Increased E-cadherin expression in the ligated kidney following unilateral ureteric obstruction

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E-cadherin expression in the kidney is used as a surrogate marker of epithelial mesenchymal transition for the testing of various antifibrotic strategies. Here we reexamined E-cadherin expression in the kidneys of rats with unilateral ureteric obstruction, which was previously reported to decrease in parallel with the development of tubulointerstitial disease in this widely used experimental model of renal fibrosis and epithelial mesenchymal transition. E-cadherin mRNA expression was consistently increased both acutely (hours) and chronically (days) in the ligated kidney compared to the cognate non-ligated kidney. Increased E-cadherin protein levels were also found in the ligated kidney particularly in dilated tubular segments. Simulation of early pressure changes in the ligated kidney by mechanical stretch of human renal epithelial cells in culture did not alter E-cadherin expression. Porcine LLCPK-1 cells subjected to hypotonic stretch, however, did have increased E-cadherin mRNA and protein levels, responses that were not prevented by transforming growth factor-β, a cytokine that promotes epithelial mesenchymal transition. Our findings question the utility of E-cadherin as a marker of epithelial mesenchymal transition in this model of renal fibrosis.

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Unilateral ureteric obstruction (UUO) causes rapid development of renal tubulointerstitial fibrosis, (reviewed in Bascands and Schanstra and Docherty *et al.*[1,2]). Transforming growth factor (TGF)-β1 is a key fibrotic mediator in obstructive (originally observation Diamond *et al.*[3]) and nonobstructive nephropathies.[4]

Epithelial mesenchymal transition (EMT) has been implicated in embryogenesis, malignancy, and fibrosis. Renal tubular EMT is proposed to contribute to fibrosis, with TGF-β1 acting as a major mediator of this process.[5] A current PubMed search using 'EMT AND renal AND fibrosis' reveals 94 references since 1997.

The early loss of the adherens junction protein E-cadherin is commonly used to characterize the presence of EMT. A current PubMed search using 'EMT AND renal AND fibrosis AND E-cadherin' finds 40 articles.

This phenomenon has been proposed to occur in the UUO model, the strongest argument for which was put forward by Iwano *et al.*[6] that claimed to demonstrate that 36% of activated fibroblasts in the UUO model were derived from the proximal tubular epithelium. Preservation of E-cadherin expression in the obstructed kidney has been cited as evidence of an inhibition of EMT in Smad3 and plasminogen null mice that are protected from UUO-induced tubulointerstitial fibrosis.[7,8] Renal E-cadherin expression has also been used as a surrogate marker of EMT on which to test the efficacy of novel antifibrotic strategies (for example, erythropoietin, paracalcitol, geranylgeranylacetone, and irbesartan[8–12]).

Two interesting recent studies contest a role for EMT in renal fibrosis, first by suggesting that fibroblast-specific protein-1 can be expressed by inflammatory cells in renal injury.[13] Specifically in the UUO model, using light and electron microscopy analyses and immunohistochemistry, Picard *et al.*[14] claim that interstitial fiber-producing cells in UUO are derived from resident fibroblasts, and provide no evidence supportive of EMT.

In light of these studies and having previously examined E-cadherin loss during EMT in the HK-2 cell line,[15] we were

interested *per se* in using E-cadherin loss as a marker of EMT in UUO.

RESULTS

Distal tubular dilatation, inflammation, and the development of tubulointerstitial fibrosis in the L kidney

We quantified distal tubular dilatation at 24 h, 3 and 10 days post-UUO, as a pathological sequela of urinary pooling. Mean distal tubular luminal area (μM^2) was not changed at any time point in non-ligated (NL) kidneys (24 h NL (521.5 ± 100.7) versus 3-day NL (572.4 ± 33.4) versus 10-day NL (672.9 ± 153.3)). Areas were increased significantly in the ligated (L) kidney as a function of time post-obstruction (24 h L (1048.2 ± 124.5) versus 3-day L (2051.2 ± 146.8, $P = 0.007$) and 3-day L (2051.2 ± 146.8 versus 10-day L (3138.4 ± 161.8, $P = 0.008$; Figure 1).

Non-ligated kidneys at 24 h, 3 and 10 days post-UUO showed a normal morphology (Figure 2a and b). L kidneys at 3 and 10 days post-UUO showed tubular dilatation with an expanded interstitium containing a predominantly lymphocytic infiltrate (Figure 2c and d).

At 10 days post-UUO, the L kidneys of rats showed evidence of mature collagen fibril deposition in the tubulointerstitium (Figure 2e arrow). Hsp47 induction provided molecular evidence of the fibrotic response (Figure 2f).

E-cadherin mRNA is consistently increased in the L kidney versus the NL kidney of rats following UUO

E-cadherin mRNA levels were not significantly altered in the NL kidney at time points between 3 h and 14 days post-UUO. Relative E-cadherin mRNA levels in L kidneys were calibrated against 3h NL values revealing a 1.7 ± 0.38-, 2.5 ± 0.92-, 2.43 ± 0.5-, and 2.56 ± 0.61-fold increase in L kidneys at 3 h, 6 h, 3 and 10 days, respectively (all $P < 0.001$), No significant difference was observed in terms of degree of fold change between L kidneys (Figure 3).

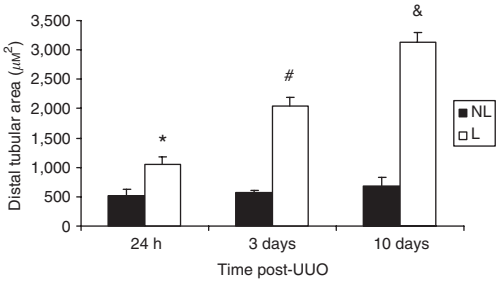

Figure 1 | L kidney distal tubular luminal area increases with time following UUO. H&E stained coronal sections of 24 h, 3 and 10-day NL and L kidneys were viewed at × 20 in Image Scope (Aperio) and distal tubular area in transverse section quantified. The mean area in μM^2 of 10 distal tubular segments per sample was calculated and group means derived. $n = 3$ per sample, $^{*}P < 0.05$ 24 h NL versus L, $^{\#}P < 0.01$ 3-day L versus 3-day NL and 24 h L, and $^{\&}P < 0.01$ 10-day L versus 10-day NL, 3-day L, and 24 h L.

Induction of E-cadherin mRNA in L kidneys coincides with increased protein expression

Densitometric quantification of western blotting signals for E-cadherin following 3 and 10 days of UUO demonstrated a twofold increase in full-length E-cadherin in L kidneys ($P = 0.05$, 3-day NL versus L and $P = 0.02$, 10-day NL versus L; Figure 4a and b).

Prolonged exposure of E-cadherin Western blots revealed the presence of a small, approximately 30 kDa C-terminal fragment and a longer 100 kDa C-terminal fragment in the L kidneys of rats (Figure 4b).

Immunohistochemical localization of E-cadherin following UUO

In the NL kidneys at both days 3 and 10, cortical staining was at the basolateral aspect of the distal tubule (Figure 5a and c).

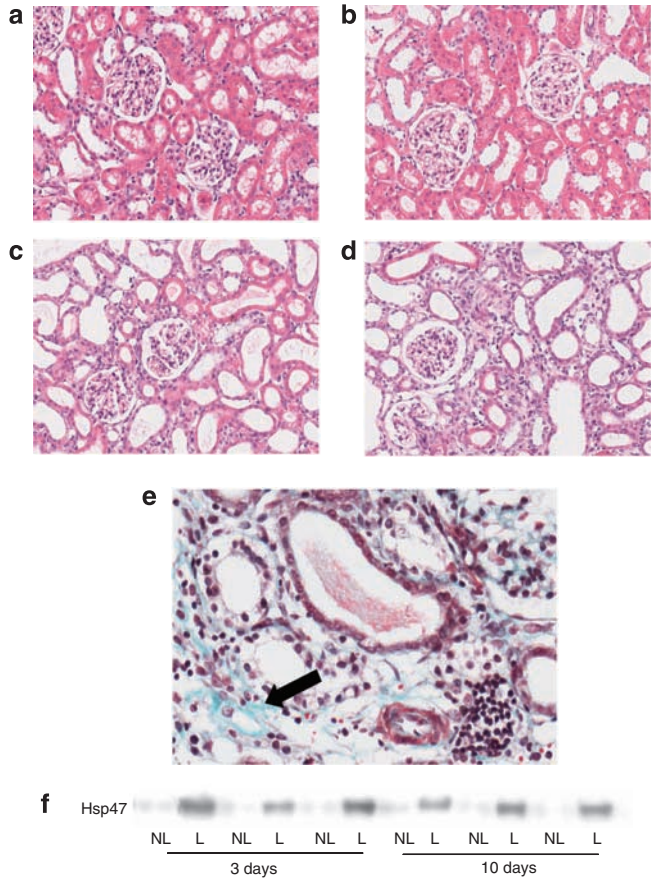

Figure 2 | Inflammatory infiltration and tubulointerstitial fibrosis in the post-UUO rat kidney. (**a**–**d**) Representative hematoxylin and eosin stained 4 μm coronal sections of rat renal cortex at 3 days post-UUO (× 20). (i) 3-day NL (ii) 10-day NL (iii) 3-day L, and (iv) 10-day L. L kidneys at both time-points present with tubular dilatation, lymphocytic infiltrate, and tubulointerstitial expansion. (**e** and **f**) (i) Gomorri's trichrome staining of a 10-day L rat kidney (× 40) illustrating mature collagen fibrils present in the expanded tubulointerstitium (arrow) Images in **a** and **b** are representative of $n = 3$ per sample type (ii) western blotting analysis of Hsp47 protein expression in paired NL and L kidneys at 3 and 10 days post-UUO, demonstrating Hsp47 induction in the L kidneys $n = 3$ per group.