Kidney International, Vol. 65 (2004), pp. 2212–2222

Matrilysin (MMP-7) expression in renal tubular damage: Association with *Wnt4*

KAMESWARAN SURENDRAN, THEODORE C. SIMON, HELEN LIAPIS, and JOHN K. MCGUIRE

Department of Pediatrics, Division of Biology and Biomedical Sciences, and Department of Pathology and Immunology, Washington University School of Medicine; and Saint Louis Children's Hospital, Saint Louis, Missouri

Matrilysin (MMP-7) expression in renal tubular damage: Association with *Wnt4*.

Background. Matrilysin, a secreted matrix metalloproteinase and target gene of Wnt signaling, functions in epithelial repair and host defense, but no role in renal injury has been described.

Methods. Matrilysin expression was assessed in human kidney specimens by immunohistochemistry, and in experimental renal injury in mice by immunohistochemistry, Northern blotting, and RNase protection assays (RPA). A relationship to *Wnt4*, which is also induced in renal injury, was determined by RPA and in situ hybridization.

Results. Matrilysin was not detected in the normal human renal tubular epithelium by immunohistochemistry. However, prominent staining was detected in sections from autosomal-dominant polycystic kidney disease in the cyst lining epithelium, atrophic tubules, and cyst micropolyps, and from hydronephrosis in dilated and atrophic tubules. Matrilysin expression was also induced by acute folic acid nephropathy and unilateral ureteral obstruction (UUO) in the mouse, and expression increased as acute injury progressed to tubulointerstitial fibrosis. Matrilysin staining was primarily localized to epithelium of distal tubule/collecting duct origin in both human and murine renal disease. Wnt signaling can induce matrilysin expression, and we found that the pattern of matrilysin expression during progression of renal fibrosis in the mouse after UUO or folic acid nephropathy, and in the *jck* model of murine polycystic kidney disease, closely paralleled that of *Wnt4*.

Conclusion. These observations suggest that matrilysin may have a role in renal tubular injury and progression of tubulointerstitial fibrosis, and that *Wnt4* may regulate matrilysin expression in the kidney.

The renal epithelium is damaged in various pathologic processes, including acute tubular necrosis, obstructive uropathy, and cystic degeneration. The initial response to tubular injury is a regenerative process to restore epithelial structure and functional integrity that is characterized by transient de-differentiation of tubular epithelial cells, migration and proliferation, and re-differentiation into an intact epithelium [1]. Renal tubular injury also stimulates inflammation and interstitial fibrosis. If repair is incomplete, progressive interstitial fibrosis leads to disruption of tubulointerstitial architecture and loss of renal tubular function [2]. Both repair and fibrosis involve deposition and turnover of extracellular matrix (ECM), and it has been proposed that the matrix accumulation seen in fibrosis results from increased deposition coupled with decreased turnover [3]. Although several proteolytic enzymes have been suggested to be involved in regulating renal responses to injury, the functions of specific proteinases in the kidney remain largely unknown.

The matrix metalloproteinases (MMPs) are a family of zinc-containing enzymes with proteolytic activity against a wide range of extracellular proteins [4]. MMP expression is typically limited to tissue remodeling associated with normal and abnormal biological processes, such as development, involution, inflammation, tumor growth, and repair. Although MMPs are expressed in normal and injured kidneys, defining roles for specific MMPs has been difficult as the levels of individual enzymes have been reported to be increased, decreased, or unchanged, depending on the disease model [3]. Although matrilysin (MMP-7) may be expressed at low levels by noninjured, noninflamed mucosal epithelia in many adult human tissues [5, 6], its expression is increased in injured epithelium of gastrointestinal tract and lung [7, 8]. For example, matrilysin is prominently expressed by airway and alveolar epithelium in a variety of human lung diseases, such as cystic fibrosis and pulmonary fibrosis [8–11]. Although first isolated from cultured mesangial cells [12], matrilysin expression has not been reported in the normal or diseased kidney.

Wnt-4, a secreted glycoprotein required for nephrogenesis and normal renal tubule development [13, 14], is expressed in the normal mouse adult kidney only in the most distal papillary collecting duct epithelium, but is induced throughout the collecting duct epithelium

Key words: matrilysin, renal tubular damage, Wnt-4, fibrosis.

Received for publication September 13, 2003
and in revised form October 22, 2003, and December 12, 2003
Accepted for publication January 9, 2004

and in surrounding interstitial cells after renal injury [15]. Activation of the canonical Wnt signaling pathway leads to translocation of cytosolic β-catenin to the nucleus, where it binds to transcription factors of the lymphocyte enhancer-binding factor-1/T-cell factor pathway (Lef-1/TCF) to regulate expression of target genes, including matrilysin [16, 17]. Because *Wnt4* is activated in tubulointerstitial disruption leading to renal fibrosis, and can stimulate the β-catenin pathway [15], and matrilysin is a target of the Wnt/β-catenin/TCF pathway, we assessed matrilysin expression in human renal tubular disease and in mouse models of experimental renal tubular injury.

METHODS

Immunohistochemistry

Blocks of formalin-fixed, paraffin-embedded normal and diseased human kidney tissue were obtained from the Department of Pathology, Washington University School of Medicine. Specimens were identified by the diagnosis recorded in the archive and included normal adult kidney ($N = 3$), autosomal-dominant polycystic kidney disease ($N = 6$), hydronephrosis with fibrosis ($N = 4$), and autosomal-recessive polycystic kidney disease ($N = 2$). Sections were stained for matrilysin as described [8]. Polyclonal antibodies against the catalytic domain of human matrilysin [6] and full-length mouse matrilysin [18] were previously generated in our laboratory. For mouse tissues, 6-μm frozen sections were air dried, fixed in acetone for 10 minutes at −20°C and methanol for 6 minutes at −20°C, blocked for 1 hour at room temperature with TBST (25 mmol/L Tris-HCL, pH 7.5, 150 mmol/L NaCl, 0.1% Tween 20) containing 1% bovine serum albumin (BSA) and 2% goat serum, and then incubated with rabbit antimouse matrilysin antiserum for 1 hour at 37°C, followed by incubation with secondary antibodies for 1 hour at 37°C. Alexa 488-conjugated antirabbit immunoglobulin (Ig)G (Molecular Probes, Eugene, OR, USA) was used at the manufacturer's recommended concentrations. For folic acid treated mice, frozen sections were fixed in Bouin's fluid and methanol, and endogenous peroxidases were quenched with 0.6% H_2O_2 in methanol, and blocked as above. After incubation with primary antibody, staining was visualized with a Vectastain *Elite* kit (Vector Laboratories, Burlingame, CA, USA) and True Blue substrate (KPL, Gaithersburg, MD, USA). For lectin staining, fixed, rehydrated sections were incubated with rhodamine-conjugated peanut agglutinin (arachis hypogaea) (Vector Laboratories) or fluorescein isothiocyanate (FITC)-conjugated winged pea (lotus tetragonolobus) lectin (Vector Laboratories) at 10 μg/mL for 30 minutes at 37°C. Indirect immunofluorescence and confocal laser-scanning microscopy were used to image fluorescent markers.

Mouse renal damage models

The Washington University Institutional Animal Care and Use Committee approved all animal experiments. Unilateral ureteral obstruction (UUO) was performed on anesthetized C57Bl/6J or FVB/N mice of 6 to 10 weeks of age by surgical cautery of the left ureter approximately 15 mm from the renal pelvis. Obstructed kidneys were harvested at 1, 2, 4, 5, 7, 14, or 28 days after obstruction. Uninjured kidneys were harvested from seven-week-old mice. Kidneys were snap-frozen in liquid nitrogen for RNA isolation with an RNeasy kit (Qiagen, Valencia, CA, USA) or frozen in OCT medium for in situ hybridization. For Northern analysis, total kidney tissue was extracted with RNA STAT-60 (Tel-test, Friendswood, TX, USA) according to manufacturer's protocol.

Mice homozygous for the juvenile cystic kidney (*jck*) mutation on the C57Bl/6J background were obtained from Jackson Laboratory (Bar Harbor, ME, USA). Kidneys from sixteen-week-old *jck* mice were used for in situ hybridization.

Folic acid nephropathy was induced by intraperitoneal injection of folic acid (250 mg/kg in 150 mmol/L sodium carbonate). All mice treated with this dose had a rise in serum creatinine of at least 2-fold above baseline at 48 hours, as assessed with creatinine kit 555-A (Sigma-Aldrich, St. Louis, MO, USA). Kidneys were harvested at 0, 24, 48, 72, and 96 hours, and at 6 and 16 days after folic acid treatment.

RNA analysis

For Northern analysis, total RNA (10 μg) was resolved by electrophoresis, transferred to Hybond+ nylon membranes (Amersham Pharmacia Biotech, Buckinghamshire, UK), and ultraviolet (UV) cross-linked. Equivalent loading and integrity of RNA was confirmed by ethidium bromide staining of 18S and 28S rRNA bands. Gel-purified cDNA probes for mouse matrilysin were labeled by random priming, hybridized to membranes overnight, and visualized by autoradiography and quantified using NIH Image software.

In situ hybridization was performed as described [15]. The template used to produce murine *Wnt4* riboprobes was a gift from Andy McMahon. The in situ *Wnt4* antisense riboprobe spans nucleotides 803 to 366 (Genbank NM009523). Specificity of the Wnt4 probe was confirmed by control experiments performed with a Wnt4 sense probe that did not hybridize to mouse tissues ([19] and data not shown). The murine *MMP7* template was subcloned from the Image Consortium EST clone AI316399. This clone was digested with XbaI and BalI and subcloned into EcoRV and XbaI sites of pZero2.1 (Invitrogen, Carlsbad, CA, USA). The *MMP7* antisense riboprobe for in situ and RPA spans nucleotides 326 to 699 (Genbank L36244).

For ribonuclease protection assays (RPA), radiolabeled antisense riboprobes were transcribed using linearized cDNA templates, ^{32}P-α-UTP (Amersham Pharmacia Biotech), and the Promega (Madison, WI) in vitro transcription system. The *Wnt4* RPA riboprobe spans nucleotides 803 to 676 (Genbank NM009523). The mouse cyclophilin A gene template was purchased from Ambion (Austin, TX, USA). Five μg of RNA from each sample was used for RPA as described [15], with one modification: following overnight hybridization at 45°C, 300 μL of RNAse digestion buffer [25 μg RNase A (Roche, Indianapolis, IN, USA); 250 U RNAse T1 (Roche); 10 mmol/L TRIS, pH 7.5; 5 mmol/L EDTA, pH 8.0; 350 mmol/L NaCl] was added to each reaction, followed by incubation at 37°C for 30 minutes. Radioactivity was visualized with a Molecular Dynamics storage phosphorimaging system and quantified using NIH Image. At each time point, at least three obstructed kidneys were analyzed by RPA, and at least two obstructed kidneys were examined by in situ hybridization.

For semiquantitative reverse transcriptase-polymerase chain reaction (RT-PCR) analysis, 1 μg of total kidney RNA was reverse transcribed with random primers using Sigma Enhanced Avian RT kit followed by PCR according to manufacturer's protocol. The primers used for specific PCR amplification were as follows: For mouse matrilysin: forward, 5′-ACAGGTGCAGCTCAGGAAGG-3′; reverse, 5′-GTGAAGGACGCAGGAGTGAAC-3′ (PCR product 292 bp); for *Wnt4*: forward, 5′-AGCCGGGCACTCATGAATCTTCACAACA-ACGAGG-3′; reverse, 5′-TCATCTGTATGTGGCTTGAACTGTGCATTCCGAG-3′ (PCR product 260 bp); for β-actin: forward, 5′-TTTTCCAGCCTTCCTTCTTGGGTATGGAA-TCCTGTG-3′, reverse, 5′- ATCTGCTGGAAGGTGGACAGTGAGGCCAGGATGGAG-3′ (PCR product 283 bp). The primers for each gene were designed from different exons so as to distinguish between products made by genomic DNA amplification versus cDNA amplification, and control PCR reactions were done without template cDNA (data not shown). PCR products were resolved on agarose gels, stained with ethidium bromide, and recorded using a Kodak EDAS 290 gel imaging system (Rochester, NY, USA).

RESULTS

Matrilysin expression in human renal tubular disease

Matrilysin expression in human renal tubular disease was assessed by immunohistochemistry. Matrilysin protein was not detected in normal adult human kidney (Fig. 1A). In samples of autosomal-dominant polycystic kidney disease (ADPKD), prominent staining for matrilysin was detected in cyst-lining epithelium and atrophic tubules (Fig. 1B and D). Lectin staining with peanut agglutinin, a marker for distal tubule/collecting duct epithelium [20], showed that matrilysin protein was detected only in epithelium of distal nephron origin (Fig. 1C). Staining of serial sections with lotus tetragonolobus lectin, a proximal tubule marker, did not overlap with matrilysin staining (data not shown). In atrophic tubules, matrilysin staining was primarily associated with the apical/lumenal surface of the epithelium and with intralumenal proteinaceous debris (Fig. 1B and E). In cyst-lining epithelium, matrilysin was diffuse throughout the cytoplasm (Fig. 1D). Matrilysin staining was also seen in the epithelium of micropolyp outgrowths from the cyst wall (Fig. 1F). In samples of hydronephrosis, matrilysin staining was seen in atrophic tubules and in cells lining some dilated tubules (Fig. 1G and H). As in ADPKD, matrilysin staining in atrophic tubules was primarily apical/lumenal. No immunoreactivity was seen in specimens processed with control rabbit IgG (Fig. 1I). Matrilysin was not detected in samples of autosomal-recessive polycystic kidney disease (ARPKD) (data not shown). As in other tissues, matrilysin immunostaining was primarily restricted to epithelial cells, and was not seen in interstitial cells or inflammatory cells.

Matrilysin expression in experimental acute renal tubular injury

Mouse models of renal tubule injury were used to determine if induction of matrilysin expression is part of the epithelial response to injury in the kidney. Matrilysin mRNA expression was assessed by Northern analysis in folic acid nephropathy in the mouse, a model of acute renal tubular injury. A single intraperitoneal injection of folic acid (250 mg/kg) causes folate crystallization in the distal tubule, and induces an acute renal tubular injury characterized by extensive cell death within 24 hours, followed by cellular de-differentiation and proliferation peaking 48 to 72 hours after injection [21]. C57Bl6 mice were treated with folic acid and sacrificed at 0, 24, 48, 72, and 96 hours, and at 6 days after treatment. Matrilysin is not developmentally expressed in the mouse kidney [5], and only low levels of matrilysin mRNA were detected in normal, uninjured adult mouse kidney (Fig. 2A). Matrilysin mRNA was detected as early as 24 hours after folic acid injection and continued to increase 72 to 96 hours after treatment. In this model, some animals develop a more severe and progressive renal injury, with interstitial inflammation beginning at 4 days after injury [22], and tubulointerstitial fibrosis apparent at 7 to 14 days [15]. We found that in some animals, matrilysin mRNA was barely detectable 5 days after folic acid treatment, but in other animals, injury was progressive, and matrilysin expression continued to increase at 6 days after initial injury (Fig. 2A and B). Matrilysin induction was not detected in the calcium carbonate vehicle-injected animals. Although the extent of injury varies from animal to

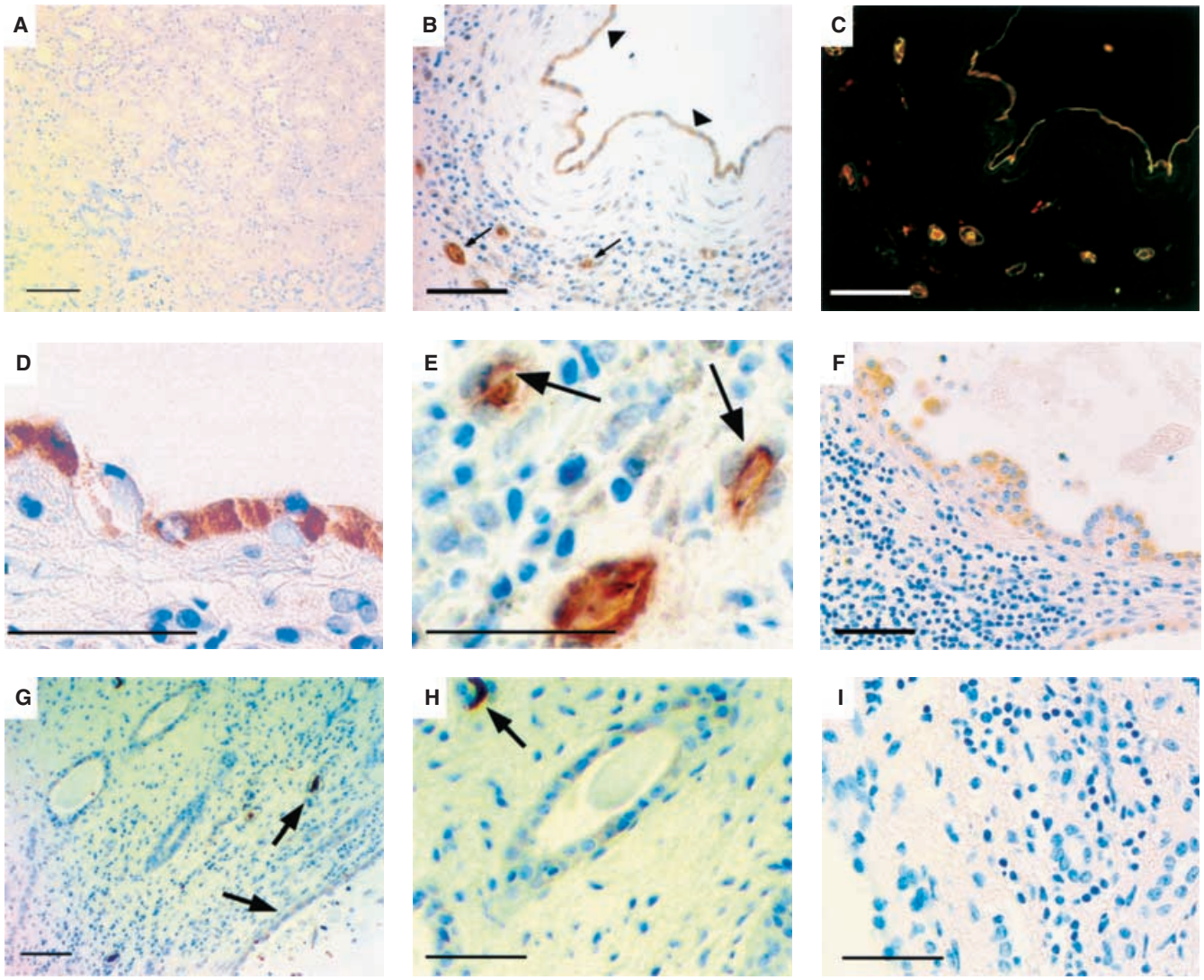

Fig. 1. Matrilysin expression in human renal tubular disease. Sections of human kidney were stained for matrilysin protein (peroxidase). (*A*) Matrilysin was not detected in normal adult human kidney tissue. In autosomal-dominant polycystic kidney disease (ADPKD), matrilysin was detected in (*B* and *D*) epithelial cells lining cysts (*arrowheads*), (B and *E*) atrophic tubules (*arrows*), and (*F*) cyst micropolyps. (*C*) Staining of serial section from (B) with peanut agglutinin (arachis hypogaea), a marker for distal tubule/collecting duct epithelium, showed that matrilysin staining was associated only with tubules of distal nephron origin. (*G* and *H*) In hydronephrosis, matrilysin staining was detected in cells lining dilated and atrophic tubules (*arrows*). Images in (E) and (H) are higher magnification images of sections in (B) and (G), respectively. (*I*) No staining is detected in ADPKD specimen incubated with rabbit IgG control. (Scale bars: A and G = 100 μm; others = 50 μm).

animal, matrilysin expression was consistently induced in all folic acid–treated animals by 48 hours. Furthermore, the degree of matrilysin mRNA induction correlated with the severity of renal tubular dysfunction as assessed by serum creatinine levels (Fig. 2C).

To determine the cellular source of matrilysin in the kidney, matrilysin protein expression in folic acid–injured kidneys was assessed by immunohistochemistry. Consistent with Northern data, matrilysin protein was not seen in normal adult mouse kidney (Fig. 3A). At 24 to 72 hours after folic acid injection, matrilysin protein was detected in the epithelium of dilated renal tubules primarily in the renal medulla and occasionally in the cortex (Fig. 3B and C). Similar to the matrilysin staining pattern in human kidney tissues, matrilysin staining was localized to the apical/luminal aspect of cells lining dilated tubules, proteinaceous debris within the tubule lumen, and also to the intracellular compartment. Peanut lectin staining indicated that matrilysin was expressed in epithelium of distal tubule/collecting duct origin (Fig. 3D to I). Because of the patchy nature of folic acid injury, not all of the peanut lectin–labeled tubules stain positive for matrilysin.

Because folic acid–induced renal injury is variable, matrilysin expression was evaluated after UUO, a more consistent and reproducible model of renal injury. UUO produces complete upper urinary tract obstruction,

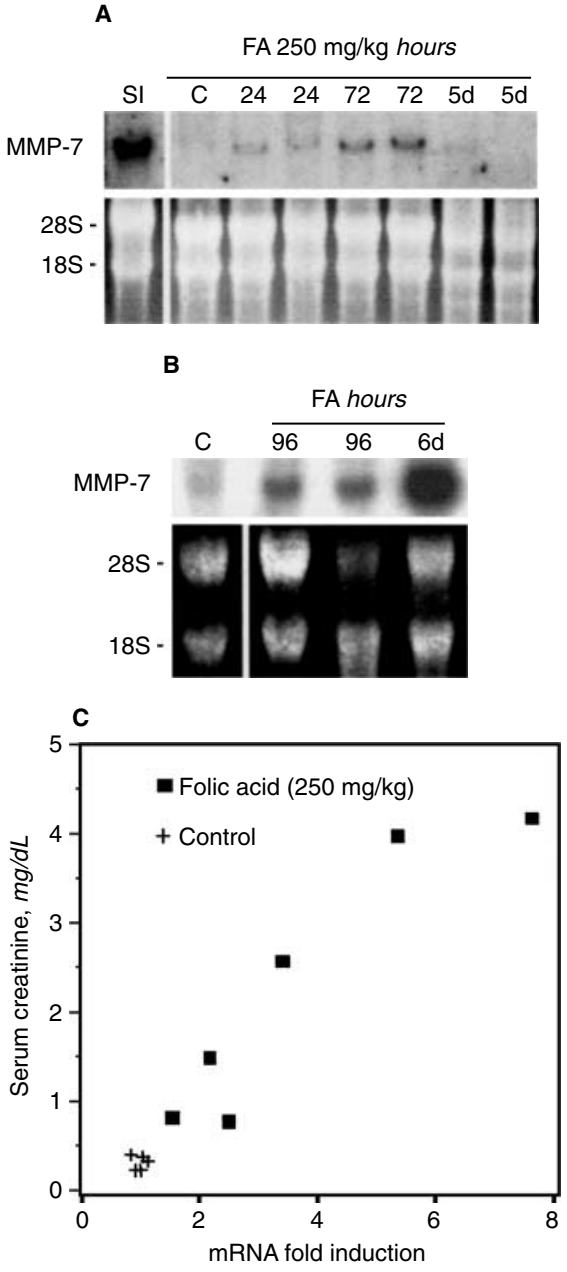

Fig. 2. Matrilysin expression is induced in acute folic acid nephropathy in the mouse. Northern analysis for matrilysin in total kidney RNA isolated from mice treated with intraperitoneal folic acid (250 mg/kg) in calcium carbonate vehicle. Ethidium bromide stained 18S and 28S rRNA are shown as a loading control. Mouse small intestine (SI) is shown as positive control. (*A*) Matrilysin mRNA is not detected in control vehicle treated animals, but is detected as early as 24 hours after folic acid treatment and peaks at 48 to 72 hours. By 5 days, matrilysin RNA is largely absent. (*B*) In some animals, a chronic injury develops, and matrilysin expression increases beyond 96 hours. (*C*) Relative matrilysin expression assessed by Northern blotting was quantified by densitometry and graphed in relation to serum creatinine. Severity of renal dysfunction is variable but matrilysin expression correlates with degree of creatinine elevation.

leading to an acute tubular injury followed by fibrosis involving the entire kidney. Induction of matrilysin expression was detected at 2 days after UUO and continued to increase at 5 days, when fibrosis begins (Fig. 4).

Association between matrilysin and *Wnt4*

Matrilysin is a target of the Wnt signaling pathway [17], and *Wnt4* is induced in kidneys after UUO and folic acid nephropathy in both C57Bl6 and FVB/N mice [15]. Therefore, the temporal relationship between matrilysin and *Wnt4* induction after UUO was assessed by RNase protection assay (RPA). FVB/N mice were subjected to UUO and sacrificed at 0, 4, 7, 14, and 28 days after obstruction. Consistent with the Northern data for C57Bl6 mice, RPA showed that matrilysin mRNA expression was induced by 4 days after injury. It then peaked at 14 days and remained elevated at 28 days (Fig. 5A). The time course of the increase in matrilysin expression paralleled closely that of the increase in *Wnt4* mRNA (Fig. 5B and C). Levels of cyclophilin A mRNA, which is ubiquitously expressed [23], remained constant. Because of the low level of detectable Wnt4 in whole kidney total RNA early after folic acid injury in C57Bl6 mice, we used semiquantitative RT-PCR, a more sensitive method, to assess *Wnt4* and matrilysin mRNA expression in the same animals at early time points. As reported by others, and as shown in Figure 2, the severity of renal injury is variable after folic acid treatment, and as predicted, the level of *Wnt4* and matrilysin mRNA expression varied between animals. However, the pattern of *Wnt4* induction correlated with that of matrilysin (Fig. 5D).

Wnt4 expression was localized in relation to matrilysin by in situ hybridization in samples of FVB/N mice. Consistent with previous findings, at 14 days after UUO, *Wnt4* was expressed in the inner medulla and in stripes that extend from the inner medulla into the cortex. *Wnt4* was also seen in the epithelium lining the dilated renal pelvis (Fig. 6A). The pattern of matrilysin expression was nearly identical to that of *Wnt4*, although the expression of matrilysin was not as diffuse as that for *Wnt4* mRNA in the renal papilla (Fig. 6B). Specificity of the matrilysin probe was confirmed by strong hybridization to small intestinal tissue in which matrilysin is highly expressed [5] and lack of hybridization to uninjured kidney (data not shown). Early after obstruction, *Wnt4* was expressed by tubular epithelial cells, but by 14 days after obstruction, expression was also seen in peritubular fibroblasts [15]. Consistent with the pattern of matrilysin expression seen in human kidney tissue specimens, and in murine kidneys after folic acid treatment, immunofluorescence microscopy for matrilysin at 14 days after UUO indicated that matrilysin was localized primarily to tubular epithelium (Fig. 6C and D).

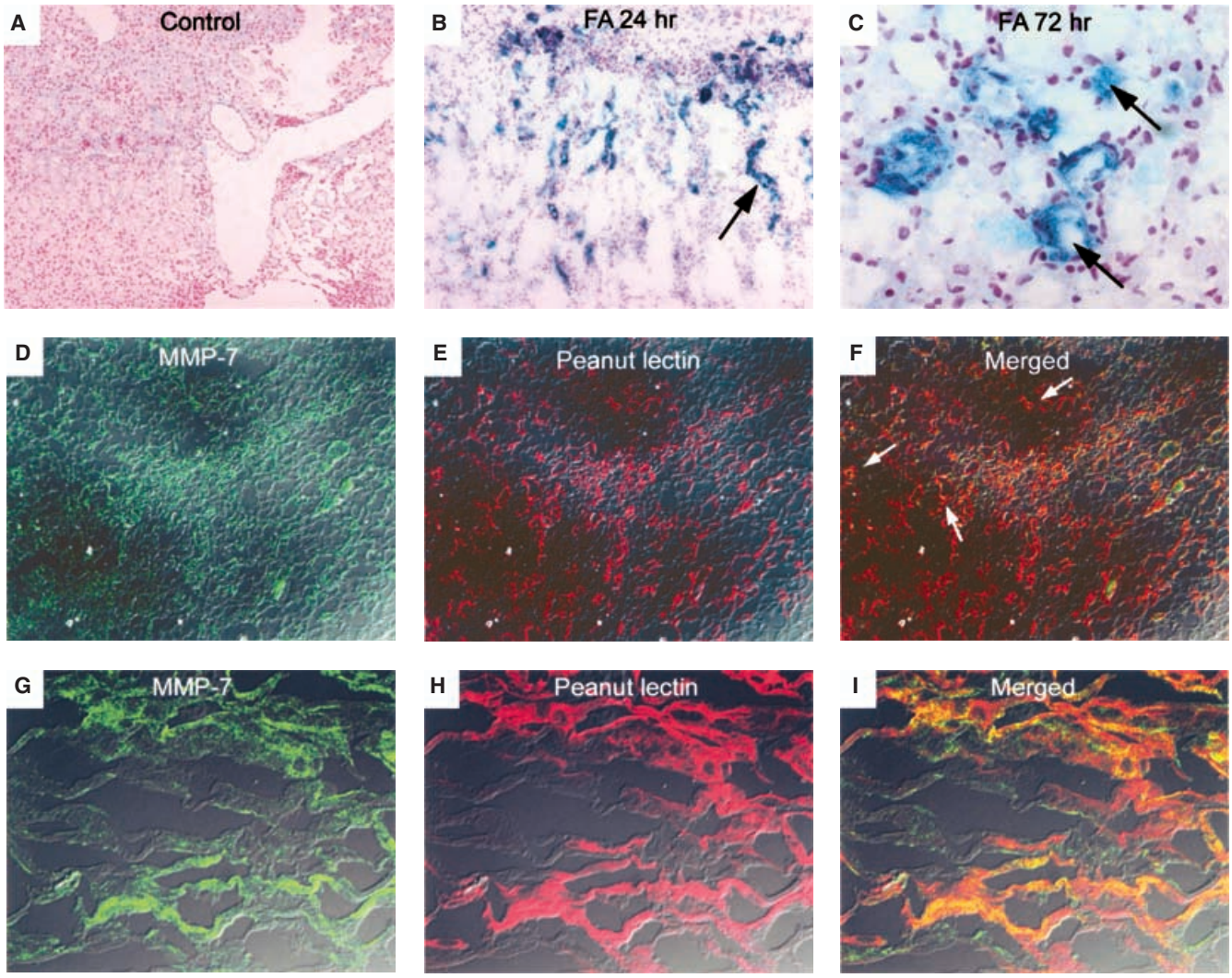

Fig. 3. Matrilysin localizes to distal nephron tubular epithelium in acute folic acid nephropathy. Sections of kidney from folic acid–treated mice were assessed for matrilysin protein expression by immunohistochemistry (peroxidase/True Blue). (*A*) Matrilysin protein was not detected in control vehicle-treated mouse kidney. (*B*) At 24 hours after folic acid treatment, matrilysin protein is detected in tubular epithelium. (*C*) Higher magnification of a 72-hour specimen shows matrilysin staining associated with luminal surface of tubule epithelium and proteinaceous debris within tubule (*arrows*). (*D–I*) Confocal immunofluorescence microscopy for matrilysin and peanut lectin (arachis hypogaea) superimposed with differential interference contrast (DIC) images in a 72-hour specimen showed that matrilysin staining was localized to epithelium of distal tubule/collecting duct origin (*arrows*). Original magnification: D–F, 10×, G–I, 40×.

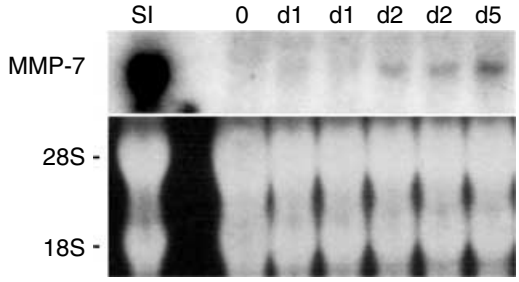

Fig. 4. Matrilysin expression is induced by acute unilateral ureteral obstruction (UUO) in the mouse and expression increases as obstruction continues. Northern blot analysis for matrilysin in total kidney RNA isolated from mice at 24 hours to 5 days after complete UUO of left kidney. Ethidium bromide stained 18S and 28S rRNA are shown as a loading control. Mouse small intestine (SI) is shown as positive control.

In mice that develop renal fibrosis after folic acid treatment, *Wnt4* expression peaks at 14 days after injury [15]. At 16 days after folic acid treatment, in situ hybridization for matrilysin and *Wnt4* identified a patchy pattern of mRNA expression consistent with the focal nature of folic acid injury (Fig. 6E). Similar to what was found for UUO, matrilysin mRNA was expressed in the same areas and in a nearly identical pattern as *Wnt4* after folic acid–induced renal fibrosis (Fig. 6F). Higher magnification views of serial sections of a kidney 16 days after folic acid treatment indicated that *Wnt4* was expressed mainly in interstitial cells adjacent to matrilysin expressing epithelial cells in damaged tubules (Fig. 6G and H). Collectively, these data indicate that *Wnt4* expression correlates

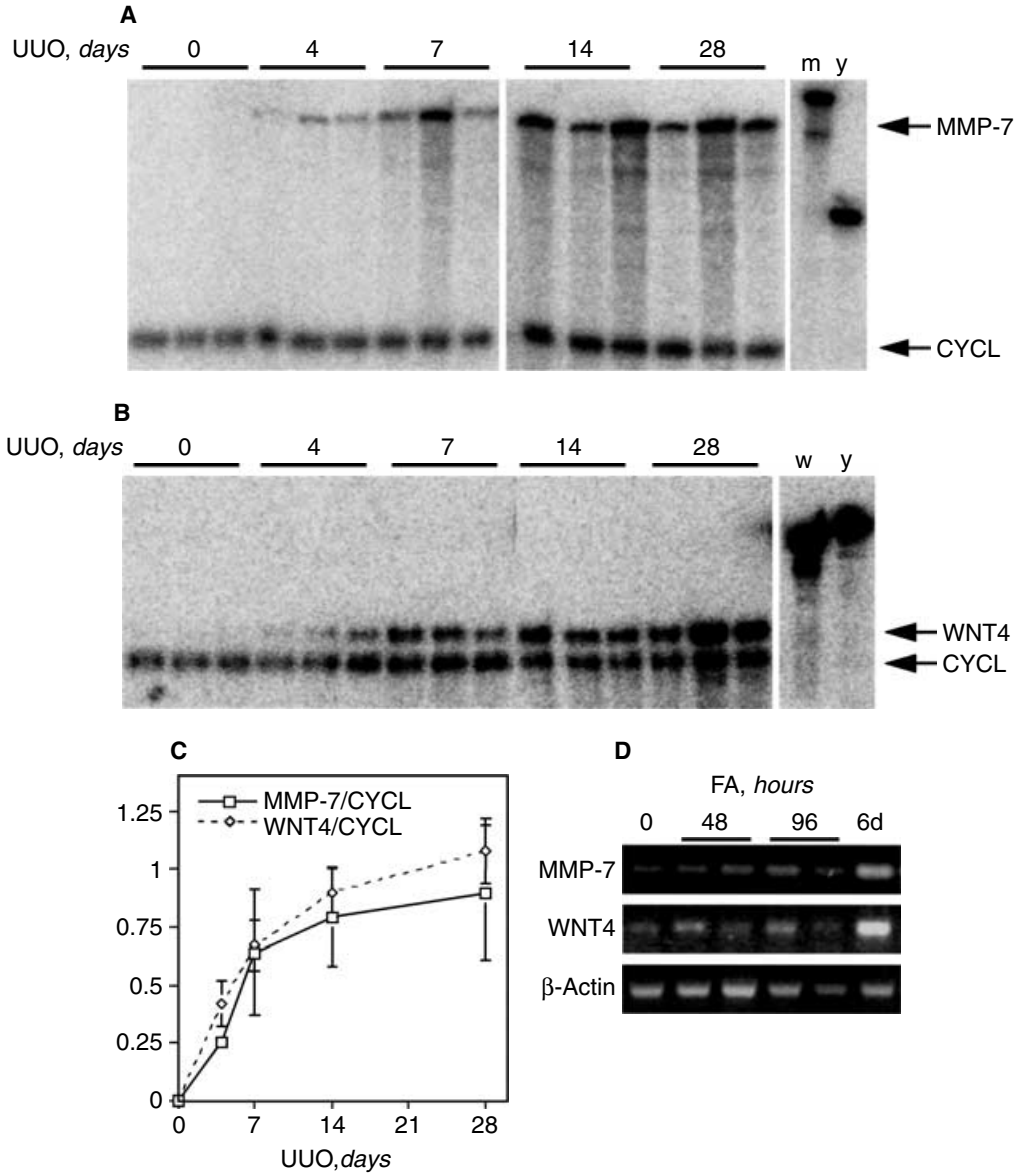

Fig. 5. Induction of matrilysin expression in relation to *Wnt4* after experimental renal tubular injury. Steady-state matrilysin and *Wnt4* mRNA levels were determined by RNAse protection assays (RPA) at various times after UUO. Five micrograms of total RNA extracted from the obstructed kidneys of FVB/N mice were used in the assays, and three separate animals were assayed at each time point. (*A*) *MMP-7* assay (*arrow*), (*B*) *Wnt4* assay (*arrow*). Both assays included probes for the ubiquitous cyclophilin A gene transcript as an internal control (arrow labeled CYCL). The undigested radiolabeled antisense riboprobes were included on each gel: *MMP-7* (A, *lane m*), *Wnt4* (B, *lane w*), and CYCL (A and B, *lane y*). Assays shown in the corresponding lanes of panels (A) and (B) were performed using the same RNA samples. (*C*) Graphical representation of the time course of matrilysin and *Wnt4* induction shown as ratios of the intensity of MMP-7 (*solid line*) or *Wnt4* (*dashed line*) signal to that of cyclophilin A (mean ratios ± SD). (*D*) RT-PCR for matrilysin (MMP-7), *Wnt4*, and β-actin performed on total RNA isolated from C57Bl6 mice up to 6 days after folic acid injury.

with that of matrilysin in two models of renal tubular injury in both strains of mice evaluated.

Cystic degeneration is characterized by renal tubular epithelial damage, interstitial fibrosis, and loss of renal function. Our previous studies found that *Wnt4* was activated in *jck* mice [15], in which spontaneous, progressive epithelial cysts develop [24]. Early in *jck* cyst formation *Wnt4* is located in cystic epithelium, with a later shift to pericystic interstitial cells [15]. In situ hybridization for *Wnt4* in kidney of a 16-week-old *jck* mouse showed that *Wnt4* localized to interstitial cells adjacent to cystic epithelium (Fig. 7A and D). In situ hybridization for matrilysin in a serial section showed that matrilysin mRNA was found only in the cyst-lining epithelium immediately adjacent to the *Wnt4* expressing cells (Fig. 7B and E). As was seen in kidneys from folic acid–treated mice, in kidneys of *jck* mice, *Wnt4* expression was seen primarily in cells adjacent to matrilysin expressing cells lining

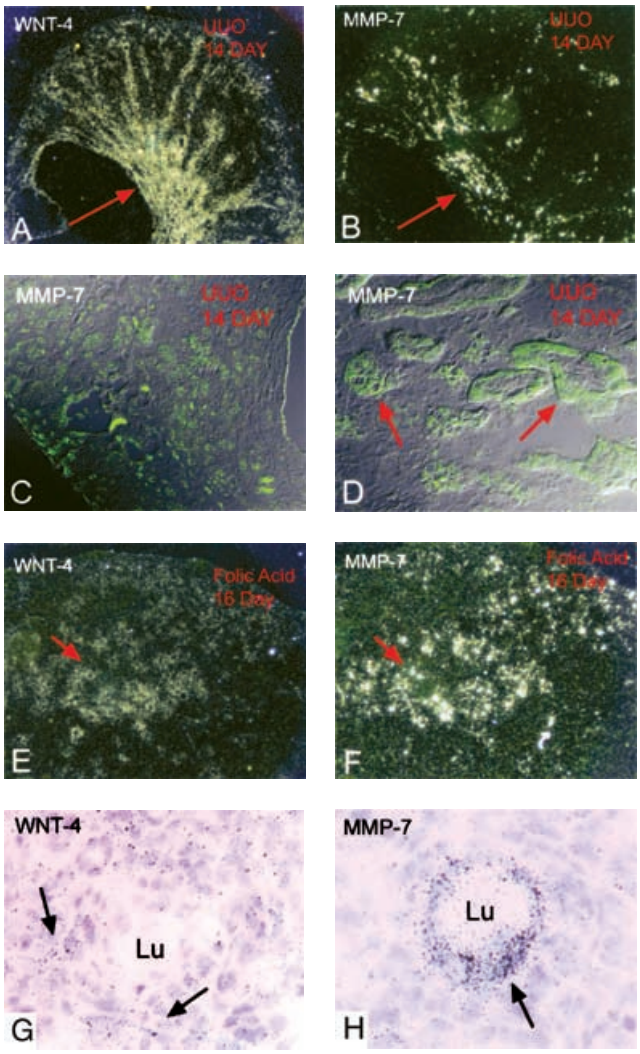

Fig. 6. *Wnt4* and matrilysin have similar spatial expression patterns after unilateral ureteral obstruction (UUO) and folic acid induced renal injury. RNA in situ hybridization was performed to compare the spatial expression patterns of *Wnt4* and matrilysin. Sections from kidneys of FVB/N mice two weeks after UUO (*A* and *B*) and 16 days after folic acid injection (*E* and *F*) were hybridized with antisense riboprobes specific for *Wnt4* (A and E) or matrilysin (B and F). Dark field images illustrate *Wnt4* mRNA expression and matrilysin mRNA expression in strikingly similar patterns on adjacent kidney sections at 14 days after UUO. Arrows identify the same inner medullary region containing both *Wnt4* and matrilysin mRNA transcripts. Panels (*C*) and (*D*) of an adjacent section show tubular epithelial expression of matrilysin protein (*green*) at 14 days after UUO (immunofluorescence superimposed with DIC). (E and F) illustrate that *Wnt4* and matrilysin have a strikingly similar pattern of mRNA expression after folic acid induced renal fibrosis. Arrows identify the same region containing both *Wnt4* and matrilysin mRNA transcripts. (G and H) are higher magnification views of serial sections from a kidney 16 days after folic acid injection showing *Wnt4* expression in interstitial cells adjacent to matrilysin expressing epithelial cells in a dilated tubule.

cysts and dilated tubules (Fig. 7F). Immunofluorescence also localized matrilysin protein to cyst-lining epithelial cells and dilated tubules (Fig. 7C). Thus, the pattern of matrilysin expression in this mouse model of cystic kidney disease was similar to that of matrilysin expression in human ADPKD.

DISCUSSION

The lack of matrilysin expression in uninjured kidneys in humans and mice, and induction after injury in three models of experimental renal tubular injury in two strains of mice, suggests that matrilysin functions in the epithelial responses to injury in the distal nephron. As in other human and mouse tissues [6, 8, 25], matrilysin expression was primarily limited to epithelial cells, and was not prominently expressed in interstitial or inflammatory cells. That matrilysin was not previously found in the kidney is not surprising. Other MMPs, such as gelatinases-A and -B (MMP-2 and -9) and stromelysin-1 (MMP-3), were identified in cultured tubules from *cpk* cystic mice kidneys [26] using gelatin zymography, which has low sensitivity for matrilysin [27]. Furthermore, matrilysin may be tightly bound to cell surface heparan sulfate proteoglycans [28], and would not likely be detected in cyst fluid or in the serum or plasma, as was reported for MMP-1 and MMP-9 in ADPKD [29].

Abnormal ECM accumulation is a common finding in renal fibrosis and cystic kidney disease [3], and altered matrix degradation may be a factor in renal fibrosis [30]. Consequently, expression of MMPs and their endogenous inhibitors, the tissue inhibitors of metalloproteinases (TIMPs), have been investigated in renal injury. MMP-2, -14 (MT1-MMP), -3, and -9, as well as TIMP-1 and -2 are variably found in human and animal models of renal tubular disease [3, 31, 32]. However, specific functions for individual MMPs have not been defined.

Studies in other systems provide insight into how matrilysin may regulate the cellular processes involved in fibrosis, including apoptosis, inflammation, chronic epithelial damage, and fibroblast migration and proliferation [3, 33]. During prostate involution and in pancreatic acinar to ductal metaplasia, matrilysin can regulate apoptosis by cleaving Fas ligand [34, 35]. Matrilysin induced by acute bleomycin lung injury in the mouse cleaves the ectodomain of syndecan-1 to shed syndecan/KC-1 complexes and generate a chemotactic gradient that directs neutrophils into the airspace [36]. In injured airways, matrilysin is secreted basolaterally to mediate shedding of E-cadherin and reorganization of intercellular junctions [11]. This is distinct from the constitutive, apical secretion seen in uninjured human airways and glandular epithelium [8], suggesting that matrilysin is likely targeted to different substrates in intact versus injured airway epithelium. Similarly, apical matrilysin localization in atrophic tubules and cytoplasmic expression in cyst lining epithelium suggests that matrilysin may have multiple substrates and functions in the kidney. Matrilysin can degrade many ECM proteins in vitro, including fibronectin

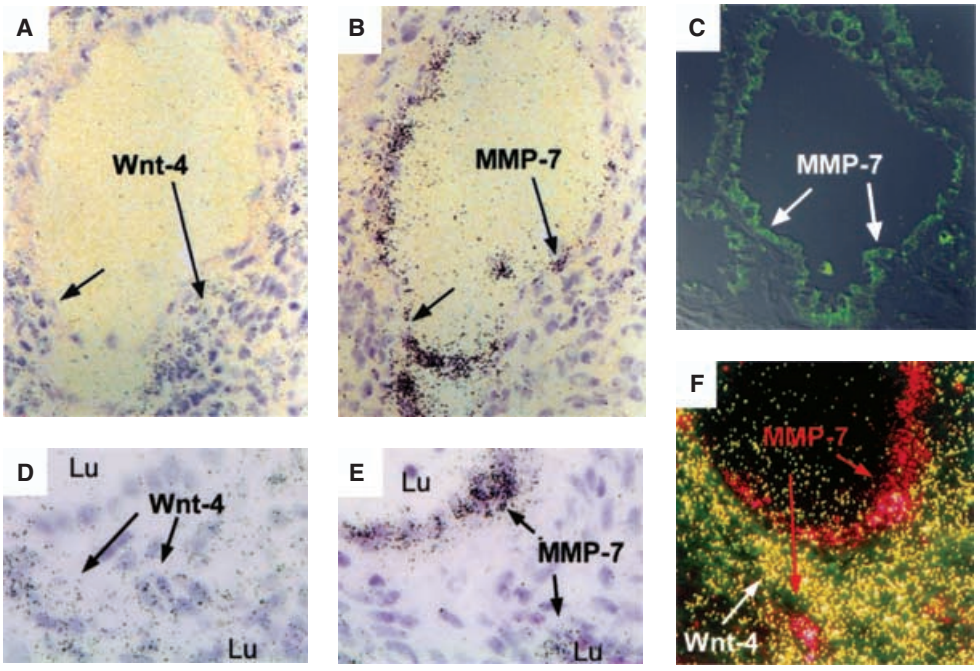

Fig. 7. In the kidneys of *jck* mice matrilysin expression is induced in the epithelial cells lining the cysts that are surrounded by *Wnt4* expressing interstitial cells. RNA in situ hybridization was performed to detect matrilysin and *Wnt4* expression patterns in 16-week-old *jck* kidneys. (*A*) and (*B*) are adjacent sections, and (*D*) and (*E*) are adjacent sections. (A) *Wnt4* mRNA expression is predominantly detected in the interstitial cells surrounding the tubular epithelial cysts in the *jck* mice. (B) Matrilysin (MMP-7) mRNA expression is detected predominantly in the epithelial cells lining the cysts. (C) Immunofluorescence for matrilysin protein is observed in the tubular epithelial cells lining the cysts (*arrows*). Higher magnification views of serial sections from a kidney from a 16-week-old *jck* mouse identify *Wnt4* expression (D) in interstitial cells adjacent to matrilysin expressing epithelial cells (E) surrounding the lumen (Lu) of medullary cysts. (*F*) Superimposed dark field images of serial sections from a kidney from a 16-week-old *jck* mouse with false color added for *Wnt 4* (*green*) and matrilysin (*red*) indicate that *Wnt4* and matrilysin are expressed in directly adjacent cells.

[27], a prominent component of the provisional matrix in injured kidneys [33]. However, MMPs are one of several classes of proteinases that mediate ectodomain shedding of transmembrane- and membrane-associated proteins [37], and therefore, secretion to the apical compartment suggests that the matrilysin substrate(s) in the kidney may include non-matrix proteins.

Although matrilysin activity is necessary for normal airway repair, matrilysin is also prominently expressed in the lung epithelium of patients with pulmonary fibrosis [8, 9], and in liver tissue of patients with chronic hepatitis C virus and hepatic fibrosis, in which matrilysin expression correlates with the extent of cirrhosis [38]. Increased matrilysin expression is also seen in experimental bleomycin lung injury in mice as acute injury progresses to fibrosis [36]. Similarly, we found matrilysin expression in human kidney diseases characterized by chronic renal tubular damage with fibrosis, and in mice that develop fibrosis after folic acid or UUO; matrilysin mRNA expression increases and persists as fibrosis progresses. Thus, one stimulus for persistent matrilysin expression may be the incomplete or impaired epithelial repair characteristic of the fibrogenic process.

Although expressed only at low levels in the normal adult mouse kidney, *Wnt4* and other developmentally regulated genes such as *Pax-2* may be re-expressed by experimental tubular injury [15, 39]. In contrast, matrilysin is not developmentally expressed in kidney or other organs in mice [5], and kidney development is normal in matrilysin-null mice [40]. Thus, our data suggest that the set of genes induced by Wnt-4 signaling in kidney injury is different from that induced during renal development. Although matrilysin is a target of the Wnt/β-catenin signaling pathway in colon cancer [17], a connection between Wnt signaling and matrilysin expression in the kidney has not been previously established. In our studies, the temporal and spatial expression pattern of matrilysin and *Wnt4* suggests that matrilysin expression in the distal nephron may be directly regulated by Wnt-4. *Wnt4* expression is induced predominantly in interstitial cells surrounding developing renal cysts in *jck* mice, and in cells surrounding collecting ducts in areas of tubulointerstitial fibrosis after folic acid injury or UUO [15]. Matrilysin expression in immediately adjacent epithelial cells suggests a paracrine effect of interstitial cell derived Wnt-4. That *Wnt4* expression is more broadly distributed than matrilysin may indicate that Wnt-4 alone is not sufficient for matrilysin induction, but rather that additional signals are necessary for matrilysin induction in injured tissue. Indeed, both β-catenin and the PEA3 subfamily of Ets

transcription factor signals are necessary for activation of the matrilysin promoter in vitro [41].

We recently identified that the gene for C-type natriuretic peptide (CNP), one of a family of related peptides that regulate fluid and electrolyte homeostasis [43], is a target of Wnt-4 in renal development and after renal tubular injury [42]. CNP may also limit fibrosis as exogenous CNP inhibits hepatic myofibroblastic stellate cell and fibroblast proliferation in vitro [44, 45], and prevents arterial neointimal fibrosis and glomerular fibrosis in vivo [46–48]. Thus, Wnt-4 may function to limit fibrosis by regulating CNP expression in renal interstitial fibroblasts and promote re-epithelialization by inducing matrilysin expression in tubular epithelium. However, a chronic injury such as UUO may provide a stimulus for continued *Wnt4* expression that may then induce ongoing matrilysin expression. It will be important to evaluate patterns of *Wnt4* and matrilysin expression in less severe models of renal tubular damage, such as reversible UUO [49].

CONCLUSION

Matrilysin is expressed in human renal tubular disease and in mouse models of acute renal tubular injury and fibrosis, and matrilysin expression temporally and spatially correlates with that of *Wnt4*. We propose that matrilysin may initially function in tubular epithelial repair, but that in chronic tubular injury, *Wnt4* stimulates persistent matrilysin expression that may then contribute to renal tubular pathology. We are now exploring how *Wnt4* and matrilysin may regulate progression of renal fibrosis.

NOTE ADDED IN PROOF

Consistent with our findings of matrilysin protein expression in human samples of hydronephrosis, Henger et al recently reported increased expression of matrilysin transcripts in human kidney RNA samples from patients with hydronephrosis and tubulointerstitial inflammation (*Kidney Int* 65:904–917, 2004).

ACKNOWLEDGMENTS

We thank Timothy Birkland, Yolanda Sanchéz López-Boado, William C. Parks, and Carole Wilson for helpful discussions; and Mary Baumann, Teresa Tolley, Hui-Qing Yin, and Darlene Stewart for technical support. This work was supported by grants HL29594, HL56419, HL 68780, and a grant from the Monsanto-Searle Biomedical Program.

Reprint requests to John K. McGuire, M.D., Washington University School of Medicine, Pediatrics, Box 8208, 660 South Euclid, St. Louis, MO 63110.
E-mail: mcguire_j@kids.wustl.edu

REFERENCES

1. Humes HD: Renal tubule cell repair following acute renal injury. *Miner Electrolyte Metab* 21:353–365, 1995
2. Becker GJ, Hewitson TD: The role of tubulointersitial injury in chronic renal injury. *Curr Opin Nephrol Hypertens* 9:133–138, 2000
3. Eddy EA: Molecular insights into renal interstitial fibrosis. *J Am Soc Nephrol* 7:2495–2508, 1996
4. Brinckerhoff CE, Matrisian LM: Matrix metalloproteinases: A tail of a frog that became a prince. *Nature Rev: Mol Cell Biol* 3:207–214, 2002
5. Wilson CL, Heppner KJ, Rudolph LA, Matrisian LM: The metalloproteinase matrilysin is preferentially expressed by epithelial cells in a tissue-restricted pattern in the mouse. *Mol Biol Cell* 6:851–869, 1995
6. Saarialho-Kere UK, Crouch EC, Parks WC: Matrix metalloproteinase matrilysin is preferentially expressed in human exocrine epithelium. *J Invest Dermatol* 105:190–196, 1995
7. Saarialho-Kere UK, Vaalamo M, Karjalainen-Lindsberg K, *et al*: Enhanced expression of matrilysin, collagenase, and stromelysin-1 in gastrointestinal ulcers. *Am J Pathol* 148:519–526, 1996
8. Dunsmore SE, Saarialho-Kere UK, Roby JD, *et al*: Matrilysin expression and function in airway epithelium. *J Clin Invest* 102:1321–1331, 1998
9. Zuo F, Kaminski N, Eugui E, *et al*: Gene expression analysis reveals matrilysin as a key regulator of pulmonary fibrosis in mice and humans. *Proc Natl Acad Sci USA* 99:6292–6297, 2002
10. Cosgrove GP, Schwartz MI, Geraci MW, *et al*: Overexpression of matrix metalloproteinase-7 in pulmonary fibrosis. *Chest* 121:25S–26S, 2002
11. McGuire JK, Li QL, Parks WC: Matrilysin mediates E-cadherin ectodomain shedding in injured lung epithelium. *Am J Pathol* 162:1861–1843, 2003
12. Marcotte PA, Kozan IM, Dorwin SA, Ryan JM: The matrix metalloproteinase pump-1 catalyzes formation of low molecular weight (pro)urokinase in cultures of normal human kidney cells. *J Biol Chem* 267, 1992
13. Stark K, Vainio S, Vassileva G, McMahon SP: Epithelial transformation of metanephric mesenchyme in the developing kidney regulated by Wnt-4. *Nature* 372:679–683, 1994
14. Vainio S, Uusitalo MS: A road to kidney tubules via the Wnt pathway. *Pediatr Nephrol* 15:151–156, 2000
15. Surendran K, McCaul SP, Simon TC: A role for Wnt-4 in renal fibrosis. *Am J Physiol Renal Physiol* 282:F431–F441, 2002
16. Miller JR, Hocking AM, Brown JD, Moon RT: Mechanism and function of signal transduction by the Wnt/β-catenin and Wnt/Ca^{2+} pathways. *Oncogene* 18:7860–7872, 1999
17. Crawford HC, Fingleton BM, Rudolph-Owen LA, *et al*: The metalloproteinase matrilysin is a target of β-catenin transactivation in intestinal tumors. *Oncogene* 18:2883–2891, 1999
18. Wilson CL, Ouellette AJ, Satchell DP, *et al*: Regulation of intestinal α-defensin activation by the metalloproteinase matrilysin in innate host defense. *Science* 286:113–117, 1999
19. Noonan FC, Goodfellow PJ, Staloch LJ, *et al*: Antisense transcripts at the *EMX2* locus in human and mouse. *Genomics* 81:58–66, 2003
20. Davila RM, Kissane JM, Crouch EC: Multilocular renal cyst: Immunohistochemical and lectin-binding study. *Am J Surg Pathol* 16:508–514, 1992
21. Baserga R, Thatcher D, Marzi D: Cell proliferation in mouse kidney after a single injection of folic acid. *Lab Invest* 19:92–96, 1968
22. Schubert GE: Folic acid-induced acute renal failure in the rat: Morphological studies. *Kidney Int* 6:S46–S50, 1976
23. Otsuka M, Terada Y, Yang T, *et al*: Localization of cyclophilin A and cyclophilin C mRNA in murine kidney using RT-PCR. *Kidney Int* 45:1340–1345, 1994
24. Atala A, Freeman MR, Mandell J, Beier DR: Juvenile cystic kidneys (*jck*): A new mouse mutation which causes polycystic kidneys. *Kidney Int* 43:1081–1085, 1993
25. Lopez-Boado YS, Wilson CL, Parks WC: Regulation of matrilysin expression in airway epithelial cells by *Pseudomonas aeruginosa* flagellin. *J Biol Chem* 276:41417–41423, 2001
26. Rankin CA, Suzuki Y, Itoh Y, *et al*: Matrix metalloproteinases and TIMPS in cultured C57 BL/6-*cpk* kidney tubules. *Kidney Int* 50:835–844, 1996

27. Wilson CL, Matrisian LM: Matrilysin, in *Matrix Metalloproteinases*, edited by Parks WC, Mecham RP, San Diego, Academic Press, 1998, pp 149–184
28. Yu W-H, Woessner JF: Heparan sulfate proteoglycans as extracellular docking molecules for matrilysin (matrix metalloproteinase-7). *J Biol Chem* 275:4183–4191, 2000
29. Nakamura T, Ushiyama C, Suzuki S, *et al*: Elevation of serum levels of metalloproteinase-1, tissue inhibitor of metalloproteinase-1 and type IV collagen, and plasma levels of metalloproteinase-9 in polycystic kidney disease. *Am J Nephrol* 20:32–36, 2000
30. Gonzalez-Avila G, Vadillo-Ortega F, Perez-Tomayo R: Experimental diffuse interstitial fibrosis. A biochemical approach. *Lab Invest* 59:245–252, 1988
31. Obermuller N, Morente N, Kranzlin B, *et al*: A possible role for metalloproteinases in renal cyst development. *Am J Physiol Renal Physiol* 280:F540–F550, 2001
32. Norman JT, Gatti L, Wilson PD, Lewis M: Matrix metalloproteinases and tissue inhibitor of matrix metalloproteinases expression by tubular epithelia and interstitial fibroblasts in the normal kidney and in fibrosis. *Exp Nephrol* 3:88–89, 1995
33. Norman JT, Fine LG: Progressive renal disease: Fibroblasts, extracellular matrix, and integrins. *Exp Nephrol* 7:167–177, 1999
34. Crawford HC, Scoggins CR, Washington MK, *et al*: Matrix metalloproteinase-7 is expressed by pancreatic cancer precursors and regulates acinar-to-ductal metaplasia in exocrine pancreas. *J Clin Invest* 109:1437–1444, 2002
35. Powell WC, Fingleton B, Wilson CL, *et al*: The metalloproteinase matrilysin proteolytically generates active soluble Fas ligand and potentiates epithelial cell apoptosis. *Curr Biol* 9:1441–1447, 1999
36. Li QL, Park PY, Wilson CL, Parks WC: Matrilysin shedding of syndecan-1 regulates chemokine mobilization and transepithelial efflux of neutrophils in acute lung injury. *Cell* 111:635–646, 2002
37. Sbarba PD, Rovida E: Transmodulation of cell surface regulatory molecules via ectodomain shedding. *Biol Chem* 383:69–83, 2002
38. Lichtinghagen R, Michels D, Haberkorn CI, *et al*: Matrix metalloproteinase (MMP)-2, MMP-7, and tissue inhibitor of metalloproteinase-1 are closely related to the fibroproliferative process in the liver during chronic hepatitis C. *J Hepatol* 34:239–247, 2001
39. Imgrund M, Grone E, Grone H-J, *et al*: Re-expression of the developmental gene *Pax-2* during experimental acute tubular necrosis in mice. *Kidney Int* 56:1423–1431, 1999
40. Wilson CL, Heppner KJ, Labosky PA, *et al*: Intestinal tumorigenesis is suppressed in mice lacking the metalloproteinase matrilysin. *Proc Natl Acad Sci USA* 94:1402–1407, 1997
41. Crawford HC, Fingleton B, Gustavson MD, *et al*: The PEA3 subfamily of Ets transcription factors synergizes with beta-catenin-LEF-1 to activate matrilysin transcription in intestinal tumors. *Mol Cell Biol* 21:1370–1383, 2001
42. Surendran K, Simon TC: CNP gene expression is activated by Wnt signaling and correlates with *Wnt4* expression during renal injury. *Am J Physiol Renal Physiol* 284:F653–662, 2003
43. Rosenzweig A, Seidman CE: Atrial natriuretic factor and related peptide hormones. *Ann Rev Biochem* 60:229–255, 1991
44. Tao J, Mallat A, Gallois G, *et al*: Biological effects of C-type natriuretic peptide in human myofibroblastic hepatic stellate cells. *J Biol Chem* 174:23761–23769, 1999
45. Chrisman TD, Garbers DL: Reciprocal antagonism coordinates C-type natriuretic peptide and mitogen-signaling pathways in fibroblasts. *J Biol Chem* 274:4293–4299, 1999
46. Ueno H, Haruno A, Morisaki N, *et al*: Local expression of C-type natriuretic peptide markedly suppresses neointimal formation in rat injured arteries through an autocrine/paracrine loop. *Circulation* 96:2272–2279, 1997
47. Furuya M, Miyazaki T, Honbou N, *et al*: C-type natriuretic peptide inhibits thickening after vascular injury. *Ann N Y Acad Sci* 748:517–523, 1995
48. Canaan-Kuhl S, Ostendorf T, Zander K, *et al*: C-type natriuretic peptide inhibits mesangial cell proliferation and matrix accumulation in vivo. *Kidney Int* 53:1143–1151, 1998
49. Chevalier RL, Kim A, Thornhill BA, Wolstenholme JT: Recovery following relief of unilateral ureteral obstruction in the neonatal rat. *Kidney Int* 55:793–807, 1999