Kid-1 expression is high in differentiated renal proximal tubule cells and suppressed in cyst epithelia

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Kid-1 expression is high in differentiated renal proximal tubule cells and suppressed in cyst epithelia. Am. J. Physiol. 275 (Renal Physiol. 44): F928–F937, 1998.—The cDNA coding for the transcriptional repressor protein Kid-1 was cloned in a screen for zinc finger proteins, which are regulated during renal development and after renal ischemia. Kid-1 mRNA levels increase in the course of postnatal renal development and decrease after acute renal injury caused by ischemia or administration of folic acid. We have raised a monoclonal anti-Kid-1 antibody and demonstrate that the Kid-1 protein is strongly expressed in the proximal tubule of the adult rat kidney. During nephron development, the Kid-1 protein appears after the S-shaped body stage concomitantly with the brush-border enzyme alkaline phosphatase. In two animal models of polycystic kidney disease, the expression of Kid-1 is downregulated. The loss of expression of Kid-1 in cyst cells correlates with the loss of alkaline phosphatase histochemical staining. Kid-1 mRNA levels are also reduced in rodent renal cell carcinomas, another condition characterized by epithelial cell dedifferentiation and increased proliferation. We propose that Kid-1 plays an important role during the differentiation of the proximal tubule.

renal development; renal cell carcinoma; polycystic kidney disease; gene regulation; transcriptional repression

THE MORPHOLOGICAL PROCESSES leading to the formation of the fully differentiated kidney have been well described (29), whereas the cellular and molecular events responsible for the ordered pattern of renal development are poorly understood. Gene targeting experiments have revealed that transcription factors play critical roles at different stages of nephrogenesis, and it can therefore be assumed that a hierarchy of genetic regulation exists. Inactivation of the WT-1 (21), Pax-2 (34), and Emx-2 (24) genes leads to renal agenesis, indicating that the proteins encoded by these genes act at a very early stage of renal development. When the BF-2 gene is inactivated, renal tissue is present but the number of nephrons that develop totals only ~10% of the number present in normal mouse kidneys (16), suggesting that BF-2 controls genes necessary at a stage of metanephrogenesis later than the stages in which WT-1, Pax-2, and Emx-2 are critical. The protein encoded by the hepatocyte nuclear factor (HNF)-1 gene appears to play a role at a very late stage of nephron development, since the inactivation of the HNF-1 gene leads to defects specifically in the proximal tubule (27).

We have cloned a rat cDNA encoding a zinc finger protein that we named Kid-1 (predominantly found in the kidney (“k”), suppressed after renal ischemia (“i”), and appearing late in renal development (“d”) (38). The mRNA for Kid-1 is barely detectable at the time of birth in the rat kidney, but levels increase after birth and reach the highest levels in the adult kidney (38). After ischemic or toxic injury to the adult kidney, at a time when many kidney cells dedifferentiate and undergo mitosis, Kid-1 mRNA levels decline to a degree comparable to that in the newborn kidney (38). Northern blot and RT-PCR analysis of a variety of rodent organs have shown that among all the organs tested, the Kid-1 mRNA is predominantly expressed in the kidney (38). This result was subsequently confirmed in the mouse (3), where in addition comparable Kid-1 mRNA levels could also be detected in the eye (expression of Kid-1 mRNA in the eye was not analyzed in the rat). A molecular analysis of the Kid-1 protein led to the finding that the non-zinc finger region of Kid-1 was able to confer transcriptional repressor activity (38). More specifically, the transcriptional repressor activity of Kid-1 is conferred by the Krüppel-associated box (KrAB)-domain at the NH₂-terminal of the protein, a widely distributed motif among Cys2His2-zinc finger proteins (23, 39).

To learn more about the potential role of Kid-1 in the kidney, we evaluated the expression of Kid-1 during nephron development, in two models of polycystic kidney disease and in rat renal cell carcinomas. Polycystic kidney disease and renal cell carcinomas are two conditions in the adult kidney that are characterized by an increased rate of epithelial cell proliferation and a loss of differentiation (4). One of the polycystic kidney disease models investigated was the C57BL/6J (cpk/ cpk) mouse, a model with an autosomal recessive pattern of inheritance (12, 13, 28); the other model was the Han:SPRD (cy/-) rat, a model with an autosomal dominant pattern of inheritance (9, 30). In addition, to

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further test our hypothesis that Kid-1 expression is suppressed in dedifferentiated epithelia, we examined the expression pattern of Kid-1 in the Eker rat model of hereditary renal cell carcinoma (11) and in transformed rat kidney epithelial cell lines (35). Our results indicate that the expression of the Kid-1 protein is induced at a late stage of differentiation of the proximal tubule and that it is downregulated in cyst wall cells of polycystic kidneys and in renal cell tumors. This downregulation is likely related to the dedifferentiated state of these cells. Kid-1 may control genes important for the abnormal phenotype in polycystic disease.

MATERIALS AND METHODS

Preparation of a monoclonal anti-Kid-1 antibody. A fragment coding for amino acids 72–173 of the rat Kid-1 protein was subcloned into the plasmid pET-21b (Novagen, Madison, WI). The resulting plasmid, pET-21b/Kid-1, was used to express a fragment of the rat Kid-1 protein without the highly conserved KRAB and zinc finger domains in Escherichia coli. Because the recombinant protein contained a tail of six histidine residues at its COOH terminal, it could easily be purified over a Ni\(^{2+}\) column according to the manufacturer’s instructions (Novagen). Approximately 75 µg of the purified protein was injected subcutaneously into BALB/c mice in 3-wk intervals. After four injections, splenic lymphocytes were harvested and hybridomas were prepared according to standard protocols (1). Culture supernatants were assayed on a dot blot with purified glutathione S-transferase (GST)/Kid-1 fusion protein. Supernatants yielding a positive signal in the dot blot assay were further tested in a Western blot. To obtain a clonal population of cells, hybridomas were subcloned by limiting dilution in a 96-well plate.

Hybridoma cell culture supernatants were combined with saturated (NH\(_4\))\(_2\)SO\(_4\) in a ratio of 55:45 (vol/vol). After an incubation of 2–4 h at 4°C, the antibody suspension was centrifuged 20 min at 4°C and 12,000 g. The antibody pellet was dissolved in PBS (\(\gamma\)_G0, of the original cell culture volume) and then dialyzed against PBS (1).

Epitope mapping. Rat Kid-1 cDNA fragments coding for amino acids 81–195 (region between the KRAB domain and the zinc finger domain), amino acids 1–195 (Kid-1 without the zinc finger domain), amino acids 53–576 (Kid-1 without the KRAB-A domain), and amino acids 174–576 (zinc finger domain only) were subcloned into the plasmid pGEX-KG (15), which resulted in the expression of fusion proteins with GST in E. coli. Equal amounts of bacterial extracts were run on SDS-PAGE gels and either stained with Coomassie brilliant blue or subjected to Western blot analysis.

Transient transfection of COS-7 cells. The cDNA coding for the full-length Kid-1 protein was subcloned into the mammalian expression vector pMT3 (pMT2 (18) modified to encode the nine-amino acid hemagglutinin (HA) epitope tag of the influenza virus at the NH\(_2\) terminal of the insert); transcription is driven by the adenovirus major late promoter. COS-7 cells were grown in Dulbecco’s minimal essential medium supplemented with 10% calf serum. One day before transfection, cells were plated at a density of 2.5–4 × 10\(^5\) cells per 100-mm dish. For transfection, cells were exposed to 20 µg of DNA in 5 ml of Dulbecco’s minimal essential medium-400 µg/ml DEAE-dextran (Sigma, Deisenhofen, Germany)-0.1 mM chloroquine (Sigma). Three hours after the addition of DNA, the medium was removed and the cells were shocked for 2 min at room temperature with 10% dimethyl sulfoxide (Sigma) in 1× PBS. After the shock treatment, the cells were washed once with PBS and new medium was added. Cells were harvested at the indicated times after transfection.

Preparation of nuclear extracts. Nuclear extracts were prepared as described by Hoppe-Seyler et al. (17). Two to three days after transfection, COS-7 cells were washed twice with PBS and harvested through a microcentrifuge tube. The cells were centrifuged for 5 min at 1,250 g, and the pellet was resuspended in lysis buffer (150 mM NaCl; 10 mM Tris, pH 7.9; 1 mM EDTA, pH 8.0; 0.6% Nonidet P-40). After another 5 min on ice, the cells were centrifuged 5 min at 1,250 g. The supernatant (corresponding to the cytoplasmic fraction) was saved, and the nuclear pellet was resuspended in nuclear extract buffer (1.5 mM MgCl\(_2\); 10 mM HEPES, pH 7.9; 0.1 mM EGTA; 0.1 mM EDTA; 0.5 mM dithiothreitol (DTT); 0.5 mM phenylmethylsulfonyl fluoride; 25% glycerol; 420 mM NaCl). The nuclear suspension was incubated 20 min on ice and then centrifuged 5 min at 14,000 g. The supernatant (corresponding to the soluble nuclear fraction) was saved, and the remaining pellet was solubilized by sonication in PBS-6 M urea.

Immunocytochemistry of kidney sections. Adult Sprague-Dawley rats (70–100 days old) were anesthetized with pentobarbital sodium (6.5 mg/100 g body wt). Anesthetized rats were injected intravenously with 1 mg/kg body wt of 18% paraformaldehyde and subsequently with PBS-18% sucrose at a pressure of 200–220 mmHg. Newborn Sprague-Dawley rats were anesthetized with ether and perfused with PBS-2% paraformaldehyde through the left ventricle at a pressure of 180–200 mmHg, before the kidneys were removed and immersed for 2 h in PBS-18% sucrose. Kidneys were snap-frozen in isopentane cooled with liquid nitrogen and stored at −80°C until further use. Kidneys were sectioned at 6- to 8-µm thickness, air-dried for 30 min, and blocked for 2 h at room temperature in PBS-2% BSA-0.1% Triton X-100. The (NH\(_4\))\(_2\)SO\(_4\)-precipitated anti-Kid-1 antibody 5D12 was applied at a dilution of 1:50–1:100 in PBS-2% BSA, after which the section was first incubated for 2 h at room temperature before being stored overnight at 4°C. The next morning, sections were washed three times with PBS and incubated 1 h at room temperature with the secondary antibody [Cy3-coupled rat anti-mouse IgG from Dianova (Hamburg, Germany)] diluted 1:100. The sections were washed again three times with PBS and then mounted. When necessary, nuclei were stained after the third wash by incubating the sections for 3 min in Hoechst stain 33258 (Sigma) at a concentration of 10 µg/ml.

Double-labeling was performed with a sheep anti-human Tamm-Horsfall protein antibody (diluted 1:200; Biotrend, Köln, Germany), which was applied simultaneously with the murine monoclonal anti-Kid-1 antibody 5D12. The primary antibodies were detected with FITC-conjugated anti-sheep IgG antibody (diluted 1:80; Sigma) and Cy3-conjugated antimouse IgG antibody (Dianova). For preabsorption experiments, the primary antibody was incubated 2 h at room temperature with 100 ng of GST or a GST/Kid-1-N (GST fused to amino acids 1–195 of the rat Kid-1 protein) fusion protein per 1 µl of antibody solution before being applied to the tissue section.

Alkaline phosphatase histochemistry. After incubation with the secondary antibody and the ensuing three washes, the sections were incubated 15 min at room temperature (or until an appropriate color development occurred) in 0.3 mM nitro blue tetrazolium chloride and 0.3 mM 5-bromo-4-chloro-3-indolyl phosphate (4-toluidine salt) in 0.2 M Tris-HCl buffer, pH 9.5. The reaction was stopped by immersing the sections for 10 min in distilled water, after which the sections were fixed again in 4% paraformaldehyde (in PBS) for 10 min, rinsed in water, and mounted.
Animal models of polycystic kidney disease and Eker rat model of hereditary renal cell carcinoma. The Han:SPRD (cy–/) rat, a model for autosomal dominant polycystic kidney disease (PKD) (9, 30); the C57BL/6j (cpl/cpl) mouse, a model for autosomal recessive PKD (12, 13, 28); and the Eker rat model of hereditary renal cell carcinoma (11, 36) have been characterized in detail in previous reports. Animals were maintained as inbred colonies in the University of Kansas Medical Center Animal Care Facility [Han:SPRD (cy–/) rats and C57BL/6j (cpl/cpl) mice], the Animal Care Facility of the Medical Research Center in Mannheim [Han:SPRD (cy–/) rats], or the Chemical Industry Institute of Toxicology in Research Triangle Park, NC (Eker rats).

Cell culture. Transformed rat kidney epithelial (TRKE) cell lines were established and characterized as described previously (19, 35). In brief, immortal rat kidney epithelial cell lines were created by exposure of primary cultures to the chemical mutagen N-methyl-N’-nitro-N-nitrosoguanidine. TRKE-8, in addition to being immortalized, forms adenocarcinomas in nude mice. Cell lines TRKE-4, TRKE-5, TRKE-7, and TRKE-8 were used in the experiments described in this report. Cells were harvested either at logarithmic growth or after having reached confluency.

Preparation of RNA. By use of a modification of the technique of Chomczynski and Sacchi (6), RNA was isolated from right kidneys of Han:SPRD rats as previously described (8, 9). Briefly, right kidneys were homogenized in GTC solution (4 M guanidine thiocyanate, 25 mM trisodium citrate, 0.1 M β-mercaptoethanol, 0.1% antifoam A, pH 7.0) using a Polytron tissue homogenizer. GTC homogenates were treated by sequential addition of 2 M sodium acetate (pH 4), phenol, and chloroform with vortexing after each addition. After centrifugation, the aqueous layer was transferred to a fresh tube, and RNA was precipitated with isopropanol. RNA was pelleted by centrifugation, redissolved, chloroform extracted, and ethanol precipitated. RNA was again pelleted by centrifugation, redissolved, and quantitated by spectrophotometry. After a final ethanol precipitation, RNA was stored at −20°C until use.

Generation of 32P-labeled DNA probes. Human full-length glyceraldehyde-3-phosphate dehydrogenase (a gift of M. Alexander-Bridges, Massachusetts General Hospital) was randomly primed according to standard protocols (1). Unincorporated nucleotides were removed by running the reaction over a spin column (Bio-Rad, Hercules, CA).

Radioabeled single-stranded antisense DNA was prepared as described by Sturzl and Roth (33). PCR buffer (final concentration 50 mM KCl; 10 mM Tris·HCl, pH 8.4; 2.5 mM MgCl2; 250 µM dATP, dGTP, and dTTP; 6.3 µM dCTP; 5 µl α-32P-dCTP (10 μCi/µl), 3,000 Ci/mmol; 250 ng primer; 2 U AmpliTaq polymerase; and 200 ng linearized template pBluescript-Kid-I (−)) (containing nucleotides 1–897 of the rat Kid-1 cDNA) (38) were combined in a final volume of 10 µl. Fifty microliters of mineral oil was added to each reaction tube. After 40 cycles of 30 s at 95°C, 1 min at 50°C, 2 min at 72°C, and a final extension of 10 min at 72°C, unincorporated nucleotides were removed over a spin column (Bio-Rad).

Northern blot and hybridization. RNA samples were run on agarose gels and blotted onto Nylon membranes according to standard protocols (1). Blots were prehybridized in 2× SDE (10× SDE is 1 M NaCl; 500 mM sodium phosphate, pH 7.0; 25 mM EDTA), 5% SDS, 100 µg/ml yeast tRNA, and 100 µg/ml denatured salmon sperm DNA at 55°C for 5 h (38). Then, 2.5 × 106 cpm of radio-labeled antisense DNA was added to each reaction tube. After 40 cycles of 30 s at 95°C, 1 min at 50°C, 2 min at 72°C, and a final extension of 10 min at 72°C, unincorporated nucleotides were removed over a spin column (Bio-Rad).

RESULTS

Characterization of the monoclonal anti-rat Kid-1 antibody 5D12. To characterize the endogenous Kid-1 protein, we generated a monoclonal antibody by immunizing BALB/c mice with a recombinant rat Kid-1 peptide (amino acids 72–173), lacking the highly conserved KRAB and zinc finger domains, to minimize the possibility of cross-reactivity of an anti-Kid-1 antibody with other zinc finger proteins. One hybridoma, 5D12, produced an antibody that reacted with the 5D12 monoclonal antibody 5D12 likely include those at position 72–81 of the peptide used to generate the antibody included only amino acids 72–173, the amino acids recognized by the 5D12 monoclonal anti-Kid-1 antibody. Since Kid-1 did not react with the antibody (Fig. 1, lane 3), this detection pattern demonstrates the specificity of the anti-Kid-1 antibody. Since the peptide used to generate the antibody included only amino acids 72–173, the amino acids recognized by the antibody 5D12 likely include those at position 72–81 of Kid-1.

The 5D12 antibody was further characterized using COS-7 cells transiently transfected with an HA epitope-tagged, full-length Kid-1 protein. Two days after transfection, the cells were harvested and lysed with nonionic detergent, and nuclear extracts were prepared by a protocol using 420 mM NaCl. Western blot analysis with the 5D12 monoclonal anti-Kid-1 antibody yielded a single band in the pellet fraction remaining after the
extraction with 420 mM NaCl, indicating that the Kid-1 protein adhered tightly to as-yet-unidentified nuclear structures (Fig. 1D). Mock-transfected cells yielded no signal, again attesting to the specificity of the anti-Kid-1 antibody 5D12.

Expression of the Kid-1 protein in adult and newborn rat kidneys. In a previous report (38), we described the temporal expression pattern of the Kid-1 mRNA during renal development and in the recovery phase after ischemic acute renal failure. Having demonstrated the
specificity of the anti-Kid-1 antibody, we then set out to
determine the spatial expression pattern of the Kid-1
protein in the rat kidney. Staining of kidney sections
from adult rats with the anti-Kid-1 antibody 5D12 resulted in a strong nuclear
labeling of many tubular profiles (A). These profiles could be identi-
fied as proximal tubules by histochemical staining for alkaline
phosphatase (B). G. glomerulus; pt, proximal tubule. Magnification, ×185.

Fig. 2. Immunohistochemical analysis of the expression of Kid-1 in
the cortex of adult rat kidneys. Staining of cortical rat kidney sections
with the anti-Kid-1 antibody 5D12 resulted in a strong nuclear
labeling of many tubular profiles (A). These profiles could be identi-
fied as proximal tubules by histochemical staining for alkaline
phosphatase (B). G. glomerulus; pt, proximal tubule. Magnification, ×185.

Fig. 3. Immunohistochemical analysis of expression of Kid-1 in the
outer stripe of adult rat kidneys. Staining of the outer stripe of adult
rat kidneys with the anti-Kid-1 antibody 5D12 shows prominent
nuclear staining in the S3 segment of the proximal tubule (A), as
demonstrated by simultaneous histochemical staining for alkaline
phosphatase activity (B). There was faint nuclear staining in collect-
ing ducts and thick ascending limbs. The identity of the thick
ascending limbs was confirmed by the simultaneous application of an
anti-Tamm-Horsfall protein antibody (C). pt, Proximal tubule; tal, thick ascending limb; cd, collecting duct. Magnification, ×190.
At time of birth, the rat kidney is not completely developed, containing a rim of metanephrogenic mesenchyme immediately beneath the capsule and early metanephrogenic forms such as comma- and S-shaped bodies deeper toward the medulla (22). In the kidney of the newborn rat only background staining could be detected with the anti-Kid-1 antibody below the capsule, whereas there was a more pronounced nuclear staining toward the medulla, which suggested that the Kid-1 protein was expressed in the tubule at latter stages of nephron development (Fig. 4A). Alkaline phosphatase activity has been described to increase progressively during postnatal renal development and can therefore serve as a marker for the differentiation state of proximal tubules (5). The histochemical appearance of alkaline phosphatase activity (Fig. 4B) and Kid-1 immunoreactivity (Fig. 4A) correlated well, indicating that the expression of the Kid-1 protein occurred in the late stages of differentiation of the proximal tubule. Comparison of immunohistochemical staining for Kid-1 (Fig. 4A) and hematoxylin and eosin staining (Fig. 4C) on the same section demonstrated that other structures, such as the ureter stem, reacted only weakly and the ureteric bud, the condensed mesenchyme, and comma- and S-shaped bodies did not react at all with the anti-Kid-1 antibody.

Expression of Kid-1 mRNA and protein in polycystic kidneys from rat and mouse. The Han:SPRD (cy+/1) rat is a model for autosomal dominant polycystic kidney disease. The cysts in the Han:SPRD (cy+/1) rats originate predominantly from the proximal tubule (26). We analyzed the expression of the Kid-1 mRNA and protein in this model of polycystic kidney disease. When polycystic kidneys from 3-, 8-, and 24-wk-old male rats were studied, Kid-1 mRNA levels in kidneys from heterozygous (cy+/1) rats were lower than those in kidneys from age-matched, wild-type (+/+) controls (Fig. 5). In polycystic kidneys from female Han:SPRD rats, however, no marked difference could be seen between the Kid-1 mRNA levels of heterozygous (cy+/1) animals and those of wild-type animals at any age (Fig. 5).

These observations were extended by analyzing the distribution of the Kid-1 protein in cyst wall cells of male Han:SPRD (cy+/1) rats using immunocytochemical staining with the anti-Kid-1 antibody 5D12. Alkaline phosphatase served as a marker for the differentiation state of cyst wall epithelia. Many cyst-lining cells showed no alkaline phosphatase activity, as demonstrated previously (26) (Fig. 6A). Staining with the anti-Kid-1 antibody was decreased in the cyst epithelial cells, but was lost completely only in a subpopulation of cyst wall cells (Fig. 6B). Thus in many cysts the Kid-1 protein was still present when alkaline phosphatase staining was absent, although in these cyst-lining cells the Kid-1 expression level was decreased relative to cells in noncystic proximal tubules or cyst wall cells that still expressed alkaline phosphatase.

To determine whether our findings were specific to polycystic kidneys of the Han:SPRD (cy+/1) rat, we also evaluated the expression of Kid-1 mRNA by Northern blot analysis in polycystic kidneys from C57BL/6J (cpk/cpk) mice, a model for autosomal recessive PKD (Fig. 7). Compared with unaffected (+/+) littermates, the levels of Kid-1 mRNA were markedly decreased,

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**Fig. 4.** Expression of Kid-1 in the kidney of the newborn rat. Kidney sections from newborn rats were subjected to immunohistochemistry with the anti-Kid-1 antibody 5D12 (A) and to histochemistry for alkaline phosphatase (B). To clearly demonstrate the morphology of the various developmental stages, the same section was subsequently stained with hematoxylin and eosin (C). Kid-1 is strongly expressed in those profiles, which are also alkaline phosphatase positive, only weakly in the stem of the invading ureter (arrowheads), and not at all in ureteric buds (arrows) and earlier stages of nephron development such as S-shaped bodies (S). Magnification, ×185.
results similar to our observations in polycystic kidneys from Han:SPRD (cy/+) rats.

Expression of Kid-1 mRNA in rat renal cell carcinomas and transformed rat renal epithelial cell lines. If decreased Kid-1 expression is a common feature of the dedifferentiated proximal tubule cell, then one might expect Kid-1 expression to be suppressed in renal cell carcinoma and transformed renal epithelial cell lines, too. The expression of Kid-1 mRNA was examined in three different Eker renal cell carcinomas (Fig. 8). Eker tumors are derived from rats carrying a mutation in the Tsc-2 tumor suppressor gene (20, 41), which predisposes them to renal cell carcinoma and transformed renal epithelial cell lines, too. The expression of Kid-1 mRNA was examined in three different Eker renal cell carcinomas (Fig. 8). Eker tumors are derived from rats carrying a mutation in the Tsc-2 tumor suppressor gene (20, 41), which predisposes them to renal cell carcinoma and transformed renal epithelial cell lines, too. 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Fig. 5. Kid-1 mRNA levels are low in polycystic kidney disease in the Han:SPRD (cy/+ rat. Northern blots with 20 µg of total RNA from Han:SPRD (cy/+ and (+/+) rat kidneys were hybridized with PCR-generated single-stranded Kid-1 antisense probe. Random-primed glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA served as control for loading of RNA. Whereas Kid-1 mRNA levels in male Han:SPRD (cy+) rat kidneys were lower than those in kidneys from wild-type (+/+) animals at 3, 8, and 24 wk of age, no difference could be detected at any age when kidneys from female (cy+) animals were compared with those from age-matched, wild-type (+/+) animals.

Fig. 6. Expression of the Kid-1 protein in cyst wall cells of a polycystic kidney from a 38-day old male Han:SPRD (cy+) rat. Alkaline phosphatase expression, as demonstrated by histochemistry, is markedly decreased in large cysts, but not in smaller cysts and in noncystic proximal tubule cells (A). The Kid-1 protein, detected with the monoclonal anti-Kid-1 antibody 5D12 and subsequent incubation with a Cy3-labeled secondary antibody (B), is expressed strongly in nondilated proximal tubules and cysts that still expressed alkaline phosphatase markedly (arrows), but only weakly in cyst wall cells lacking alkaline phosphatase activity (arrowheads). The same section was also stained with the DNA-binding dye Hoechst 33258 to identify nuclei (C). Magnification, ×185.
Expression of Kid-1 in the adult and newborn rat kidney. In the adult rat kidney, the Kid-1 protein is strongly expressed in all segments of the proximal tubule, a localization confirmed by simultaneous histochemical analysis of alkaline phosphatase activity. By the same measure, Kid-1 appears at a relatively advanced stage of nephron development, that is, shortly after the S-shaped body stage at a time when alkaline phosphatase activity is also expressed. Kid-1 is a transcriptional repressor (38), and it can therefore be hypothesized that Kid-1 shuts off genes that are dispensable or even inhibitory for the establishment of the phenotype of the proximal tubule. Alternatively, similar to the hypothesis set forth for the transcription factors HNF-1 (27) and AP-2α (25). The HNF-1 protein appears to be necessary for the transcription of certain genes in proximal tubular cells. An inactivation of the HNF-1 gene results in a renal Fanconi syndrome caused by a dysfunction of the proximal tubule, because target genes of HNF-1 are no longer transcribed at sufficiently high levels (27). Whether Kid-1 is one of those target genes has yet to be evaluated.

Expression of Kid-1 in kidney disease. After renal injury, Kid-1 mRNA levels decline rapidly and transiently (38) at a time that the surviving tubular cells dedifferentiate and divide (37). Kidney Kid-1 mRNA levels increase with time after birth and are highest in the adult rat (38). These findings suggest that transcription of the Kid-1 gene and/or the stability of the transcript are decreased in cells that are less differentiated. The expression pattern of the Kid-1 protein during nephron development is consistent with this concept. In addition, our results with polycystic kidneys, renal cell carcinomas, and immortal rat kidney epithelial cell lines lend more support to this hypothesis. Both polycystic kidneys and renal cell carcinomas are characterized by a lower degree of cellular differentiation, higher mitotic rates, and lower Kid-1 mRNA levels than the adult normal kidney. It is interesting that in the polycystic kidneys of heterozygous female Han:SPRD (cpk+/−) rats, in which the polycystic phenotype is milder than in male (cpk+/−) rats (14), the mRNA levels of Kid-1 are the same as in age-matched, wild-type animals.

Similar to human autosomal dominant PKD, only a subset of nephrons in the Han:SPRD (cpk+/−) rat become cystically dilated (26). Thus Northern blot analysis, in which total kidney RNA is examined, underestimates the marked decrease of Kid-1 protein expression in cyst-lining cells demonstrated by immunohistochemical staining. The loss of Kid-1 protein expression occurred in the same cells in which there was loss of alkaline phosphatase activity, which is consistent with the appearance of Kid-1 late during nephron development when the epithelial cells are more differentiated. During nephron development, Kid-1 protein appears approximately at the same time as alkaline phosphatase activity; in cyst wall epithelia, Kid-1 disappears after or at the same time as the loss of alkaline phosphatase. Dedifferentiation in cyst wall cells in kidneys from the Han:SPRD (cpk+/−) rat likely is a graded process and appears first to be associated with a loss of expression of alkaline phosphatase and decrease in Kid-1 protein, followed by further loss of the Kid-1 protein.

The pattern of Kid-1 mRNA expression in polycystic kidneys is not specific to the Han:SPRD (cpk+/−) rat model of autosomal dominant PKD, because a similar pattern can be seen in polycystic kidneys from the
C57BL/6J (cpk/cpk) mouse, a model for autosomal recessive PKD. Kid-1 mRNA levels were much lower in kidneys from homozygous (cpk/cpk) mice than in kidneys from control animals of the same age. The affected gene in Han:SPRD rats is located on rat chromosome 5 (2), whereas in the cpk/cpk model the affected gene lies on mouse chromosome 12 (10). Both loci are different from the locus of the Kid-1 gene (chromosome 10 in the rat (40) and chromosome 11 in the mouse (32)), so that a mutation in the Kid-1 gene cannot be the cause of the disease in either animal model. It is possible that the decrease of Kid-1 expression contributes to a pattern of epithelial dedifferentiation common to both models. Because Kid-1 is a transcriptional repressor, it is likely that a regulated gene is repressed when Kid-1 is expressed. When Kid-1 expression is suppressed there may be enhanced production of a protein, which then may contribute to the cystic phenotype.

In transformed epithelial cell lines from the rat kidney, mRNA levels of Kid-1 are low. This observation is consistent with the hypothesis that rapidly dividing and dedifferentiated cells have downregulated the expression of Kid-1. We could, however, see no difference between cells in a logarithmic growth state or at confluency. The mechanisms, however, by which growth arrest and differentiation are brought about probably share certain features, but also are different in other aspects. Growing these cell lines to confluence may not have been sufficient to achieve a level of differentiation associated with increases in Kid-1 mRNA levels.

In summary, Kid-1 is expressed prominently in the proximal tubule. Levels of Kid-1 increase during nephron development and are markedly reduced in two animal models of PKD, in rat renal cell carcinomas, and in TRKE cell lines. Each of the latter conditions is characterized by a proliferative phenotype with a low level of differentiation. We propose that this decreased Kid-1 expression may contribute to the derepression of genes, which in turn may contribute to the abnormal cyst phenotype of PKD and perhaps the dedifferentiated highly proliferative state of renal tumor cells and transformed cell lines.

We acknowledge the skillful introduction to hybridoma technology by Peter Mundel and the kind gifts of materials from Jack Dixon (pGEX-KG) and John Kyriakis (pMT3), without which this work would not have been possible. Michael Kramer kindly established the isotype of the hybridoma 5D12. The expert photographic work of Ingrid Ertel and graphics of Rolf Nonnenmacher are gratefully acknowledged. We also thank Karen Delvo and Alessandro Alessandrini for help with the figures. The experiments described were made possible by financial support from the Deutsche Forschungsgemeinschaft to R. Witzgall (Wi 1042/2-1 and Wi 1042/2-2) and National Institutes of Health (cpk) is located on proximal chromosome 12. Genomics 9: 778–781, 1991.


Fig. 1. Kid-1 expression in normal and cystic proximal tubules. (A) The transcription factor, Kid-1, is highly expressed in both eye and kidney of the mouse. Exp. Eye Res. 64: 287–290, 1997.


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