Kidney Injury Molecule-1 (Kim-1) expression in murine polycystic kidney disease

E. Wolfgang Kuehn¹, Kwon Moo Park¹, Stefan Somlo², and Joseph V. Bonventre¹,³

¹Renal Unit and Department of Medicine, Massachusetts General Hospital, Charlestown, MA 02129. ²Section of Nephrology, Yale University School of Medicine, New Haven, CT 06519. ³Harvard–Massachusetts Institute of Technology Division of Health Sciences and Technology, Cambridge, MA 02139.

Running head: KIM-1 in polycystic kidney disease.

Contact Information: Joseph V. Bonventre, MD, PhD
Massachusetts General Hospital East
Renal Unit 4th floor, Room 4002
149, 13th Street
Charlestown, MA 02129
Tel.: 617 726 3770
Fax: 617 726 4356
E-mail: joseph_bonventre@hms.harvard.edu
Abstract

Kidney Injury Molecule-1 (Kim-1) is a type 1 membrane protein maximally upregulated in proliferating and de-differentiated tubular cells after renal ischemia. Since epithelial de-differentiation, proliferation and local ischemia may play a role in the pathophysiology of autosomal dominant polycystic kidney disease (ADPKD), we investigated Kim-1 expression in a mouse model of this disease. In the Pkd2^{WS25/-} mouse model for ADPKD, cystic kidneys show markedly upregulated Kim-1 levels compared to 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 de-differentiation of epithelial cells and may play a role in the development of interstitial fibrosis.

Keywords: Kidney Injury Molecule-1, KIM-1, polycystic kidney disease, PKD, Na,K-ATPase, cell polarity, fibrosis.
Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is the most commonly inherited monogenic renal disease, affecting more than 1 in 1000 live births. In the US it leads to end-stage renal disease in over 1500 patients per year (24). In most patients the disease is caused by an inherited defect in one of the two polycystin genes, \( PKD1 \) (6) or \( PKD2 \) (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 de-differentiation 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 transformation are unknown. Epithelial cells in cysts have been shown to be de-differentiated and to have increased proliferative indices (18,21). The growth of cysts, leading to massively enlarged kidneys are thought to be responsible for regional ischemia, which contributes to elevations in plasma renin and hypertension (5). Less than 1 percent of all nephrons are directly affected by cysts, and therefore direct nephron loss through cyst formation does not explain the decline of renal function. Progressive nephron loss is thought to involve apoptosis (30) and fibrosis (19) in areas adjacent to cysts, but this process is not very well characterized.

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 hours after exposure of the kidney to transient ischemia (12). It is expressed in human kidneys with ATN and can be detected in the urine of these patients (10). In the postischemic kidney Kim-1 is expressed in vimentin positive cells and cells which take up BrdU (12), suggesting a role for the protein in the de-differentiation and proliferation of epithelial cells. As epithelial de-differentiation, proliferation and ischemia may play a role in the pathophysiology of ADPKD, we examined the pattern of Kim-1 expression in cystic kidneys in the Pkd2\(^{WS25/-}\) mouse model. We found that Kim-1 is expressed in polycystic kidneys, but not in normal kidneys. Kim-1 expression is 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 non-cystic 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:

\[Pkd2^{WS25}\] mice (31) and wild type (WT) littermates were genotyped by Southern analysis. Unilateral ureteral obstruction and renal ischemia with reperfusion were performed as previously described from 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 PLP solution containing 2% paraformaldehyde, 38 mM phosphate buffer, 60 mM lysine, 10mM sodium periodate and 5% sucrose. Subsequently kidneys were immersed in 2% paraformaldehyde for 4 hours, washed in PBS and cryoprotected by overnight submersion in 30% sucrose in 1x PBS. Five micron frozen sections were cut in a cryostat.

Antibodies:

Primary antibodies: AKG7 is a mouse monoclonal antibody against the extracellular domain of human KIM-1 (Bailly et al., submitted for publication). R9 is a rabbit polyclonal antibody, which was raised to the intracellular domain of rat KIM-1 (Fig. 2) (12). Monoclonal anti-Na,K-ATPase 6F was obtained from the Developmental Studies Hybridoma Bank, University of Iowa. It recognizes the alpha1 subunit and has been well characterized (1). Monoclonal anti-E-cadherin was purchased from BD transduction laboratories. Mouse anti-proliferative cellular nuclear antigen (PCNA) was purchased from DAKO, and mouse anti-smooth muscle \(\alpha\)-actin from Sigma. Polyclonal rabbit anti-polycystin 2 YCC is a gift from Dr.
Yiqiang Cai, Yale University School of Medicine (4). Polyclonal rabbit anti-villin and rabbit anti-aquaporin 2 are gifts from Dr. Dennis Brown of the Massachusetts General Hospital (MGH) (2). Polyclonal rabbit anti-aquaporin 1 is a gift from Dr. Alfred Van Hoek, MGH (23). Secondary antibodies were Cy3 conjugated goat anti-rabbit and FITC conjugated goat anti-mouse from Sigma. Actin was visualized using TRITC labeled phalloidin (Sigma).

**Apoptosis:**

TdT-mediated dUTP-X nick end labeling (TUNEL) staining was performed with materials obtained from Boehringer according to instructions supplied by the manufacturer.

**Subcloning:**

EST AA547594 was identified by BLAST search to have significant sequence homologies to rat KIM-1 and was obtained from the I.M.A.G.E consortium (clone 960204) (14). After further sequencing it was found to contain the full open reading frame (ORF) of putative mouse *Kim-1*. Subcloning of the entire ORF was performed by polymerase chain reaction (PCR) into pCR2.1 (Invitrogen), using primers 5’-CGCGTGGACCATGAATCAGATTC-3’ and 5’-CTGCCCTCAAGGTCTATCTTC-3’. Subsequently the insert was subcloned into the EcoR1 site of pCDNA3 (Invitrogen). The sequence was confirmed through double stranded sequencing. Sequence analysis was performed with MacVector 6.5 software (Oxford Molecular).
Cell cultures and transient transfection:

COS1 cells were maintained in Dulbecco’s Modified Eagle’s Medium (DMEM) supplemented by 10% fetal calf serum. Transient transfections were carried out with the superfect reagent (Qiagen), according to instructions by the manufacturer.

Proteins and Western Blot:

For SDS PAGE shock frozen kidney tissue was homogenized in sample buffer containing 125 mM TRIS pH 6.8, 4.1% SDS, 20% glycerol and 2% mercaptoethanol. Lysis of cells for protein analysis, electrophoresis, transfer and Western Blotting were performed as previously described (20).

Immunohistochemistry:

Tissue sections were boiled in 0.1M Na citrate buffer for 10 min, incubated with 1% SDS in PBS for 5 minutes, washed with 1x PBS and blocked in 1x PBS with 2% FCS, 5% sucrose, and 0.1% IPEGAL CA-630 (Sigma). Incubation with primary antibody was done overnight at 4°C or for one hour at room temperature. Secondary antibodies were applied for 1 hr at room temperature. For immunofluorescence, slides were mounted with Vectashield (Vector Labs) in 1:1 dilution with 1.5M TRIS pH 8.9 and examined under an epifluorescence microscope (Nikon)
Microphot FXA). Images were captured with a Hamamatsu Orca charge-coupled device camera and processed with IPLab Spectrum software (Scanalytics, Vienna, VA).
Results:

Cloning of a mouse ortholog to rat KIM-1 and confirmation of crossreactivity of the anti-rat KIM-1 antibody, R9, with mouse Kim-1

With the aim of studying Kim-1 expression in a mouse model of ADPKD we searched the database for mouse genes with homologies to human and rat Kim-1. A putative mouse ortholog of rat Kim-1 was identified by 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 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^WS25/- mouse model of ADPKD

Expression of mKim-1 is markedly increased in kidneys of Pkd2^WS25/- mice, in comparison with minimal basal expression in kidneys from wild-type 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^WS25/- 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 non-cystic tubules are seen in areas adjacent
to Kim-1 negative cysts (Fig. 4D). No staining for Kim-1 is present in normal kidneys from wild-type 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). In order to evaluate if 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 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). As 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 tubular segment 48 hours after transient ureteral obstruction in wild-type mice (Fig. 5I),...
a pattern markedly different from the pattern of sporadic cell staining within a given tubule in cystic kidneys.

**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.**

Since KIM-1 is expressed in de-differentiated 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 lost after ischemic injury (33). In tubules adjacent to cysts, single Kim-1 positive cells (Fig. 6A,D) exhibit a loss of complexity (Fig. 6E) and decreased quantity (Fig. 6B,E) of Na,K-ATPase expression, whereas Kim-1 negative cells in the same tubule have a normal staining pattern for Na,K-ATPase(Fig. 6C,F). We additionally examined the staining pattern of actin and villin, which have been shown to redistribute from the apical brush border of epithelial cells to the basolateral membrane after renal ischemia (2,3). A normal distribution of apical brush border staining for actin (not shown) and villin is present in Kim-1 expressing pericystic tubules (Fig. 6G,H). Since 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. 6I-L). These results indicate that Kim-1 expression in epithelial cells is associated with a partial loss of polarity. This is not likely due to ischemia
Different distribution patterns of Na,K-ATPase are found in Kim-1 expressing cysts.

Since Kim-1 expressing tubular cells show alterations of Na,K-ATPase expression which are consistent with a state of partial de-differentiation, and de-differentiation 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. 7A-C), whereas cystic epithelia with cytoplasmic Kim-1 expression show diffuse staining for Na,K-ATPase (fig. 7D-F). Diffuse as well as basolateral staining for Na,K-ATPase in cysts are 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. 7G-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, where 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). Since 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 which 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. 8A,C,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). By contrast, very few proliferating cells are found in the interstitial areas adjacent to Kim-1 negative tubules (Fig. 8B,D,F).

Since 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). It’s 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. 9A,C,E). Smooth muscle α−actin expression is not observed in the interstitium adjacent to Kim-1 negative tubules (Fig. 9B,D,F). Since nephron loss in ADPKD may be due to apoptosis we checked TUNEL staining. There is no increase in the numbers of apoptotic cells in either Kim-1 positive cysts or tubules, nor in the surrounding interstitium, as compared to Kim-1 negative cysts or areas without Kim-1 expressing tubules (not shown). Thus Kim-1 is expressed in tubules which 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 de-differentiation of epithelial cells. Hypothetically this could explain Kim-1 expression in cysts. However, 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 non-cystic tubules clustered near cysts. These express polycystin 2 by immunostaining (data not shown) and hence Kim-1 positive tubular cells are not characterized by loss of heterozygosity, which is associated with cyst formation in this model. Since 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.

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. The expression pattern of Kim-1 in post-obstructed kidneys is markedly different, however, from the pattern in ADPKD. In post-obstructive 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 suggest that Kim-1 expression in proximal tubules adjacent to cysts is associated with interstitial proliferation and fibrosis. This is particularly important, as 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), 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 (MCP-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 (unpublished data) 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 which 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, 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.

Acknowledgements

The authors thank Dr. Sayoko Nishimura and Dr. Xin Tian for assistance with the Pkd2^{ws25/-} mice. We thank Dr. Dennis Brown for helpful discussions. EWK was supported by awards from the Deutsche Forschungsgemeinschaft (DFG - Ku 1322/1-1) and the National Kidney Foundation. SS is supported by DK 54053 and DK 57328. JVB is supported by NIH DK 39773, DK 38452, DK 46267, and NS 10828. The core facility used for the immunohistochemistry was partially supported by NIH CSIBD center grant # DK 43351.
References:


Figure Legends:

Fig. 1. Alignment of the rat Kim-1 and mouse Kim-1 amino acid sequences. Identical amino acids are shaded in dark grey, similar ones in light grey. The peptide region in the rat sequence, to which the rabbit polyclonal antibody R9 was made, is underlined in red. Note the high degree of homology in this region.

Fig. 2. Western Blot analysis of mouse Kim-1, with polyclonal anti-rat Kim-1 antibody (R9), demonstrates crossreactivity of the R9 antibody between species. COS cells were transfected with a mouse Kim-1 expression construct or a GFP control construct and compared to protein lysates of a post-ischemic mouse kidney, which were obtained 24 hours after 30 minutes of renal ischemia and subsequent reperfusion (20). Kim-1 is highly glycosylated, resulting in three bands, which represent different degrees of glycosylation (12). The predicted weight of the unglycosylated protein is 32 kDa. The bands are shifted downwards in the COS cell lysates compared to the bands in the postischemic kidney. This likely represents differences in glycosylation patterns between fibroblasts in culture and epithelial cells in-vivo (12).

Fig. 3. Kim-1 expression is increased in cystic kidneys of Pkd2^{WS25/-} mice, compared to wild-type littermates. Kidneys from two 4 week old Pkd2^{WS25/-} or two wild-type mice were removed and shock frozen. The post-ischemic control kidney was removed and shock frozen 24 hours after 40
min of unilateral ischemia in a WT mouse. The kidneys were homogenized in sample buffer and analyzed by SDS PAGE and Western Blot with the R9 anti Kim-1 antibody. Only the 64 kDa band of Kim-1 is shown. Equal loading was demonstrated by stripping the membrane and probing with anti-P38 antibody.

Fig. 4. Immunofluorescence staining for mouse Kim-1 with polyclonal anti-Kim-1 (R9) antibody and anti-rabbit Cy3 antibody. 10-week-old Pkd2^{ws25/-} mice were sacrificed and perfused with PLP fixative solution. Five micrometer cryostat sections were analyzed by indirect immunofluorescence staining. (A) Intermediate sized cyst with a diameter of 550 µm shows nearly circumferential staining for Kim-1 in cyst lining cells. Staining for Kim-1 is found in cysts of various sizes. Most cysts are negative for Kim-1 staining (not shown) Bar: 100 µm. (B,D) The pattern of Kim-1 expression differs among cysts. (B) Cyst epithelium with apical expression of Kim-1. Bar: 10 µm. (C) Cyst epithelium with intracellular staining for Kim-1. Bar: 10 µm. (D) Kim-1 expressing tubules are clustered around cysts (arrows). Counter-staining with monoclonal anti-E-cadherin antibody and anti-mouse FITC antibody. Bar: 50µm.

Fig. 5. Expression of a proximal and a distal segmental marker in Kim-1 positive cysts. Kim-1 staining cysts were examined by immunofluorescence for aquaporin 1, which is expressed in the proximal tubule and aquaporin 2 which is expressed in the collecting duct. Secondary staining with anti-rabbit Cy3 antibody.
(A-C) A Kim-1 expressing cyst is derived from the proximal tubule. (A) Kim-1 expressing cyst. (B) Cyst epithelium stains positive for aquaporin 1. Nearby proximal tubules also express aquaporin 1. (C) No staining for aquaporin 2 is visible in the cyst lining epithelium. 2 adjacent collecting ducts stain positive for aquaporin 2.

(D-E) A Kim-1 positive cyst is derived from collecting duct. (D) Kim-1 staining cyst. (E) Staining for aquaporin 1 is negative in the cyst epithelium. The surrounding proximal tubules stain positive for aquaporin 1. (C) Staining for aquaporin 2 in the cyst lining epithelium is positive. No surrounding collecting ducts are seen in the visualized portion of the cortex. Bars: 50 µm. (G) Kim-1 expression in pericystic tubules is apical and sometimes lateral. Bar: 10µm.

(H,I) Kim-1 staining in kidneys from Pkd2<sup>WS25/−</sup> mice in proximal tubules occurs in subsets of tubular cells and differs from postobstructive kidneys. (H) The staining pattern for Kim-1 in individual tubules is discontinuous, frequently involving less than half of the cells in an individual tubule (arrows). Bar 10µm. (I) A wild-type mouse was subjected to unilateral ureteral obstruction for the duration of 24h. The kidneys were harvested by perfusion with PLP fixative solution 48 h after the release of obstruction. R9 staining involves all cells of Kim-1 expressing tubules. Bar: 10µm.

Fig. 6. Kim-1, Na,K,-ATPase, E-cadherin, and villin expression in tubules of cystic kidneys from Pkd2<sup>WS25/−</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 in red. (B) Staining for Na,K-ATPase in green demonstrates individual cells which have strongly reduced staining of Na,K-ATPase at the basal and lateral membrane (arrows) (C) The combined image shows that the cells which express Kim-1 have greatly diminished Na,K-ATPase expression at the basal and lateral membrane (arrows). Bars: 10 µm.

(D-F) Another example of reduced amounts and complexity of Na,K-ATPase in Kim-1 expressing tubule cells. (D) Half of the cells of this tubule stain for Kim-1. (E) the cells in the upper half of the tubule show a strong reduction in the complexity and quantity of staining for Na,K-ATPase. (F) in the combined image reduced expression of Na,K-ATPase in individual cells coincides with apical Kim-1 expression. Bars: 10 µm.

(G,H) Apical villin staining is preserved in Kim-1 expressing proximal tubules. (G) a 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 brushborder, but not in the basolateral membrane as has been described after ischemia/reperfusion (2). Bars: 10 µm.

(I-L) Lateral E-cadherin staining is preserved in Kim-1 expressing proximal tubular cells in adjacent sections to (G) and (H). (I) Kim-1 expressing proximal tubule showing apical
staining in red (K). E-cadherin in green localizes to the lateral membranes. (L) in the combined image staining for anti-E-cadherin in the lateral membrane is preserved in the Kim-1 expressing tubule. Bars: 10 µm.

Fig. 7. Expression of Kim-1, Na,K,-ATPase and E-cadherin in Pkd2^{WS25/-} kidney cysts. Sections were stained by indirect immunofluorescence with polyclonal anti-Kim-1 (R9), and monoclonal anti-Na,K-ATPase (6F), and monoclonal anti-E-cadherin antibodies. Secondary antibodies were Cy3-coupled anti-rabbit and FITC-coupled anti-mouse antibodies. The pattern of Na-,K-ATPase differs between cysts with apical vs. intracellular expression of Kim-1. (A-C) Cyst epithelia with apical expression of Kim-1 have preserved basolateral staining for Na,K-ATPase. (A) Cyst epithelium expressing Kim-1 at the apical membrane in red. (B) Na,K-ATPase staining in green is mostly lateral. (C) In the combined image Na-K-ATPase staining is localized to the lateral membrane of a Kim-1 expressing cyst epithelium.

(D-F) Cyst epithelia with cytoplasmic Kim-1 expression lack an organized pattern of Na-K-ATPase staining. (D) cyst with cytoplasmic staining for Kim-1. (E) Staining for Na-K-ATPase in green is diffuse. (F) No organized pattern of Na,K-ATPase expression is seen in this cyst with cytoplasmic Kim-1 expression.

(G-I) Kim-1 expressing epithelia in cysts with a cytoplasmic pattern of Kim-1 expression have preserved lateral staining for E-cadherin. (G) A Kim-1 expressing cyst epithelium is shown in red. (H) The same section using a FITC filter shows E-cadherin staining in the lateral
membranes in green. (I) the combined image shows preserved expression of E-cadherin in the lateral membrane of a cyst epithelium with cytoplasmic Kim-1 expression. Bars: 10 µm.

Fig. 8. Kim-1 and PCNA staining in Pkd2^{WS25/-} 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,E), relative to areas of absent Kim-1 staining (B,D,F). (A) clusters of tubules near a cyst (*) stain positive for apical Kim-1 expression in red. (B) In a different area adjacent to a cyst no tubular staining for Kim-1 is observed. (C) A large number of PCNA positive nuclei in green is present in the interstitium, as well as in some tubules. (D) PCNA staining shows markedly fewer proliferating nuclei (arrows) compared to (C). (E) In the combined image the proliferating cells in the interstitium and the tubules are near Kim-1 expressing tubules. (F) the combined image shows that the absence of Kim-1 expression in tubules coincides with a markedly reduced proliferative activity in the interstitium. Bars: 50 µm.

Fig. 9. Kim-1 and smooth muscle α-actin staining in Pkd2^{WS25/-} kidneys. Sections were stained with polyclonal anti-Kim-1 (R9) and monoclonal anti-smooth muscle α-actin antibodies respectively. Secondary antibodies were Cy3-coupled anti rabbit and FITC-coupled anti-mouse
antibodies. Clusters of Kim-1 expressing proximal tubules adjacent to cysts are seen in areas of interstitial myofibroblast activity, as indicated by the presence of smooth muscle α-actin (A,C,E). No interstitial smooth muscle α-actin expression is seen in Kim-1 negative areas (B,D,F). (A) clusters of tubules near a cyst (*) stain positive for apical Kim-1 in red. (B) a different area adjacent to the same cyst does not show Kim-1 expression in surrounding tubules. (C) smooth muscle α-actin in green is present in the interstitium. (D) staining for smooth muscle α-actin is strong in arteries and present only in minimal amounts in the interstitium. (E) The combined image demonstrates that smooth muscle α-actin is present in the interstitium around Kim-1 expressing tubules. (F) The combined image demonstrates the absence of Kim-1 expression and interstitial smooth muscle α-actin. Bars: 50µm.
Fig. 1

mouse Kim-1
rat KIM-1


mouse Kim-1
rat KIM-1


mouse Kim-1
rat KIM-1


mouse Kim-1
rat KIM-1


mouse Kim-1
rat KIM-1


mouse Kim-1
rat KIM-1

Fig. 2

<table>
<thead>
<tr>
<th>Cos cells</th>
<th>post ischemic</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ mouse Kim-1</td>
<td>+ mouse kidney</td>
</tr>
</tbody>
</table>

- 130 kDa
- 90
- 64
- 53
- 37
Fig. 3

WT       Pkd2       Post-ischemic WT mouse
WS25/-

Kim-1

P38