

Activation of the Extracellular-Signal Regulated Protein Kinase Pathway in Human Glomerulopathies

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Abstract. Examined was extracellular-signal regulated kinase (ERK) activation in normal human kidney ($n = 2$) and a cohort of glomerulopathies by immunohistochemistry staining for the dual-phosphorylated form of ERK (p-ERK). Cell proliferation was determined by expression of the proliferating cell nuclear antigen (PCNA). In normal human kidney, p-ERK was largely restricted to the cytoplasm of cells of the collecting duct (CD). In glomerulopathies, glomerular ERK activation was highly variable. However, there was colocalization of cell proliferation and ERK activation in the glomerular tuft and crescents. Tubular ERK activation in the different glomerulopathies was confined to the CD in areas with normal architecture. In contrast, ERK activation was prominent in tubules and interstitial cells in areas of tubulointerstitial damage. ERK activa-

tion was observed in glomerular and interstitial α -smooth muscle actin-positive myofibroblasts, but few macrophages or T cells showed ERK activation. There was a significant correlation between glomerular p-ERK+ and PCNA+ cells and between tubular p-ERK+ and PCNA+ cells. Glomerular p-ERK+ cells correlated with glomerular cellularity and the percentage of glomeruli with segmental lesions. Tubular p-ERK+ cells correlated with renal dysfunction and interstitial fibrosis and tubular atrophy. In conclusion, activation of the ERK pathway in human glomerulopathies correlates with cell proliferation, histologic lesions, and renal dysfunction. ERK activation may promote renal repair through tubular proliferation while promoting renal fibrosis via proliferation of glomerular and interstitial myofibroblasts.

Cell proliferation is an important feature in the development of renal disease. Proliferation of glomerular mesangial cells leads to mesangial expansion and glomerulosclerosis (1), whereas proliferation of myofibroblasts contributes to the development of interstitial fibrosis and the irreversible fibrous organization of glomerular crescents (2–4). In addition, local macrophage proliferation contributes to glomerular and interstitial inflammatory lesions and renal injury (5).

Many studies have examined the factors that drive pathologic cell proliferation in human and experimental renal disease. A number of growth factors have been identified that induce proliferation of glomerular mesangial cells and myofibroblasts in cell culture and animal disease models (6). However, the intracellular mechanisms by which these growth factors induce cells to enter the cell cycle remain poorly understood.

Activation of the extracellular-signal regulated kinase (ERK) pathway plays an important role in the response of

many cell types to extracellular stimuli, such as growth factors, cytokines, osmotic stress, stretch, reactive oxygen species, and high glucose levels (7). After binding of ligands, such as platelet-derived growth factor and angiotensin II, to their respective receptors on the cell surface, a cascade of phosphorylation events leads to dual phosphorylation of the Thr-Glu-Try motif in the two isoforms of ERK (ERK1 and ERK2). The dual-phosphorylated form of ERK (p-ERK) is then an active kinase and is able to phosphorylate a number of transcription factor targets and thus alter the pattern of gene transcription. The best characterized feature of this pathway is that it provides the link between the binding of a growth factor to its cell-surface receptor and movement of the cell from G_0 to the G_1 phase of the cell cycle (8). *In vitro* studies have identified an important role for the ERK pathway in the proliferation of cultured mesangial cells, tubular epithelial cells, and fibroblasts (9–13). Recently, we identified an association between ERK activation and proliferation of tubular epithelial cells and interstitial myofibroblasts in a time-course study of the obstructed rat kidney (14). Also, ERK activation has been identified in proliferating cystic tubules in rats with autosomal-dominant polycystic kidney disease (15). Furthermore, glomerular ERK activation occurs in the rat Thy-1 model of mesangioproliferative nephritis and blockade of the ERK pathway results in a significant reduction in mesangial cell proliferation in this disease model (16,17). However, nothing is known of ERK activation in normal adult human kidney or human renal disease.

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The aims of the study presented here were to identify the cell types in which the ERK pathway is activated in a variety of human glomerulopathies and to determine whether ERK activation is associated with cell proliferation, histologic damage, and renal dysfunction.

Materials and Methods

Patients

Forty patients undergoing diagnostic renal biopsy at Monash Medical Centre were examined. Patients gave informed consent, and this study was approved by the Monash Medical Centre Human Ethics and Research Committee. Disease categories were based on histologic examination of biopsy specimens. Details of the patients are summarized in Table 1. The tissue of two patients who underwent biopsy for minimal proteinuria (<0.26 g/d) and hematuria ($<25 \times 10^3$ /L urinary glomerular red cells) was designated as normal upon microscopic examination, and these were used as normal controls. Serum creatinine, creatinine clearance, and 24-h protein excretion were determined at the time of biopsy with assays performed by the Department of Biochemistry, Monash Medical Centre. In addition, renal tissue from the uninvolved pole of two carcinoma nephrectomy specimens were analyzed by Western blot test.

Antibodies

Antibodies used in this study were rabbit antibody against the dual phosphorylated forms of ERK1 and ERK2 (Cell Signaling Technology, Beverly, MA); K-23, a rabbit antibody recognizing ERK2 and, to a lesser extent, ERK1 (Santa Cruz Biotechnology, Santa Cruz, CA); PC 10, antiproliferating cell nuclear antigen (PCNA), which recognizes cells in G1, S, and G2 phases of cell cycle (Dako, Glostrup, Denmark); 1A4, anti- α -smooth muscle actin (α -SMA; Sigma-Aldrich, Castle Hill, New South Wales, Australia); KP1, anti-CD68, label monocytes, and macrophages (Serotec, Oxford, UK); rabbit anti-aquaporin 2 (AQP-2; Calbiochem, San Diego, CA) was used as a marker of collecting ducts (CD); and UCHL1 was used recognizing human T cells (Serotec). Secondary polyclonal antibodies used were biotinylated goat anti-rabbit IgG and avidin-biotin complexes (ABC) (Vector Laboratories, Burlingame, CA); horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG and rabbit anti-goat IgG (Dako); HRP-conjugated goat anti-rabbit IgG (Silenus Laboratories, Melbourne, Australia); and complexes of HRP-conjugated mouse, goat, or rabbit anti-HRP IgG complexes (PAP) (Dako).

Western Blotting

Normal human kidney tissue (the uninvolved pole from carcinoma nephrectomies) was homogenized on ice in 2 ml of lysis buffer (10 mM Tris-HCl, pH 7.4, 100 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM NaF, 2 mM Na_2VO_4 , 1% Triton X-100, 10% glycerol, 0.5% deoxycholate, 1 mM PMSF, and 10% protease inhibitor cocktail [Sigma-Aldrich]), left on ice for 10 min with regular vortexing, and tissue debris removed by centrifugation at $15,000 \times g$ for 20 min and the supernatant aliquotted and stored at -80°C . As a positive control for ERK activation, rat NRK49F fibroblasts were cultured in 6-ml wells, starved overnight in 0.25% FCS, and then were either left untreated (Nil) or stimulated for 10 min with 10 ng/ml human IL-1 β (PeproTech, London, UK) before being lysed with the lysis buffer as described above.

Lysates (10 μg per lane) were run on 12.5% SDS-PAGE and then transferred onto nitrocellulose membranes (Amersham, Buckinghamshire, UK) by electroblotting in 25 mM Tris-HCl, pH 8.5, 192 mM glycine, 20% methanol overnight with a Bio-Rad Transblot apparatus (Bio-Rad Laboratories, Hercules, CA). Blots were blocked for 2 h in 5% nonfat milk powder in 20 mM Tris-HCl, pH 7.6, 137 mM NaCl, 1% BSA, and 0.05% Tween 20, and washed five times in 20 mM Tris-HCl, pH 7.6, 137 mM NaCl, and 0.05% Tween 20. Blots were then incubated overnight at 4°C with rabbit anti-p-ERK antibody in the blocking solution, washed, and then incubated for 1 h with HRP-conjugated sheep anti-rabbit IgG in binding buffer. After washing, bound antibody was detected with SuperSignal chemiluminescent substrate (Pierce, Rockford, IL). Chemiluminescent emissions were captured on Kodak XAR film. Blots were stripped and reprobed with the rabbit anti-ERK2 antibody (K-23).

Immunohistochemistry

Detection of p-ERK in 4- μm sections of formalin-fixed, paraffin-embedded tissue used the ABC method. Sections were dewaxed, microwaved for 10 min in 0.1 M sodium citrate buffer pH 6.0, washed in PBS, and then blocked in 10% normal sheep serum, 10% FCS, and 5% BSA in PBS for 30 min. A 1/100 dilution of rabbit anti-p-ERK1/2 antibody in 10% normal human serum and 1% BSA in PBS was added to sections overnight at 4°C . Sections then were washed three times in PBS, endogenous peroxidase inactivated in 0.6% H_2O_2 in methanol for 20 min, washed in PBS, and a biotin block applied (Vector Laboratories). After washing in PBS, sections were incubated with biotinylated goat anti-rabbit IgG for 45 min, washed, and incubated with the ABC complex for 45 min at room temperature. After washing

Table 1. Classification and clinical parameters of patients^a

Group	Patients (n)	Age (yr)	Gender (M:F)	Serum Creatinine ($\mu\text{mol/L}$)	Creatinine Clearance (ml/min)	Proteinuria (g/d)
Normal	2	47 \pm 4	2:0	83 \pm 21	133 \pm 54	0.19 \pm 0.1
TMD	8	40 \pm 9	1:7	80 \pm 23	122 \pm 22	0.26 \pm 0.3
MCD	8	46 \pm 30	4:4	209 \pm 199	57 \pm 29	9.9 \pm 8.1
FGS	5	49 \pm 25	1:4	191 \pm 128	63 \pm 49	6.8 \pm 4.4
SLE III/IV	8	40 \pm 12	4:4	136 \pm 84	79 \pm 31	4.0 \pm 4.3
Vasculitis	9	56 \pm 18	4:5	487 \pm 380	28 \pm 41	1.8 \pm 1.3

^a TMD, thin membrane disease; MCD, minimal change disease; FGS, primary focal and segmental glomerulonephritis; SLE, systemic lupus erythematosus Class III and IV; Vasculitis, pauci-immune segmental necrotizing crescentic glomerulonephritis. Data are shown as the mean \pm 1 SD.

Table 2. Histopathological features of renal biopsies^a

Group	Patients (n)	Glomerular Cellularity (cells/gcs)	Glomerular Seg Lesions (%)	Crescents (%)	Interstitial Fibrosis & Tubular Atrophy (0–3+)
Normal	2	67.5 ± 10.9	0	0	0
TMD	8	67.5 ± 7.5	0	0	0
MCD	8	66.7 ± 9.0	0	0	0.38 ± 0.52
FGS	5	75.2 ± 22.7	0	0	1.3 ± 0.52
SLE III/IV	8	73.6 ± 15.5	29.4 ± 21	8.5 ± 7	0.89 ± 0.78
Vasculitis	9	88.4 ± 13.5	49.8 ± 25.6	43.4 ± 27.1	1.4 ± 0.73

^a Glomerular lesions are segmental proliferation and/or necrosis. Data are shown as the mean ± 1 SD.

in PBS, sections were developed with the diaminobenzidine substrate (Sigma-Aldrich) to produce a brown color. The specificity of p-ERK staining was confirmed by a lack of staining with no primary antibody or an isotype-matched irrelevant antibody. In addition, the anti-p-ERK antibody was incubated with the immunizing phospho-ERK peptide that abolished the staining reaction.

Detection of PCNA, macrophages, α-SMA, T cells, and AQP-2 used a three-layer PAP method. Sections underwent microwave heating (except for α-SMA staining), washed in PBS, and blocked for 30 min as above. Primary antibodies were applied to sections overnight at 4°C. Sections then were washed in PBS, and then incubated sequentially with HRP-conjugated secondary antibodies followed by PAP complexes. HRP was developed with Vector SG (Vector Laboratories) to give a blue-gray color.

For two-color immunostaining, sections were stained with p-ERK or α-SMA antibodies, treated with microwave oven heating to prevent antibody cross-reactivity (18), and then stained with p-ERK, KP1, or T cell antibodies with a different color development.

Samples from all cases were immunostained for p-ERK and PCNA. However, because of limitations in tissue availability, double staining for the other antibody combinations was performed on approximately three cases per disease group.

Quantification of Immunostaining

The number of cells stained positive for p-ERK or PCNA were counted in glomerular cross sections under high power (×400). All tissues contained at least 6 glomeruli, with most containing 10 to 15 glomeruli. Glomerular counts included both the capillary tuft and crescents. The number of tubular cells stained positive for p-ERK or PCNA were counted in 50 to 100 consecutive high-power (×400) cortical fields (avoiding glomeruli and large vessels) with a graticule and was expressed as the number of positive tubular cells per square millimeter.

Histopathology

Periodic acid–Schiff–stained sections were scored for the number of nuclei per glomerular cross section (tuft and crescent), the number of glomeruli with segmental proliferative and/or necrotic lesions, and the number of glomeruli with crescent formation (Table 2). The area of the tubulointerstitium with interstitial fibrosis and tubular atrophy was assessed by a semiquantitative score: 0 (<5%), 1 (6% to 20%), 2 (20% to 40%), and 3 (>40%). All analysis was performed on blinded slides.

Statistical Analyses

Statistical differences were analyzed by the one-way ANOVA by Tukey’s multiple-comparison test with GraphPad Prism 3.0 (Graph-

Pad Software, San Diego, CA). Correlation analyses used the Pearson or Spearman single correlation coefficient, as specified. Data are shown as the mean ± 1 SD.

Results

ERK Activation in Normal Human Kidney

Western blotting of normal human kidney from the uninvolved pole of carcinoma nephrectomy samples with the phospho-ERK antibody detected bands of 44 and 42 kD, corresponding to the presence of phosphorylated ERK1 and -2

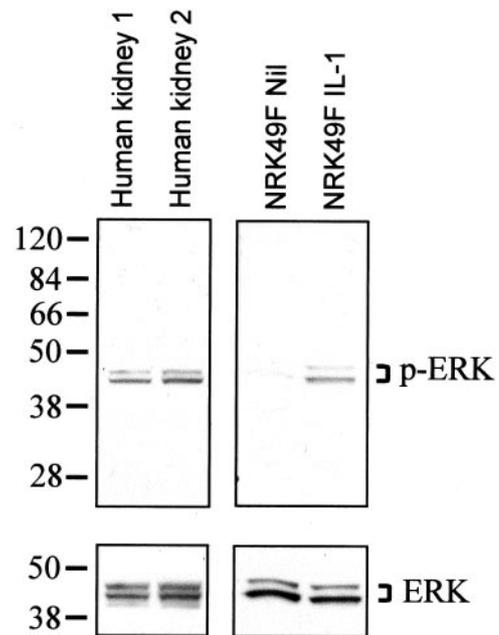


Figure 1. Extracellular-signal regulated kinase (ERK) phosphorylation in normal human kidney. (top) Western blot of two different samples of normal human kidney (uninvolved pole of two carcinoma nephrectomies) in which the dual-phosphorylated form of ERK (p-ERK) antibody identified two bands of approximately 44 and 42 kD (corresponding to p-ERK1 and p-ERK2, respectively). (bottom) Blot reprobed with an antibody that detects total ERK1 and -2 (i.e. detects both phosphorylated and nonphosphorylated forms of ERK1 and -2). As a positive control, a marked induction of ERK phosphorylation was seen in cultured fibroblasts (NRK49F) stimulated with IL-1 with the p-ERK antibody (top), whereas a reprobe for total ERK shows that IL-1 did not affect the level of ERK protein within the cells (bottom).

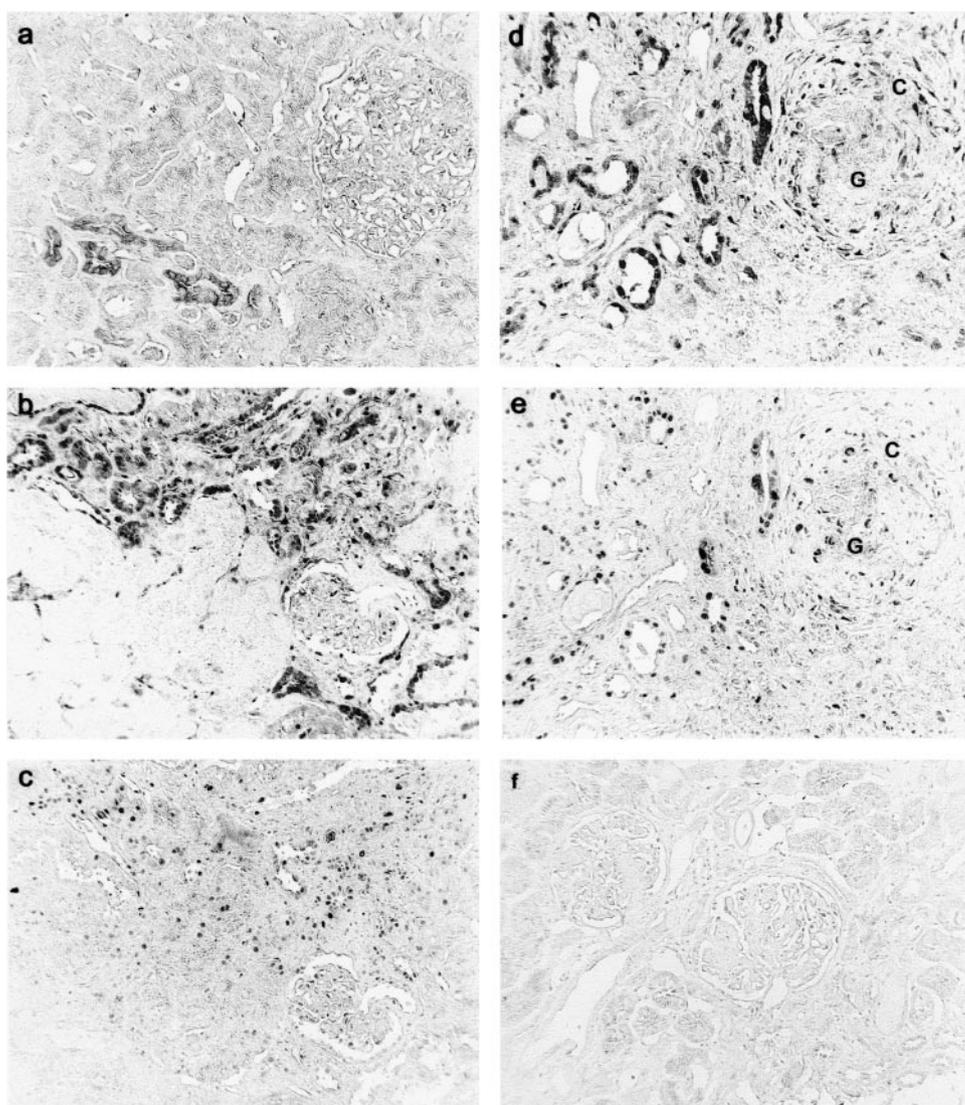


Figure 2. Immunohistochemistry staining of extracellular-signal regulated kinase (ERK) activation and cell proliferation in normal and disease human kidney. (a) Normal human kidney showing dual-phosphorylated form of ERK (p-ERK) staining in some cortical collecting ducts (CD); glomeruli and other tubular segments are negative. (b) Lupus IV showing tubular and interstitial p-ERK staining in an area of tubulointerstitial damage. There is little p-ERK staining in the adjacent area of intact tubulointerstitium. Some p-ERK-stained cells are also present in the glomerulus. (c) Serial section to (b) showing proliferating cell nuclear antigen (PCNA) staining in tubules and interstitial cells in the area of tubulointerstitial damage, with little proliferation in the adjacent intact area. (d) Vasculitis showing p-ERK staining of cells in a glomerulus (G), a glomerular crescent (C), and in tubules and interstitial cells in an area of tubulointerstitial damage. (e) Serial section to (d) showing PCNA+ cells in the glomerulus (G), crescent (C), and in tubules and interstitial cells in the area of tubulointerstitial damage. (f) Specificity control in which the p-ERK antibody was incubated with the p-ERK peptide before being applied to a section of a case of lupus IV. No staining is seen in damaged glomeruli or in an area of tubulointerstitial damage. Original magnification, $\times 160$ (b, c, f) and $\times 250$ (a, d, e).

(Figure 1). Localization of phosphorylated ERK was performed on biopsy samples from histologically normal kidney. Immunostaining identified p-ERK in the cytoplasm of some CD in the cortex (Figure 2a). Colocalization of immunostaining for p-ERK with AQP-2 confirmed that ERK activation was restricted to CD (not shown). Occasional glomerular cells were positive for p-ERK immunostaining, whereas Bowman's capsule was negative.

ERK Activation in Glomerulopathies

Similar to normal human kidney, p-ERK staining in thin membrane disease (TMD) and minimal change disease (MCD) was largely restricted to the CD, with only a small number of positive cells seen in glomeruli. Double staining of p-ERK and AQP-2 in CD in TMD is shown in Figure 3a. In some glomeruli, p-ERK staining of parietal epithelial cells was seen (Figure 3a). Although infrequent, tubular ERK activation was seen in focal areas of tubulointerstitial damage in MCD and TMD.

Glomerular p-ERK staining was quite variable in primary focal and segmental glomerulosclerosis (FGS), systemic lupus

erythematosus World Health Organization class III/IV (lupus) and, in particular, in pauciimmune segmental necrotizing crescent glomerulonephritis (vasculitis). In lupus and vasculitis, some glomeruli contained large numbers of p-ERK+ cells, which were present within the capillary tuft and in crescents. On the basis of light microscopy, ERK activation was apparent in podocyte-like cells, mesangial-like cells, and parietal epithelial cells, as well as in elongated fibroblast-like cells within crescents. Double immunostaining showed nuclear p-ERK staining in α -SMA+ cells within both the tuft and the crescent (Figure 3b). However, very few glomerular macrophages or T cells showed ERK activation.

Tubular p-ERK immunostaining in FGS, lupus, and vasculitis was restricted to CD in areas of intact tubulointerstitium. However, p-ERK staining was evident in damaged tubules (Figure 2, b and d), especially those containing casts. In addition, interstitial p-ERK stained cells were seen—again, predominantly in areas of tubulointerstitial damage (Figure 2, b and d). Double immunohistochemistry identified nuclear p-ERK staining in most interstitial α -SMA+ myofibroblasts

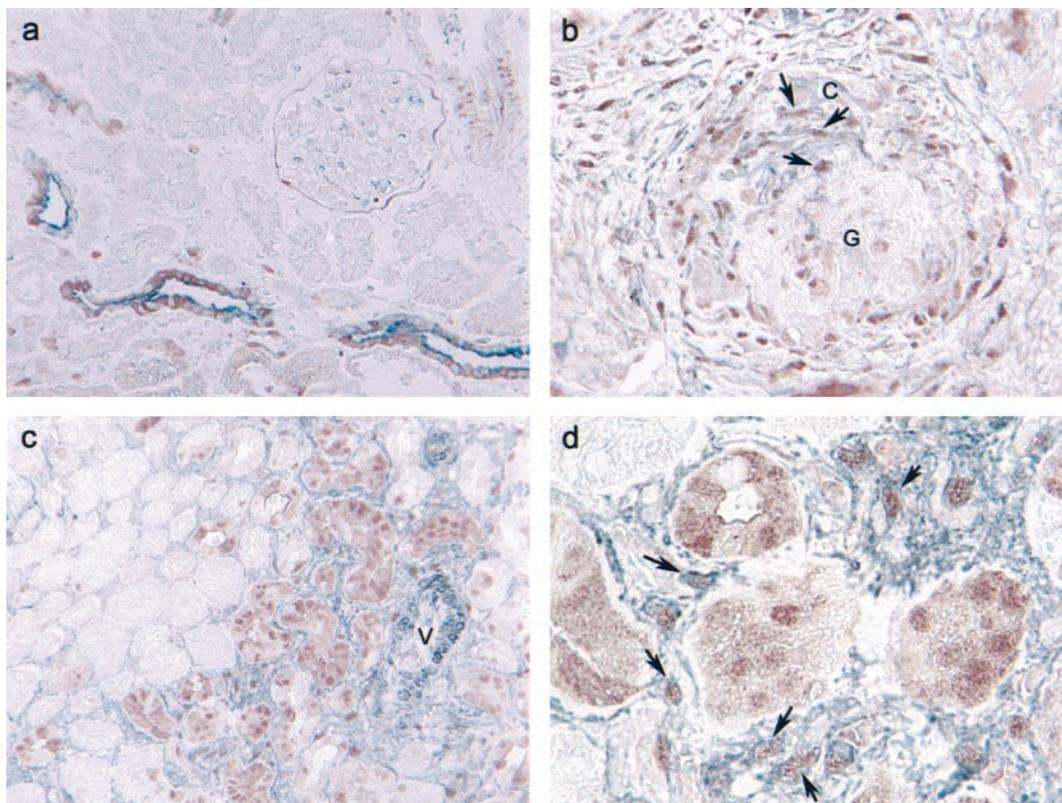


Figure 3. Two-color immunostaining of extracellular-signal regulated kinase (ERK) activation in human glomerulopathies. (a) Thin membrane disease showing dual-phosphorylated form of ERK (p-ERK) staining (brown) in collecting ducts that are also stained positive for aquaporin-2 (blue/gray). p-ERK staining is also seen in parietal epithelial cells of Bowman's capsule and in occasional interstitial cells. (b) Vasculitis showing double staining for p-ERK (brown) and α -smooth muscle actin (α -SMA, blue/gray). Many p-ERK+ cells are seen in the glomerulus (G) and crescent (C). Some double stained p-ERK+ α -SMA+ cells are seen in the crescent and glomerular tuft (arrows). (c) Vasculitis showing p-ERK staining (brown) of tubules in an area of tubulointerstitial damage that has many α -SMA+ (blue/gray) interstitial myofibroblasts. Note the lack of p-ERK staining in the adjacent intact area. Smooth muscle cells of a blood vessel (V) are also stained. (d) High power of (c) showing nuclear p-ERK staining (brown) of interstitial α -SMA+ (blue/gray) myofibroblasts (arrows). Original magnification, $\times 160$ (c), $\times 250$ (a), $\times 400$ (b), and $\times 1000$ (d).

(Figure 3, c and d), whereas only rare interstitial macrophages or T cells were positive for p-ERK (data not shown). The specificity of p-ERK antibody was verified by absorption with the immunizing ERK phosphopeptide (Figure 2f) and by Western blotting (Figure 1). The highly variable nature of the glomerular and tubular p-ERK staining across the different biopsy samples is reflected in the large SD seen in each disease group in Figure 4.

Cell Proliferation in Glomerulopathies

Cell proliferation was determined by immunostaining for PCNA. As with p-ERK immunostaining, the number of glomerular PCNA+ cells varied widely between individual glomeruli within a single biopsy sample and between different cases in the same disease group. Indeed, in the two normal biopsy samples examined, significant glomerular and tubular cell proliferation was evident in one case, perhaps reflecting a response to acute renal injury. Small numbers of proliferating glomerular cells were seen in all glomerulopathies, with proliferation most marked in vasculitis in which the presence of PCNA+ cells was particularly prominent in cellular crescents

(Figure 2e). Indeed, the majority of glomerular p-ERK+ and PCNA+ cells in the vasculitis group were present within crescents.

Proliferation of tubular epithelial cells was also highly variable across the cohort. Indeed, significant tubular cell proliferation was seen in the two "normal" cases and in some cases of TMD. This was usually present in areas of focal interstitial damage. Substantial numbers of proliferating tubular cells were also seen in the more aggressive diseases, in which proliferation of interstitial cells was also observed (Figure 2e).

Staining of serial sections identified colocalization of p-ERK+ cells and PCNA+ cells in areas of tissue damage (Figure 2, b and c). A case of vasculitis is shown in Figure 2, d and e, in which a crescentic glomerulus and an area of tubulointerstitial damage contain numerous p-ERK+ and PCNA+ cells. Whether ERK activation and PCNA expression were present within the same individual cell was not examined because ERK activation is a generally an early event that induces resting cells to move from G₀ into G₁ phase of the cell cycle (8), whereas PCNA is not usually expressed until cells have already entered G₁ phase. However, it should be pointed

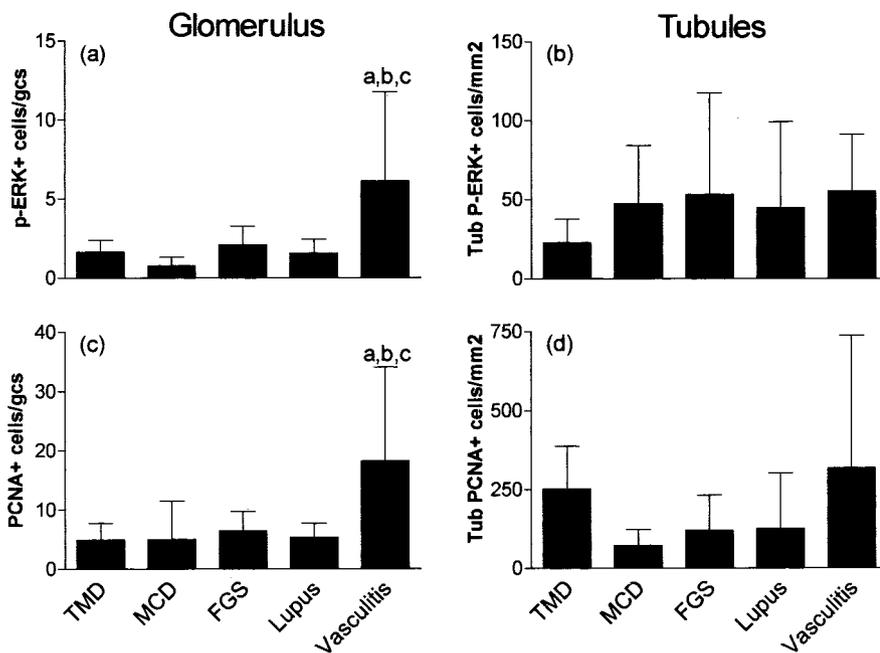


Figure 4. Quantification of extracellular-signal regulated kinase (ERK) activation in human glomerulopathies. Immunostained tissue sections were scored for the number of (a) dual-phosphorylated form of ERK (p-ERK)+ cells per glomerular cross-section (gcs); (b) p-ERK+ tubular cells per mm²; (c) proliferating cell nuclear antigen (PCNA)+ cells per gcs; and (d) PCNA+ tubular cells per square millimeter. Abbreviations as in Table 1. Data are mean \pm SD. ^a $P < 0.05$ versus thin membrane disease (TMD); ^b $P < 0.05$ versus minimal change disease (MCD); ^c $P < 0.05$ versus lupus by ANOVA with Tukey's multiple-comparison posttest.

out that not all glomerular or tubulointerstitial areas with prominent p-ERK staining had substantial numbers of PCNA+ cells.

Correlation of ERK Activation with Cell Proliferation and Renal Dysfunction

Analyzing all disease groups as a single cohort found a significant correlation between the number of glomerular p-ERK+ and PCNA+ cells, and a significant correlation between the number of tubular p-ERK+ and PCNA+ cells (Figure 5). The number of glomerular p-ERK+ cells correlated with glomerular cellularity and the percentage of glomeruli with segmental lesions, but was of borderline significance in relation to renal function and did not correlate with proteinuria (Table 3). In contrast, the number of p-ERK+ tubular cells gave a significant correlation with renal function and interstitial damage (fibrosis and tubular atrophy), but not with proteinuria (Table 3).

Discussion

To our knowledge, this is the first study to examine ERK activation in normal and diseased adult human kidney. ERK activation was detected within the CD in normal human kidney. This localization was confirmed by double immunostaining for AQP-2. This pattern of ERK activation was largely unaltered in TMD and MCD. Activation of the ERK pathway in the CD in normal human kidney is consistent with studies in normal rat kidney (14). The reason for ERK activation in this segment of the kidney is unclear. It may reflect a response to osmotic stress as suggested by studies in which urea and sodium chloride induced hypertonic stress activate ERK in cultured inner medullary CD cells (19,20). This postulate is supported by studies in rats in which hypertonic stress caused by water restriction induced activation of ERK in the papilla

(21), and by *in vitro* studies in which hypertonicity-induced transcription of AQP-1 in inner medullary CD cells operates, in part, via ERK (22).

A clear association between ERK activation and cell proliferation was seen in the different glomerulopathies examined. This was evident by the significant correlation between the number of p-ERK+ and PCNA+ cells in glomeruli and in tubules, and by colocalization of p-ERK+ and PCNA+ cells within focal glomerular and tubulointerstitial lesions. A feature of the study was the variability of the number of p-ERK+ cells within individual biopsy samples and between individual patients with same diagnosis. This is, perhaps, not surprising, given that cell proliferation in the pathologic state is a dynamic event that occurs in bursts within focal areas. It is of note that although PCNA has been verified as a good indicator of cell proliferation, this marker has some limitations, such as a small proportion of cells expressing PCNA may not complete cell division, and that PCNA expression may be detectable in some cells for a short period after division has been completed.

Studies of cultured mesangial cells and in the rat Thy-1 model of mesangioproliferative nephritis have provided evidence of a causal link between ERK activation and mesangial cell proliferation (9,10,16,17). In the study presented here, double staining identified ERK activation in α -SMA+ cells within the glomerular capillary tuft in FGS, lupus, and vasculitis. This probably represents activation of the ERK pathway in "activated" mesangial cells, providing evidence that ERK signaling may play an important role in mesangial cell proliferation in human glomerulopathies. However, it needs to be appreciated that in some glomeruli, the α -SMA+ cells within the glomerular tuft could be myofibroblasts that have migrated from the periglomerular area through ruptures in Bowman's capsule.

We observed p-ERK staining in α -SMA+ myofibroblasts in

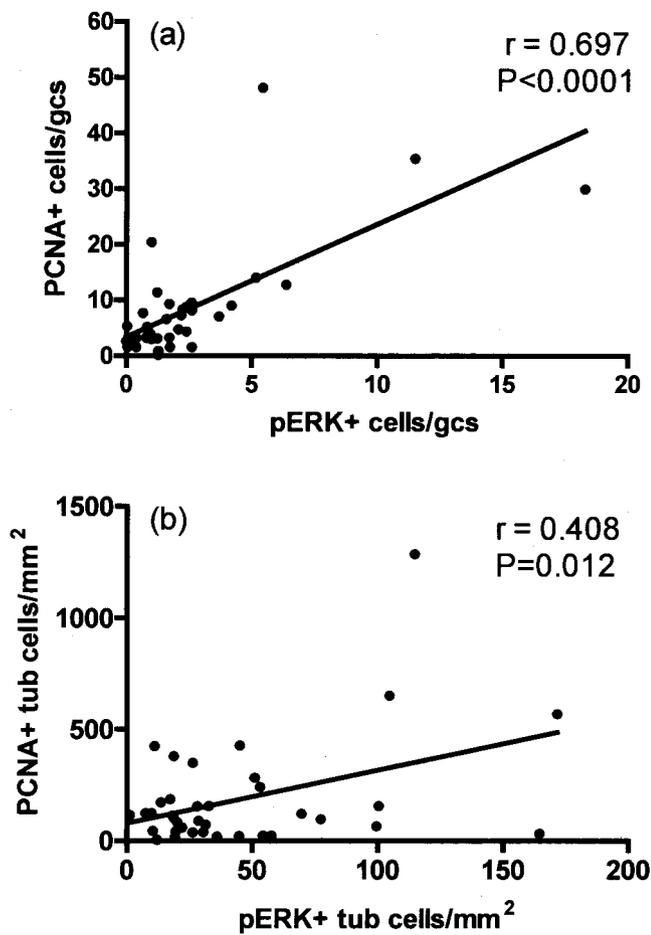


Figure 5. Correlation of extracellular-signal regulated kinase (ERK) activation with cell proliferation. Examining all patients as one cohort by the Pearson correlation coefficient found a significant correlation between the number of dual-phosphorylated form of ERK (p-ERK)+ cells and proliferating cell nuclear antigen (PCNA)+ cells in glomeruli (a), and between the number of tubular p-ERK+ cells and PCNA+ cells (b).

both glomerular crescents and the interstitium in human glomerulopathies, suggesting that ERK signaling plays a role in myofibroblast proliferation, an important step in myofibroblast accumulation and development of renal fibrosis. This is con-

sistent with studies in which PDGF-BB induced proliferation of hepatic myofibroblasts operates in an ERK-dependent fashion (13), and the finding that administration of PDGF-BB to normal rats leads to the proliferation and accumulation of α -SMA+ myofibroblasts and mild fibrosis in the renal interstitium (23). However, ERK signaling may mediate other functions in renal myofibroblasts, such as the synthesis of collagen and fibronectin (24,25), and the production of metalloproteinase-1, cyclooxygenase-2, and nitric oxide (26–28).

One interesting finding was the lack of ERK activation in infiltrating macrophages and T cells. *In vitro* studies have shown that macrophage-colony stimulating factor (M-CSF) driven macrophage proliferation operates via the ERK pathway (29), and we have previously demonstrated the presence of local macrophage proliferation in human glomerulonephritis in association with up-regulation of renal M-CSF production (30). One possible reason for this unexpected result may be the transient nature of ERK activation after M-CSF stimulation of macrophages (31).

ERK activation in tubular epithelial cells was restricted to the CD in normal human kidney and in the different glomerulopathies where the tubulointerstitial area remained intact. However, ERK activation was prominent in damaged tubules and colocalized with tubular cell proliferation. Indeed, there was a significant correlation between tubular ERK activation and tubular cell proliferation. This is consistent with a time-course study in the obstructed rat kidney in which ERK activation preceded tubular proliferation (14), and a study in autosomal-dominant polycystic kidney disease in which ERK activation is closely associated with dysregulated tubular cell proliferation in cysts (15). In addition to proliferation, ERK activation in damaged tubules may enhance cell survival by inhibition of apoptosis (32), and promoting cell adhesion (33).

ERK activation may be a double-edged sword in renal disease. On one hand, ERK-mediated proliferation of mesangial cells and myofibroblasts may promote renal fibrosis and disease progression. Conversely, ERK activation in damaged tubules may be desirable to promote renal repair. Therefore, therapies targeting the undesirable ERK-mediated proliferation in renal disease may need to focus on the events upstream of ERK activation.

In summary, this study has localized ERK activation in

Table 3. Correlation analysis of ERK activation with clinical and pathological disease parameters^a

	Serum Creatinine (μ mol/L)	Creatinine Clearance (ml/min)	Proteinuria (g/d)	Glomerular Cellularity (cells/gcs)	Glomerular Seg Lesion (%)	Interst Damage (0–3+)
p-ERK+ cells/gcs	0.33 ($P = 0.0497$)	−0.32 NS	−0.20 NS	0.35 ($P = 0.042$)	0.37 ($P = 0.04$)	—
p-ERK+ tubular cells/mm ²	0.34 ($P = 0.04$)	−0.46 ($P = 0.0038$)	−0.03 NS	—	—	0.5 ($P = 0.0036$)

^a All patients with glomerulopathies were analysed as a single cohort. Analysis of serum creatinine, creatinine clearance, proteinuria, and glomerular cellularity used the Pearson correlation coefficient. Analysis of the percentage of glomeruli with segmental lesions (proliferative and/or necrotic), and the score of interstitial damage (fibrosis and tubular atrophy) used the Spearman correlation coefficient. Data are shown as the correlation coefficient r and statistical values given in parenthesis. NS, not significant.

normal human kidney and in a variety of glomerulopathies. ERK activation in human glomerulopathies correlates with cell proliferation, histologic lesions, and renal dysfunction. However, signaling through the ERK pathway may promote renal repair through tubular proliferation on one hand, while inducing renal fibrosis via proliferation of glomerular and interstitial myofibroblasts on the other.

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