are grossly normal until about 3 weeks of age, when in males, they begin to become pale and symmetrically enlarged. Kidneys with fully developed lesions are pale tan with finely stippled cortical surfaces. The sectioned kidneys show multiple, small, closely arranged cysts containing clear fluid (Figure 1A). The pelvises are dilated and distorted, but no hydroureter is present. The average weight of a pair of kidneys is 3 g compared with 0.5 in control animals. Hemizygous females do not develop these lesions.

Microscopically, the lesions are first recognized between the 18th day of gestation and birth. The lesions in the newborn (Figure 1, B and C) consist of focal dilatation of tubules within the renal cortex. The tubular epithelium at this point is cuboidal with slightly increased nuclear/cytoplasmic ratios and a brush border defined by periodic acid-Schiff staining, consistent with proximal tubular epithelium. The dilated tubules at birth are lined by approximately 3 times as many epithelial cells as nondilated tubules in age-matched controls. Some of the tubules exhibit stratification and cytoplasmic vacuolization of the lining epithelium with occasional papillary projections extending into the tubular lumina (Figure 1D). In addition, some of the kidneys show focal microadenomas that are either solid or contain a cystic center (Figure 1E). Rare mitotic figures are seen. In more advanced lesions, the distal tubules, collecting ducts, and Bowman's spaces also become dilated, and the lining epithelium is often flattened and attenuated (Figure 1F). Pyelonephritis was present in several moribund animals that were sacrificed. No carcinomas were identified.

The kidney lesions progress more rapidly in 16499 animals than in 16500 animals. Involved 16499 animals become moribund at about 8 months of age, whereas 16500 animals usually survive until about 15 months. Terminally, the animals from both lines show polyuria, and serum chemistry shows azotemia and hypernatremia. The blood urea nitrogen measured in moribund animals averaged 390 mg/dl (nontransgenic control 24 mg/dl) and the sodium averaged 161 mmol/L (control 145 mmol/L). The creatinines averaged 1.4 mg/dl (control 0.3 mg/dl).

Homozygous mice in each family can be identified on the basis of coat color (Overbeek, personal communication). When homozygous 16499 mice are obtained by crossing hemizygous transgenic animals, the animals die within the first month of life. Microscopically, these animals show advanced polycystic kidney disease and microadenomas similar to the lesions seen in the kidneys of moribund hemizygous animals. Additionally, female homozygous transgenic mice also develop cystic and hyperplastic renal lesions.

Electron micrographs of early cystic and hyperplastic lesions show the lining epithelium to be rich in mitochondria and contain numerous microvilli along the cell surface (Figure 2), features of proximal tubular epithelium that are absent in other renal epithelial cell types. Some of the lesional cells show cytoplasmic vacuolizations, consistent with lipid.

Enzyme histochemistry for alkaline phosphatase (Figure 3A) and γGT (Figure 3B) in the early lesions show staining of the lining epithelium within the cystic lesions. Within a particular dilated tubule, the staining intensity of individual cells varies from negative to intense. Foci of hyperplastic epithelium also stain positively for alkaline phosphatase and γGT. Enzyme histochemistry of advanced lesions shows staining in some but not all of the dilated tubules.

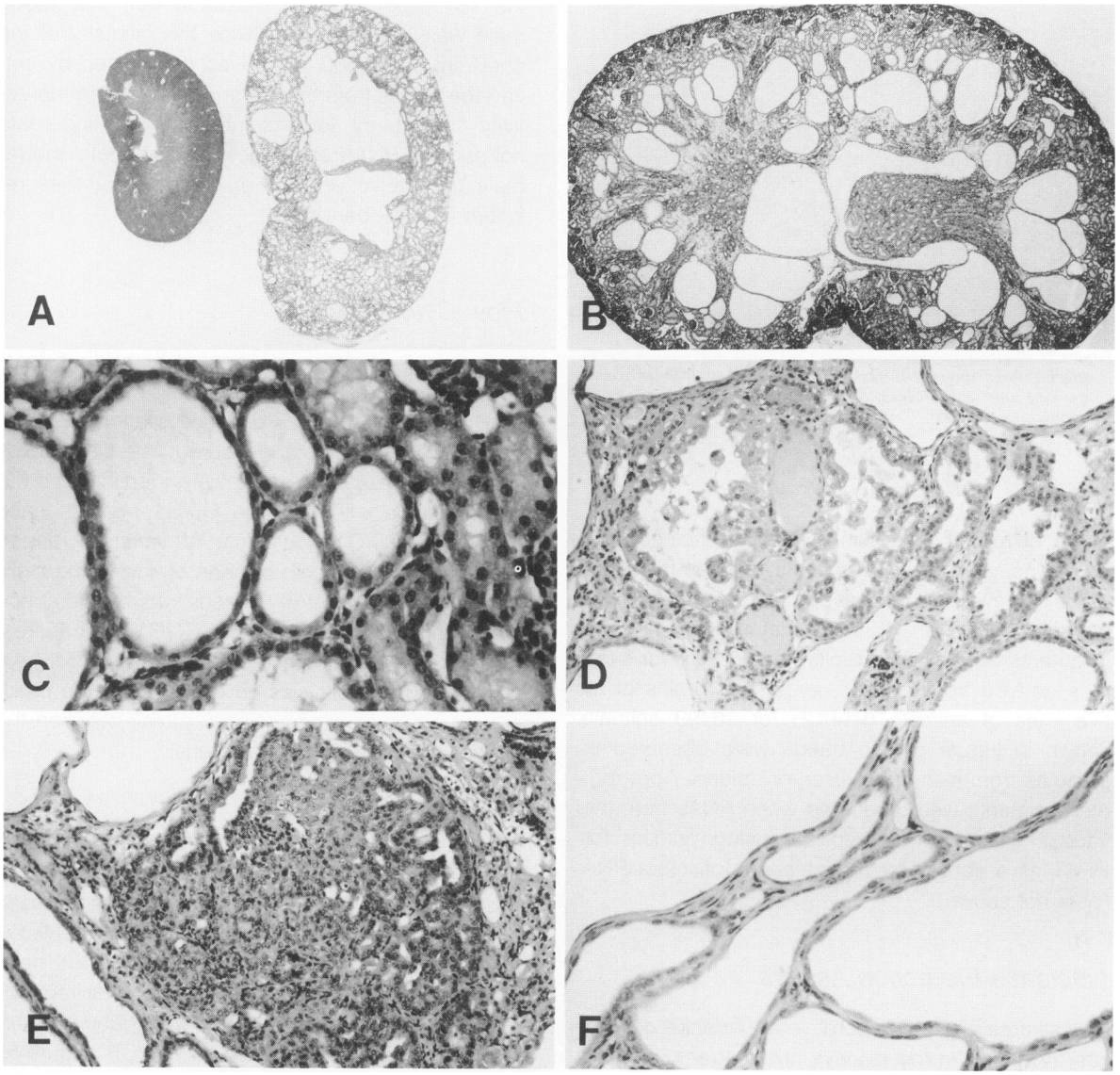

Figure 1. **A:** *Whole mount sections of an 8-month-old male nontransgenic control kidney* **(left)**, *and a transgenic kidney from an 8-month-old male 16499 animal* **(right)** *showing diffuse enlargement, distortion of cortical and medullary architecture by cystic lesions, and hydronephrosis in the transgenic kidney (H & E, ×1).* **B:** *Kidney from a newborn 16499 transgenic male showing early cystic lesions confined to the proximal tubules (H & E, ×20).* **C:** *Newborn 16499 male transgenic animal showing cystic renal proximal tubules lined by a single layer of cuboidal epithelium (H & E, ×400).* **D:** *Renal tubule from an 8-month-old 16499 transgenic male showing stratification of the hyperplastic tubular lining epithelium (H & E, ×100).* **E:** *A microadenoma from an 8-month-old 16499 transgenic male showing solid and tubular architecture (H & E, ×200).* **F:** *Distal nephron of an 8-month-old 16499 transgenic male showing cysts lined by a flattened and attenuated layer of epithelium (H & E, ×400).*

In Situ *Hybridization*

In situ hybridization for *ras*T24 mRNA was performed on kidneys from newborn, 2-month-, and 7-month-old 16499 animals, as well as an 18-month-old 16500 animal. Intensely positive staining for *ras* mRNA was observed within the cytoplasm of cells from cystic and hyperplastic tubular lesions and microadenomas in the newborn and 2-month-old animals (Figure 4, A and B), but no staining was observed in the glomeruli or more distal segments of the nephron. Positive staining for *ras* mRNA was also observed in the cystic proximal renal tubules of the 7-month- and 18-month-old animals. Again there was no staining of the glomeruli, tubules of Henle's loop, distal tubules, or collecting tubules, even though all of these components showed cystic dilatation. No staining was observed in transgenic kidney sections hybridized with a sense riboprobe or in nontransgenic albino kidneys hybridized with the anti-sense *ras* riboprobe.

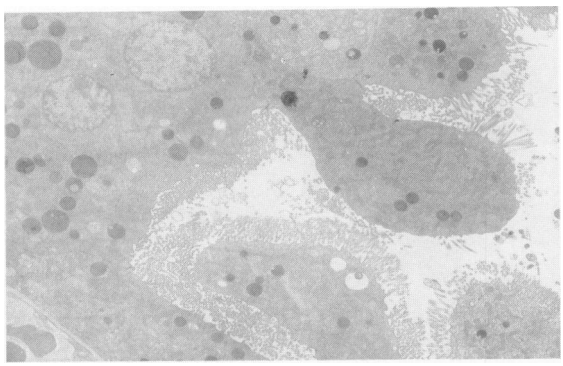

Figure 2. *Electron micrograph of a renal cystic lesion from a 16499 transgenic male showing lining epithelium derived from proximal convoluted tubular epithelium with characteristic surface microvilli (magnification ×2800).*

Assay of RNA by RT-PCR

RNAs extracted from skin, lung, liver, spleen, kidney, eyes, and brain of animals from all five lines were surveyed for expression of the γGT/*ras*T24 transgene by RT-PCR. Three hundred fifty-nine-bp fragments were obtained following RT-PCR for samples derived from kidneys, eyes, and brains of all five lines of animals (RT-PCR for 16499 animals shown in Figure 5). No bands were observed in samples from skin, lung, liver, or spleen. Appropriate fragments were observed when RNAs from the various tissues were amplified using primers for HPRT as a control for the presence of intact RNA (data not shown).

Nuclease Protection Assays

An estimate of the comparative abundance of γGT/*ras*T24 mRNA in the kidneys of transgenic animals was obtained using nuclease protection assays. We expected to protect a 375-nt fragment from the 3′ untranslated region of *ras*T24. Prominent bands of ~270 nt and less intense bands of ~375 nt were present in samples from all pigmented transgenic mice as well as in the sample from C5 *ras*-transformed liver cells (positive control, Figure 6). We attribute the apparent discrepancy in the expected and observed band size to the adenine and uridine-rich 3′ region of the mRNA that may focally denature and be digested by ribonuclease A under the conditions used in this assay.[34] No band was identified in the lane containing RNA from 228 liver cells (which do not carry *ras*T24) or RNA from non-transgenic albino animals (negative controls). The most intense bands were present in the 16499 and 16500 males. The band in the 16499 male was approximately three times the intensity of the one from the 16499 female, and the band from the 16500 male was approximately twice the intensity of the one from the 16500 female, as determined by cutting the bands from the gel and quantitating the activity in each by liquid scintillation counting (data not shown). RNA from kidneys of both male and female transgenic animals of the other families resulted in weak bands.

Flow Cytometry

Kidney cell nuclei were prepared, and at least 10,000 events were detected for each specimen except for one low event rate preparation in which only 5,000 events were measured. The DNA ploidy histograms on gated events were identical and superimposable with a single G0/G1 peak in each case. The G0/G1 coefficient of variation ranged from 3.4% to 6.5% with a mean of 4.2%. The highest G0/G1 coefficient of variation was on the specimen with a low event rate. The G0/G1 peak fluorescence:CEN peak fluorescence ratio was 2.5 or 2.6 in each case. There was no difference in the proliferative phase fractions or DNA ploidy between the control and transgenic specimens.

Discussion

Type I γGT mRNA has been shown to be expressed predominantly in the proximal convoluted tubules of the kidney.[19,21] In the experiments reported here, we have used the putative type I promoter region[23] to target expression of a *ras*T24 transgene to proximal tubules as demonstrated by RT-PCR (Figure 5), RNAse protection (Figure 6), and *in situ* hybridization (Figure 4). In addition, most of the early lesions observed in these animals involve proximal tubules as demonstrated by alkaline phosphatase and γGT histochemistry (Figure 3) as well as electron microscopy (Figure 2). γGT is also known to be expressed in the ciliary body of the eye,[35] and the presence of eye lesions in all five of our transgenic lines suggests that it is the γGT type I promoter that is active in this location. RT-PCR assays for γGT/*ras* mRNA also suggest that the type I promoter may be active in the brain, though no brain lesions have been detected at present. This positivity may be attributable to the choroid plexus and/or capillary endothelium where γGT activity has been previously reported.[36,37]

The development of the kidney lesions in our transgenic animals appears to correlate with the

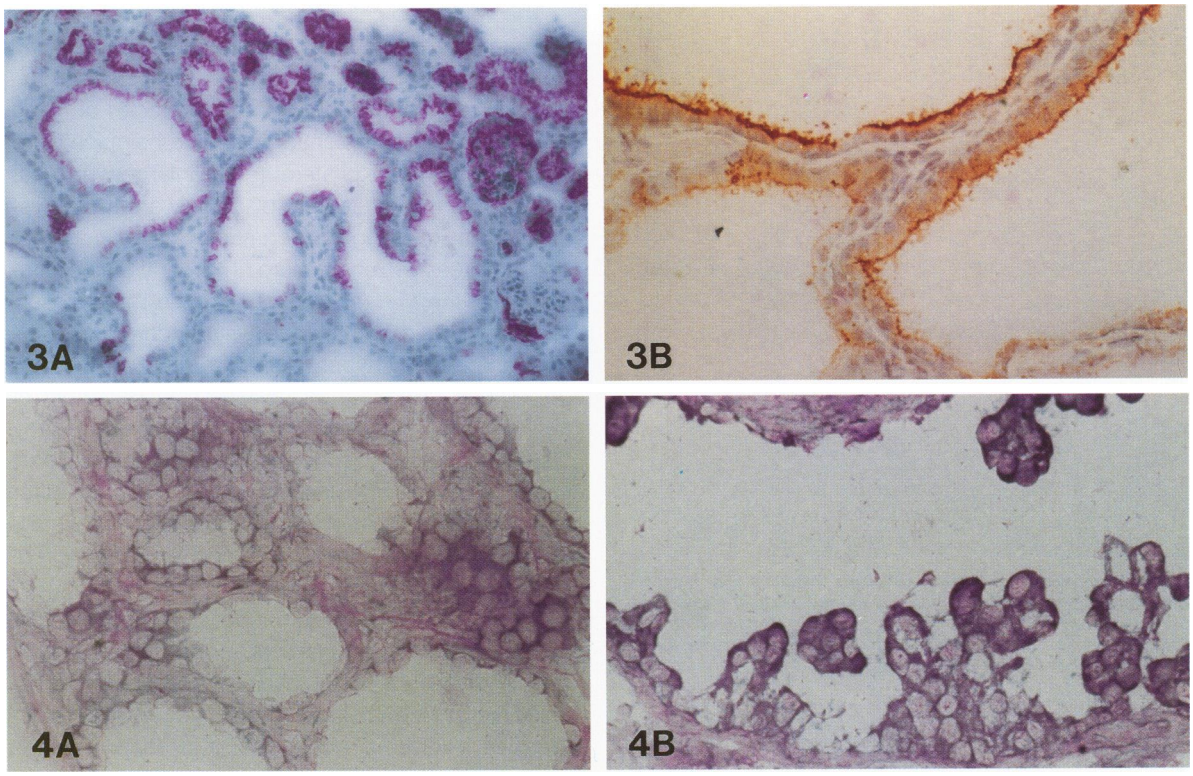

Figure 3. **A:** *Histochemical stain for alkaline phosphatase showing positive staining of the cystic proximal convoluted tubules in a 2-month-old 16499 transgenic male (×20).* **B:** *Immunohistochemical stain for γGT showing positive immunostaining of the cystic proximal tubular epithelium brush border in a 2-month-old 16499 transgenic male (×20).*
Figure 4. **A:** In situ *hybridization for γGT/rasT24 showing positive staining overlying the cystic proximal tubules in a 6-month-old 16499 transgenic male (×20).* **B:** In situ *hybridization for γGT/rasT24 showing positive staining within the hyperplastic epithelium in a 6-month-old 16499 transgenic male (×40).*

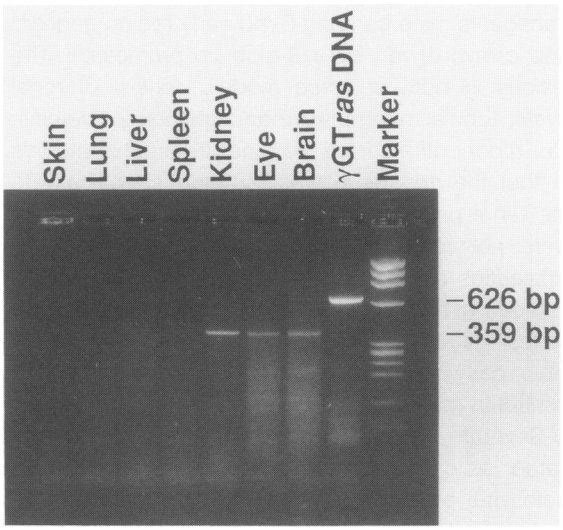

Figure 5. *RT-PCR for γGT/rasT24 in organs of a 16499 animal showing positive bands (359 bp) in the kidney, eye, and brain. Plasmid DNA, which contains an intron, results in a 626-bp band.*

amount of γGT/*ras*T24 mRNA present. The two families that exhibit cystic kidney lesions, 16499 and 16500 males, are also the two families that exhibit the highest steady-state levels of the transgenic mRNA (Figure 6). The difference in band intensity between the male animals that develop kidney lesions and the female animals that do not is only 2- to 3-fold, suggesting that the threshold for development of these lesions lies within a rather narrow range. Additional evidence for a dosage effect in the development of the kidney lesions is derived from observations on homozygous 16499 animals. These animals, which should exhibit higher levels of expression due to higher numbers of transgene copies, show more rapid development of their kidney lesions, and homozygous 16499 males die of renal failure within the first postnatal month. Furthermore, homozygous 16499 female animals develop kidney lesions, whereas hemizygous 16499 female animals do not. Because these observations are based on homozygous animals from only one line, it remains possible that this early mortality in homozygous animals may be related to interruption of a critical gene. It seems more likely, however, that their deaths result from renal failure because the females usually die 3 to 4 weeks later than homozygous males and the timing of their deaths corresponds to the severity of the kidney lesions.

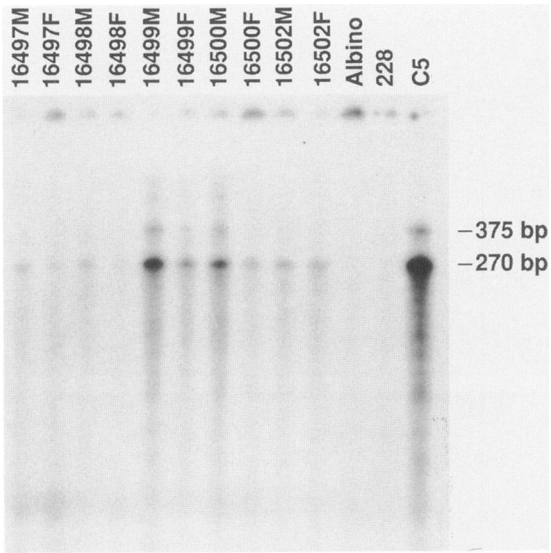

Figure 6. *Nuclease protection assay showing protection of a* ras *probe with kidney RNA from male and female animals of all five lines. Positive protected bands of 375 bp and more intense secondary bands at 270 bp are present in all transgenic animals and the C5 control, but absent in the 228 and albino negative controls.*

The basis of the observed sex difference in levels of transgenic mRNA is unknown. Although a steroid hormone-responsive element is present in the γGT promoter sequence used in the γGT*ras*T24 transgene (S. Rajagopalan, personal communication), the possible contribution of this element to the observed mRNA levels has not been studied.

Pathogenetic theories for the development of human polycystic kidney disease include tubular hyperplasia, distal nephron obstruction, abnormal tubular compliance, and abnormal tubular secretion.[38–41] The early lesions in our animals appear to arise by hyperplasia of the proximal tubular lining epithelium and consist of both cystic tubules and microadenomas. The cystic tubules are lined by approximately three times the number of cells lining corresponding tubules in control animals, and some of the cysts show stratification of their lining epithelium with occasional intraluminal papillary tufts of hyperplastic tubular cells. Furthermore, the lining epithelial cells are cuboidal and not flattened and attenuated as would be expected if the cysts arose by distension of the lumina with fluid. Some of the cystic tubules in older animals do not stain with proximal tubule markers and exhibit histochemical and ultrastructural features of distal tubules (R. Barrios et al, in preparation). These lesions may result from distortion of the renal architecture by the cystic proximal tubules compressing, and possibly obstructing, more distal segments of the nephron.

The pathological phenotype observed in our transgenic animals show many similarities to some of the human polycystic kidney diseases. The similarities are most striking in the end stages of these diseases when they share the morphological and clinical features of bilateral renal enlargement, distortion of renal architecture by closely arranged cystic lesions involving all segments of the nephron, and progressive renal failure. The early lesions observed in the γGT*ras* transgenic mice most closely resemble those human polycystic kidney diseases characterized by hyperplasia of the tubular epithelium. These diseases, as reviewed by Bernstein et al,[39] include autosomal dominant polycystic kidney disease, tuberous sclerosis, and acquired renal cystic disease. The lesions in our animals differ from these diseases, however, in their initial localization to the proximal tubules and lack of progression to renal cell carcinoma.

Several animal models for polycystic kidney disease have been previously described. The *cpk*/*cpk* murine disease is an autosomal recessive disorder resulting in neonatal polycystic kidneys with progressive involvement culminating in death from renal failure by about 3 weeks of age.[42,43] Similar to our model, the early lesions in *cpk* mice consist predominantly of proximal tubular hyperplasia and cysts with later secondary involvement of the distal portions of the nephron. More recently, features of polycystic kidney disease have been reported in transgenic mice carrying SV40 early region genes[44] and c-*myc* driven by a β-globulin promoter.[45] The lesions in both of these models consist of renal cysts, tubular hyperplasia, and glomerulosclerosis. Our model differs from the other transgenic models in that the transgene was targeted specifically to the renal proximal tubules, and glomerular lesions were not observed. Based upon the phenotypic similarities observed in these various models with different genetic alterations, it seems likely that cystic lesions are a common end-point of multiple possible pathways that may produce altered growth control in the kidney.

Several transgenic mouse models carrying activated *ras* oncogenes have been developed, using both viral promoters[8,10,12] and tissue-specific promoters.[6,7,9,11] Various malignant neoplasms were induced in these animal models, usually following a latency period of many months. The effect of the *ras*T24 oncogene in our kidney model seems to be limited to proximal tubular hyperplasia. This observation is consistent with *in vitro* studies showing that the *ras* oncogene is capable of inducing prolifera-

tion in quiescent cells.[46,47] It is also similar to the alterations observed in the Harderian glands of transgenic mice carrying activated *ras* oncogenes driven by either the mouse mammary tumor long terminal repeat[12] or by the murine c-H-*ras* promoter[7] in which only hyperplasia of the gland was observed.

None of the animals in our experiments developed renal cell carcinomas. It has previously been observed both *in vitro*[48] and *in vivo*[7,8,12] that different tissues appear to have different susceptibility to transformation by activated *ras* oncogenes. It seems that even a high level of *ras*T24 expression is insufficient to transform proximal convoluted tubular cells *in vivo*.

Acknowledgments

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