Kidney injury molecule-1 expression in murine polycystic kidney disease

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Kidney injury molecule-1 (Kim-1) is a type 1 transmembrane protein that is expressed at very low levels in normal kidneys and is maximally upregulated in the S3 segment of the proximal tubule 24–48 h after exposure of the kidney to transient ischemia (12). It is expressed in human kidneys with acute tubular necrosis (19) and can be detected in the urine of these patients (30) and fibrosis (19) in areas adjacent to cysts, suggesting a role for the protein in the development of interstitial fibrosis. Na-K-ATPase; cell polarity; fibrosis; Tim; ischemia; kidney obstruction

AUTOSOMAL DOMINANT POLYCYSTIC kidney disease (ADPKD) is the most commonly inherited monogenetic renal disease, affecting >1 in 1,000 live births. In the United States, it leads to end-stage renal disease in >1,500 patients/yr (24). In most patients, the disease is caused by an inherited defect in one of two polycystin genes, PKD1 or PKD2 (10a, 16). Random loss of heterozygosity in individual tubular epithelial cells is thought to be responsible for the transformation of epithelial cells through somatic inactivation of the second copy of the affected gene. The resulting dedifferentiation and proliferation causes progressive cyst formation (31). The PKD2 gene product polycystin-2 is a nonselective cation channel, and polycystin-1 may be required for its proper localization and function (8, 11, 13). The molecular mechanisms underlying the epithelial transmembrane expression in cystic kidneys in the Pkd2WS25/H11022 mouse model for autosomal dominant polycystic kidney disease, cystic kidneys show markedly upregulated Kim-1 levels compared with noncystic control kidneys. Kim-1 is present in a subset of cysts of different sizes and segmental origins and in clusters of proximal tubules near cysts. Kim-1-expressing tubular cells show decreased complexity and quantity of basolateral staining for Na-K-ATPase. Other changes in polarity characteristic of ischemic injury are not present in Kim-1-expressing pericystic tubules. Polycystin-2 expression is preserved in Kim-1-expressing tubules. The interstitium surrounding Kim-1-expressing tubules shows high proliferative activity and staining for smooth muscle α-actin, characteristic of myofibroblasts. Although the functional role of the protein in cysts remains unknown, Kim-1 expression in tubules is strongly associated with partial dedifferentiation of epithelial cells and may play a role in the development of interstitial fibrosis.

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1 In Ref. 12, rat KIM-1 was capitalized. We have modified the nomenclature and reserved the capitalized form for human KIM-1. We refer to the rodent rat or mouse form as Kim-1 throughout this manuscript.
found in a small subset of cysts of varying sizes and different segmental origins. Additionally, we found a striking pattern of Kim-1 expression in clusters of noncystic tubules adjacent to cysts in regions characterized by interstitial cell proliferation and fibrosis. In tubular cells, Kim-1 expression is associated with a decrease in complexity and quantity of Na-K-ATPase expression, without other features of loss of cell polarity, as reflected by actin, villin, and E-cadherin staining patterns. The functional role of Kim-1 expression in cysts is unclear. The tubular and peritubular findings support the possibility that Kim-1 may play a role in interstitial fibrosis and nephron loss in ADPKD.

**METHODS**

**Animals and tissues.** Pkd2<sup>WS25/−</sup> mice (31) and wild-type (WT) littermates were genotyped by Southern blot analysis. Unilateral ureteral obstruction and renal ischemia with reperfusion were performed as previously described by our laboratory (20). For total protein lysates, the kidneys were harvested unfixed and shock frozen in liquid nitrogen. For immunohistochemical analysis, mice were perfused via the left ventricle with paraformaldehyde-lysine-periodate solution containing 2% paraformaldehyde, 38 mM phosphate buffer, 60 mM lysine, 10 mM sodium periodate, and 5% sucrose. Subsequently, kidneys were immersed in 2% paraformaldehyde, 38 mM phosphate buffer, 60 mM lysine, 10 mM sodium periodate, and 5% sucrose. Subsequently, kidneys were immersed in 2% paraformaldehyde for 4 h, washed in PBS, and cryoprotected by overnight submersion in 30% sucrose in 1× PBS. Five-micrometer frozen sections were cut in a cryostat.

**Antibodies.** Primary antibodies included the following. AKT7 is a mouse monoclonal antibody against the extracellular domain of human KIM-1 (1a). R9 is a rabbit polyclonal antibody, which was raised to the intracellular domain of rat KIM-1 (1a). R9 is a rabbit polyclonal antibody with mouse Kim-1. With the purpose of studying Kim-1 expression in a mouse model of ADPKD, we searched the database for mouse genes with homologies to human KIM-1 and rat Kim-1. A putative mouse ortholog of rat Kim-1 was identified by BLAST search of the mouse EST database. EST AA547594 was found to contain the full coding region of putative mouse Kim-1 (mKim-1). Comparison of the deduced amino acid sequence with rat Kim-1 shows 58% identity. There is a high degree of homology in the protein region to which a rabbit anti-rat-Kim-1 antibody, R9, had been raised (Fig. 1). Binding of R9 to mKim-1 was demonstrated by Western blot analysis of total cell lysates of COS cells transfected with the mKim-1 expression construct (Fig. 2).

**Mouse Kim-1 is expressed in the Pkd2<sup>WS25/−</sup> mouse model of ADPKD.** Expression of mouse Kim-1 is markedly increased in kidneys of Pkd2<sup>WS25/−</sup> mice, compared with minimal basal expression in kidneys from WT mice (Fig. 3). Kim-1 staining is present in 2–5% of cysts and can be seen in cysts of different sizes (Fig. 4A). Two patterns of Kim-1 expression are observed in Pkd2<sup>WS25/−</sup> kidneys. One pattern of Kim-1 staining in cyst epithelia is apical (Fig. 4B), whereas the other is intracellular (Fig. 4C). In addition to Kim-1-staining cysts, occasional clusters of Kim-1-staining noncystic tubules are seen in areas adjacent to Kim-1-negative cysts (Fig. 4D). No staining for Kim-1 is present in normal kidneys from WT mice.

**Kim-1-expressing cysts are derived from different segmental origins.** Kim-1 expression after ischemia-reperfusion injury is localized to the proximal tubule (12). To evaluate whether Kim-1 expression occurs only...
in cysts of proximal tubular origin, we examined Kim-1-expressing cysts for staining with the proximal tubular marker aquaporin-1 and the collecting duct marker aquaporin-2. Some Kim-1-positive cysts express aquaporin-1 (Fig. 5B) but not aquaporin-2 (Fig. 5C), whereas others stain for aquaporin-2 (Fig. 5F) but not aquaporin-1 (Fig. 5E). Some Kim-1-positive cysts do not express either aquaporin-1 or aquaporin-2 (data not shown). These findings indicate that Kim-1 expression in cysts is not related to their segmental origin.

The staining pattern for Kim-1 differs between pericystic tubules and obstructed WT kidneys. In tubules adjacent to cysts, the staining pattern is apical and occasionally lateral (Fig. 5G). Staining for Kim-1 is often discontinuous, with only some of the epithelial cells in a given tubule expressing detectable amounts (Fig. 5H). Because it has been proposed that tubular obstruction plays a role in the pathophysiology of ADPKD (25), we compared the Kim-1 expression pattern in ADPKD to that observed in obstructed kidneys. We found Kim-1 uniformly expressed in all cells of a
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Kim-1-expressing tubular epithelial cells have decreased complexity and quantity of Na-K-ATPase expression but maintain normal expression of actin, villin, and E-cadherin. Because Kim-1 is expressed in dedifferentiated epithelial cells after ischemic injury (12), we analyzed Kim-1-expressing tubular cells for the distribution of Na-K-ATPase. Basolateral expression of Na-K-ATPase is a marker of terminal differentiation of epithelial cells (28) and is decreased after ischemic injury (33). In tubules adjacent to cysts, single Kim-1-positive cells (Fig. 6, A and D) exhibit a loss of complexity (Fig. 6E) and decreased quantity (Fig. 6, B, C, E, and F) of Na-K-ATPase expression, whereas Kim-1-negative cells in the same tubule have a normal staining pattern for Na-K-ATPase. We additionally examined the staining pattern of actin and villin, which have been shown to partially redistribute from the apical brush border of epithelial cells to the basolateral membrane after renal ischemia (2). A normal distribution of apical brush-border staining for actin (not shown) and villin is present in Kim-1-expressing pericycstic tubules (Fig. 6, G and H). Because ischemia has been shown to result in breakdown of the adherens junction and loss of lateral E-cadherin expression in epithelial cells (3), we also examined E-cadherin staining in Kim-1-expressing tubules. E-cadherin staining is preserved in the lateral membrane of Kim-1-expressing cells (Fig. 6, I–L). These results indicate that Kim-1 expression in epithelial cells is associated with a partial loss of polarity. This is not likely to be due to ischemia.

Different distribution patterns of Na-K-ATPase are found in Kim-1-expressing cysts. Because Kim-1-expressing tubular cells show alterations of Na-K-ATPase expression that are consistent with a state of partial dedifferentiation and dedifferentiation of epithelial cells has been implicated to play a role in cyst formation, we investigated the staining pattern for Na-K-ATPase in Kim-1-expressing cysts. Cysts with apical Kim-1 expression have preserved basolateral staining for Na-K-ATPase (Fig. 7, A–C), whereas cystic epithelia with cytoplasmic Kim-1 expression show diffuse staining for Na-K-ATPase (Fig. 7, D–F). Diffuse, as well as basolateral, staining for Na-K-ATPase in cysts is also found in the absence of Kim-1 expression. Lateral staining for E-cadherin is preserved in all Kim-1-expressing cysts, as reflected in an example of a Kim-1-positive cyst with diffuse expression of Na-K-ATPase (Fig. 7, G–I). These findings of apical Kim-1 expression with basolateral staining for Na-K-ATPase in cysts are different from the findings in Kim-1-positive tubular cells, wherein the expression of Kim-1 is associated with loss of quantity and complexity of basolateral Na-K-ATPase staining.

Kim-1-expressing tubular cells are surrounded by proliferating cells and interstitial myofibroblasts. Kim-1 is expressed in proliferating tubular epithelial cells after ischemic injury (12). Because increased cell proliferation has been observed in ADPKD (18, 21), we examined cellular proliferation in Kim-1-expressing epithelial cells by PCNA staining. We observed PCNA staining in only a few positive cells that express Kim-1,
either in cysts or in pericystic tubules (data not shown), but found a greater number of PCNA-positive cells in the peritubular interstitium surrounding Kim-1-expressing tubules (Fig. 8, A, C, and E). Increased numbers of PCNA-positive cells were also found in some non-Kim-1-expressing tubules in these same areas, consistent with a “field effect,” as previously described (18). In contrast, very few proliferating cells are found in the interstitial areas adjacent to Kim-1-negative tubules (Fig. 8, B, D, and F).

Because cellular proliferation in the renal interstitium is observed during the development of interstitial fibrosis in ADPKD (21), we evaluated these areas for the presence of myofibroblasts by staining for smooth muscle α-actin. Smooth muscle α-actin is found in vascular smooth muscle cells and in fibrogenic myofibroblasts but not in resident interstitial fibroblasts (7). Its expression has been associated with the progression of interstitial fibrosis (22). We found that smooth muscle α-actin staining is enhanced in regions of the kidney surrounding Kim-1-expressing clusters of pericystic tubules (Fig. 9, A, C, and E). Smooth muscle α-actin expression is not observed in the interstitium adjacent to Kim-1-negative tubules (Fig. 9, B, D, and F). Because nephron loss in ADPKD may be due to apoptosis, we checked TdT-mediated dUTP-X nick-end-labeling...
Fig. 6. Kim-1, Na-K-ATPase, E-cadherin, and villin expression in tubules of cystic kidneys from Pkd2<sup>WS25/H11002</sup> mice. Sections were stained with polyclonal anti-Kim-1 (R9), monoclonal anti-Na-K-ATPase (6F), monoclonal anti-E-cadherin, and polyclonal anti-villin antibodies. Secondary antibodies were Cy3-coupled anti-rabbit and FITC-coupled anti-mouse antibodies. 

**A–C:** Basal membrane expression of Na-K-ATPase is greatly diminished in individual Kim-1-expressing proximal tubular cells. 

- **A:** Kim-1 is expressed in individual epithelial cells of proximal tubules (red).
- **B:** Staining for Na-K-ATPase (green) demonstrates individual cells that have strongly reduced staining of Na-K-ATPase at the basal and lateral membrane (arrows).
- **C:** Combined image of A and B shows that the cells that express Kim-1 have greatly diminished Na-K-ATPase expression at the basal and lateral membrane (arrows).

**D–F:** Another example of reduced amounts and complexity of Na-K-ATPase in Kim-1-expressing tubule cells. 

- **D:** One-half of the cells of this tubule stain for Kim-1. 
- **E:** Cells in the upper one-half of the tubule show a strong reduction in the complexity and quantity of staining for Na-K-ATPase. 
- **F:** In the combined image of D and E, reduced expression of Na-K-ATPase in individual cells coincides with apical Kim-1 expression. 

**G and H:** Apical villin staining is preserved in Kim-1-expressing proximal tubules. 

- **G:** Pair of Kim-1-expressing tubules (arrows) show apical staining for R9 in red. 
- **H:** In a neighboring section, apical villin expression in the Kim-1-expressing tubules (arrows) is present only in the apical brush border but not in the basolateral membrane, as has been described after ischemia-reperfusion (2). 

**I–L:** Lateral E-cadherin staining is preserved in Kim-1-expressing proximal tubular cells in sections adjacent to **G** and **H**. 

- **I:** Kim-1-expressing proximal tubule cells show apical staining (red). 
- **K:** E-cadherin (in green) localizes to the lateral membranes. 
- **L:** In the combined image of I and K, staining for anti-E-cadherin in the lateral membrane is preserved in the Kim-1-expressing tubule. Bars = 10 μm.
staining. There is no increase in the numbers of apoptotic cells in either Kim-1-positive cysts or tubules or in the surrounding interstitium, compared with Kim-1-negative cysts or areas without Kim-1-expressing tubules (not shown). Thus Kim-1 is expressed in tubules that are surrounded by early fibrogenic activity.

**DISCUSSION**

Despite important insights into the molecular genetics of ADPKD (24) and recent data about the function of the polycystins (8, 11, 13), the precise mechanism of cyst formation and the pathophysiology leading to interstitial fibrosis and nephron loss in ADPKD remain unknown. In this report, we demonstrate that Kim-1 is expressed in a subset of cysts, without a clear association with cyst size, segment of origin, or proliferative activity. Kim-1 expression in tubules is associated with a partial loss of polarity, as indicated by a loss of complexity and quantity of Na-K-ATPase expression, but preserved staining for actin, villin, and E-cadherin. These findings raise the possibility that Kim-1 plays a role in the dedifferentiation of epithelial cells. Hypothetically, this could explain Kim-1 expression in cysts. The close relationship between apical Kim-1 staining in tubules and disordered Na-K-ATPase expression is not found in cysts as might be expected if Kim-1 expression was tightly coupled to Na-K-ATPase expression. Our findings do not rule out a more complex...
relationship between Kim-1 and Na-K-ATPase expression in cysts or an effect of Kim-1 expression on the differentiation status of epithelial cells in cysts.

In addition to Kim-1 expression in cysts, we found a striking pattern of Kim-1-expressing noncystic tubules clustered near cysts. These express polycystin-2 by immunostaining (data not shown); hence, Kim-1-positive tubular cells are not characterized by loss of heterozygosity, which is associated with cyst formation in this model. Because Kim-1 expression in proximal tubules is most pronounced after ischemia (12), and ischemia has been proposed to play a role in the pathophysiology of ADPKD (5), we looked for evidence of ischemic injury in Kim-1-expressing pericystic tubular cells. Ischemia is associated with breakdown of the adherens junction and disruption of the apical brush-border actin and villin localization pattern (2, 3). Our findings of normal distribution patterns for E-cadherin, actin, and villin make it very unlikely that tubular Kim-1 expression near cysts is a consequence of ischemic injury.

Fig. 8. Kim-1 and PCNA staining in Pkd2<sup>W825/−</sup> kidneys. Sections were stained with polyclonal anti-Kim-1 (R9) and monoclonal anti-PCNA antibodies. Secondary antibodies were Cy3-coupled anti-rabbit and FITC-coupled anti-mouse antibodies. Clusters of Kim-1-expressing proximal tubules adjacent to cysts coincide with a high degree of cell proliferation in the surrounding interstitium (A, C, and E) relative to areas of absent Kim-1 staining (B, D, and F). A: clusters of tubules near a cyst (*) stain positive (red) for apical Kim-1 expression. B: in a different area adjacent to a cyst, no tubular staining for Kim-1 is observed. C: large number of PCNA-positive nuclei (green) are present in the interstitium, as well as in some tubules. D: PCNA staining shows markedly fewer proliferating nuclei (arrows) compared with C. E: in the combined image of A and C, the proliferating cells in the interstitium and the tubules are near Kim-1-expressing tubules. F: combined image of B and D shows that the absence of Kim-1 expression in tubules coincides with a markedly reduced proliferative activity in the interstitium. Bars = 50 μm.
Tubular obstruction has also been proposed to play a role in progressive nephron loss in ADPKD (25). We found that obstructed kidneys express Kim-1. However, the expression pattern of Kim-1 in postobstructed kidneys is markedly different from the pattern in ADPKD. In postobstructed kidneys, Kim-1 is expressed in entire tubular segments, involving all cells of a given tubule, as opposed to the intermittent epithelial cell staining pattern in cystic kidneys. It is therefore unlikely that Kim-1 expression in pericystic tubules in ADPKD is a consequence of tubular obstruction.

The large number of PCNA-positive cells in the interstitium surrounding Kim-1-expressing tubules together with the presence of smooth muscle α-actin-positive cells suggests that Kim-1 expression in proximal tubules adjacent to cysts is associated with interstitial proliferation and fibrosis. This is particularly important because the loss of renal function in patients with ADPKD is not believed to be due solely to
the cysts themselves. Interstitial fibrosis and inflammation have been implicated in nephron loss (32). It is possible that pericystic Kim-1 expression in ADPKD kidneys is a consequence of epithelial injury caused by a proliferative and fibrogenic interstitial process. Alternatively, Kim-1 expression in tubular epithelial cells could be a primary event leading to interstitial fibrosis. Proximal tubular cells have been previously invoked in the pathogenesis of tubulointerstitial damage and fibrosis in various animal models of chronic renal disease, such as systemic lupus erythematosus (26), diabetic nephropathy (29), and ureteral obstruction (27), and in studies of human tissues from patients with chronic renal disease (9). The mechanisms resulting in interstitial fibrosis are not understood, but chemokine expression, such as monocyte chemoattractant protein-1, by tubular epithelial cells has been implicated in the recruitment of interstitial macrophages and the development of interstitial fibrosis (26, 27). Recent data from our laboratory that the extracellular portion of Kim-1 is shed from the cell (1a) raise the possibility that shed Kim-1 reaches the interstitium and plays a role in the appearance of myofibroblasts and the proliferative and fibrogenic response. Further support for the concept that Kim-1 is an immunologically active molecule comes from recently published evidence that suggests that molecules of the Kim family are involved in T cell differentiation and macrophage activation (15, 17).

In summary, we demonstrate that Kim-1 is expressed in murine ADPKD but not in normal kidneys. It is expressed in a small number of cysts and in a pattern of proximal tubule clusters adjacent to cysts. Kim-1 expression in single tubule cells is associated with a partial loss of polarity, which is likely unrelated to ischemic injury. Kim-1-expressing tubules are surrounded by a highly proliferative and fibrogenic interstitial response. In conclusion, we propose that Kim-1 expression may play a pathogenic role in the development of interstitial fibrosis and subsequent nephron loss in ADPKD.

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