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Tubular expression of heat-shock protein 27 inhibits fibrogenesis in obstructive nephropathy

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Morphological changes that occur during kidney injury involve actin skeleton remodeling. Here we tested whether heat-shock protein 27 (HSP27), a small stress response protein involved in cytoskeletal remodeling, protects the kidney from tubulointerstitial fibrosis in obstructive nephropathy. Tubular cell HSP27 immunostaining was significantly increased in human kidneys with ureteropelvic junction obstruction, supporting the clinical relevance of our studies. To develop an animal model for mechanistic studies, we generated transgenic mice that specifically overexpress human HSP27 in renal tubules, under the kidney androgen–regulated protein promoter, and determined the effects of HSP27 overexpression on epithelial-to-mesenchymal transition and tubulointerstitial fibrosis following unilateral ureteral obstruction. This was associated with decreased fibrogenesis as evidenced by significant declines in phosphorylated p38MAPK, collagen III, α-smooth muscle actin, 4-hydroxynonenal, and reduced trichrome staining following obstruction. Notably, E-cadherin and β-catenin remained at the cell membrane of tubular cells in transgenic mice with an obstructed ureter. Monocyte/macrophage infiltration, however, was not significantly affected in these transgenic mice. Thus, tubular HSP27 inhibits fibrogenesis in obstructive nephropathy. Further studies are needed to determine pathways regulating the interactions between HSP27 and the E-cadherin–β-catenin complex.

Kidney International (2012) **83,** 84–92; doi:10.1038/ki.2012.336; published online 12 September 2012

KEYWORDS: fibrosis; HSP27; unilateral ureteral obstruction

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Received 6 January 2012; revised 18 July 2012; accepted 26 July 2012; published online 12 September 2012

Renal tubulointerstitial fibrosis or interstitial fibrosis and tubular atrophy (IFTA) is the point of pathological commonality for native and transplant kidney disease.[1] Fibrotic damage is associated with the presence of interstitial fibroblasts and myofibroblasts. A distinguishing characteristic of myofibroblasts is their expression of α-smooth muscle actin (α-SMA). These cells are also responsible for the deposition of extracellular matrix proteins such as collagens I, III, IV, and fibronectin. Tubular atrophy entails the loss of tubular epithelial cells through apoptotic and necrotic pathways, as well as through transition to a mesenchymal phenotype. A better understanding of the cellular and molecular mechanisms involved in IFTA would result in the development of preventive and therapeutic interventions that improve long-term outcomes in patients with native and transplant kidney disease.

Epithelial-to-mesenchymal transition (EMT) is an important profibrotic process and a surrogate marker of native and transplant kidney fibrosis.[2–5] Transforming growth factor-β1 is the primary cytokine that initiates and maintains EMT by activating signaling pathways and transcriptional regulators such as Smad 2/3 molecules. During EMT, tubular epithelial cells are transformed into myofibroblasts through processes involving loss of cell–cell adhesion molecules (e.g., E-cadherin) and *de novo* expression of mesenchymal markers (e.g., α-SMA). These events are followed by tubular basement membrane disruption, cell migration, and fibroblast invasion of the interstitium with production of fibrotic molecules including collagen and fibronectin. Iwano *et al.*[6] demonstrated that up to one-third of interstitial fibroblasts could originate from the epithelium during renal injury, suggestive of EMT *in vivo*. Another recent fate-mapping study by Humphreys *et al.*[7] suggests that pericytes may be another important source of interstitial myofibroblasts. Although the exact contribution of EMT to renal fibrosis remains unknown, this pathway has been extensively studied as a reversible injury process and a surrogate marker of native and transplant kidney fibrosis.[2,3,5]

Heat-shock protein 27 (HSP27) is a small-molecular-weight stress response protein originally characterized as a molecular chaperone induced by heat shock.[8] Over time, HSP27 has emerged as a dynamic protein with diverse

roles in regulation of actin cytoskeletal remodeling,[9,10] apoptosis,[11,12] and oxidative stress.[9,13,14] Renal fibrogenesis and EMT include several processes that may involve HSP27.[10,15] For example, HSP27 is activated *via* phosphorylation by p38MAPK signaling, an important injury pathway in inflammation and oxidative stress.[16,17] Similarly, reactive oxygen species, known inducers of HSP27, have an important role in the pathogenesis of EMT and IFTA.[16,17] In addition, the morphological changes that occur during EMT entail actin skeleton remodeling,[2–5] suggesting that HSP27 regulates actin filament dynamics and actin–cadherin junction interactions during this process.

Previous studies from our laboratory have examined the role of tubular HSP27 in experimental models of transplant and native kidney disease.[18,19] These studies demonstrated that cortical HSP27 was upregulated in response to allograft injury, suggestive of a local stress response to chronic rejection.[19] Similarly, we observed that tubular HSP27 was upregulated in the *in vitro* model of transforming growth factor-β1-induced EMT and during experimental obstructive nephropathy.[16] In these studies, the overexpression of human HSP27 (Hu27) in rat tubular epithelial cells preserved E-cadherin protein levels during EMT, leading us to hypothesize that HSP27 has an active and protective role during renal fibrogenesis.[18,19] In the current manuscript, we hypothesize that elevated tubular HSP27 reduces fibrogenesis. We test our hypothesis *in vitro* and *in vivo* using human samples and an animal model of obstructive nephropathy, a condition that is an important cause of end-stage renal disease in the pediatric population.[16]

RESULTS

HSP27 was increased in tubules of pediatric kidneys with congenital ureteropelvic junction obstruction

To determine the relevance of our studies in the clinical setting, we examined HSP27 expression in five obstructed and five control human and mouse kidneys. Controls included preimplantation biopsies from deceased donor kidneys before transplantation in humans, and baseline unobstructed kidneys in mice. By using the Nuance digital analysis software system, we found that HSP27 was significantly increased in tubules of obstructed kidneys (Figure 1).

Development of HSP27 transgenic mice

We developed HSP27 transgenic mice that overexpress human HSP27 in the proximal tubule, using the KAP2 promoter and human angiotensinogen (hAGT) enhancer complex as described previously.[20] The presence of the KAP2-HSP27 transgene was first determined by PCR of genomic DNA from tail snips (Figure 2). The transgene was evidenced by a distinct band migrating between 0.5 and 0.7 kb (Figure 2b). KAP2-HSP27 transgenic male mice expressed the transgene in the cortex of the kidney. Next, we examined the expression of the KAP2-HSP27 gene by imaging enhanced green fluorescent protein (eGFP) fluorescence using the Caliper IVIS Spectrum optical imaging system (Figure 2c). Spectral analysis indicated that 465-nm excitation and 520-nm emission wavelengths were optimal for the detection of eGFP. Transgenic animals showed significantly greater fluorescence in the kidney but not in the heart compared with wild-type animals, consistent with the specificity of the KAP2 promoter and hAGT enhancer.[20]

HSP27 upregulation was greater in KAP2-HSP27 transgenic mice after ureteral obstruction

To determine whether HSP27 was differentially upregulated by unilateral ureteral obstruction (UUO) in the transgenic mice, we performed western blot and immunohistochemical analyses in obstructed kidneys of transgenic and wild-type littermates (Figure 3). Animals were killed at 7 and 14 days post UUO for time-course analyses. Total and phosphory-

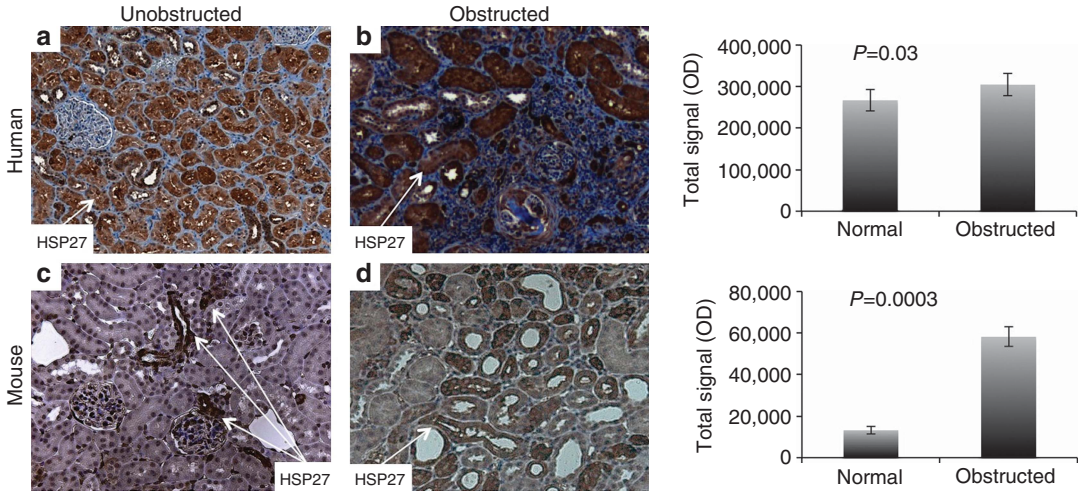

Figure 1 | Heat-shock protein 27 (HSP27) was increased in tubules of human and mouse kidneys with obstruction. Tissue sections prepared from obstructed and unobstructed human and mouse kidneys ($n = 5$ each group) were stained for HSP27 (dark brown). Digital image analyses using the Nuance multispectral imaging system showed that obstructed kidneys exhibited significantly more intense HSP27 staining in renal tubules. OD, optical density.