see commentary on page 242

Nestin expression in the kidney with an obstructed ureter

T Sakairi[1], K Hiromura[1], S Yamashita[1], S Takeuchi[1], M Tomioka[1], H Ideura[1], A Maeshima[1], Y Kaneko[1], T Kuroiwa[1], M Nangaku[2], T Takeuchi[3] and Y Nojima[1]

[1]Department of Medicine and Clinical Science, Gunma University Graduate School of Medicine, Maebashi, Gunma, Japan; [2]Division of Nephrology and Endocrinology, University of Tokyo School of Medicine, Tokyo, Japan and [3]Department of Molecular Medicine, Institute for Molecular and Cellular Regulation, Gunma University Graduate School of Medicine, Maebashi, Gunma, Japan

Nestin is an intermediate filament protein originally identified in neuroepithelial stem cells. This cytoskeletal-associated protein is also expressed in some non-neuronal organs including renal tubular cells and glomerular endothelial cells during kidney development. Little is known, however, about nestin expression in the kidney during injury. In this study, we find nestin expression induced in renal tubular and interstitial myofibroblasts in the adult rat kidney following unilateral ureteral obstruction. The degree of nestin expression was well correlated with the degree of tubulointerstitial fibrosis. Immunohistochemical identification of specific nephron segments showed that nestin was primarily expressed by proximal tubules, partially by distal tubules and thick ascending limbs of Henle but not by collecting ducts. The nestin-positive tubular cells also expressed vimentin and heat-shock protein 47 (HSP47) suggesting these cells reverted to a mesenchymal phenotype. Not all vimentin- or HSP-expressing cells expressed nestin; however, suggesting that nestin is distinct from these conventional mesenchymal markers. Nestin expression was also found associated with phenotypical changes in cultured renal cells induced by hypoxia or transforming growth factor-β. Nestin expression was located in hypoxic regions of the kidney with an obstructed ureter. Our results indicate that nestin could be a novel marker for tubulointerstitial injury.

Kidney International (2007) **72,** 307–318; doi:10.1038/sj.ki.5002277; published online 11 April 2007

KEYWORDS: fibroblast; hypoxia; interstitial fibrosis; obstructive nephropathy; TGF-β

Correspondence: *K Hiromura, Department of Medicine and Clinical Science, Gunma University Graduate School of Medicine, 3-39-22 Showa, Maebashi, Gunma, 371-8511, Japan. E-mail: hiromura@med.gunma-u.ac.jp*

Received 31 October 2006; revised 23 February 2007; accepted 28 February 2007; published online 11 April 2007

Nestin is a cytoskeleton-associated class VI intermediate filament protein, which was originally identified in neuroepithelial stem cells.[1,2] In contrast to microfilaments and microtubules, whose components are highly evolutionarily conserved and very similar within cells of a particular species, intermediate filaments display much diversity in their numbers, sequences, and abundance.[3] In humans, there are more than 60 different intermediate filament genes, which are differentially expressed in nearly all cells of the body.[4,5] Changes within the spatial and temporal expression of intermediate filament proteins were thought to regulate remodeling of the cell cytoskeleton during development.[6]

Although nestin was initially identified as a marker for neural stem and neural progenitor cells, a wider range of nestin expression has been found in non-neuronal organs than previously thought.[7] The expression of nestin was reported in developing organs in the fetus, including skeletal muscle cells,[8] cardiomyocytes,[9] pancreatic epithelial progenitor cells,[10] and vascular endothelium.[11] Compared to embryonic tissues, nestin expression is limited in normal adult tissues. However, re-expression of nestin has been reported during injury of adult organs, such as in central nervous system,[12,13] skeletal muscle,[14] liver,[15] pancreas,[16] and teeth.[17]

Recently, nestin expression in embryonic and adult kidney has been reported.[18–20] In immature glomeruli, nestin is expressed in the progenitors of glomerular endothelial cells. Nestin is also transiently expressed by epithelial cells of immature proximal tubules in the newborn kidney.[11] In contrast, in the mature adult kidney, podocytes are the only cells that exhibit persistent nestin expression.[18] However, nestin expression in injured kidneys has been poorly investigated, especially in tubulointerstitial injury.

Tubular damage and interstitial fibrosis are considered a final common pathway leading to end-stage renal disease.[21–23] Irrespective of the nature of initial renal injury, the degree of tubular damage correlates well with the decline of renal function and long-term prognosis.[21,22] Therefore, it is important to understand the molecular mechanisms underlying the progression of interstitial fibrosis. Recently, phenotypic change of renal tubular cells, so-called epithelial-

to-mesenchymal transition, was considered important in the progression of tubulointerstitial injury.[24–26] During injury, tubular epithelial cells lost the expression of epithelial cell marker, E-cadherin, and acquired mesenchymal features such as vimentin and α-smooth muscle actin (α-SMA).[25] Vimentin is a type III intermediate filament, which is found in cells of mesenchymal origin. Vimentin is known to assemble with nestin, *in vivo* or *in vitro*,[8,27–29] suggesting the possibility of nestin expression in vimentin-positive cells in injured kidneys.

In this study, we hypothesized that nestin is re-expressed in tubular cells during tubulointerstitial injury. We examined an adult rat unilateral ureteral obstruction (UUO) model and found that increased nestin expression was detected in renal tubular cells and tubulointerstitial myofibroblasts during the progression to tubulointerstitial fibrosis. We further demonstrated that increased nestin expression was associated with hypoxia and transforming growth factor-beta (TGF-β) stimulation in cultured renal cells. Hypoxia and TGF-β are recognized as important mediators for aggravating tubulointerstitial injury.[23,30–34] We propose that nestin could be a novel marker for tubulointerstitial injury.

RESULTS

Nestin expression in normal adult rat kidney and UUO kidney

We first examined the localization of nestin by immunohistochemical analysis in normal adult rat kidney. As previously reported,[18,19] nestin was predominantly detected in the glomeruli. No staining was observed in the tubulointerstitial area (Figure 1a and b). In the glomeruli, co-expression of nestin with synaptopodin, a marker for podocytes, was observed by double-immunofluorescent staining, but not with rat aminopeptidase P, a marker of endothelial cells (data not shown), consistent with the previous report that nestin was predominantly expressed in podocytes in normal adult kidney.[18,19] In contrast, nestin was strongly expressed by tubular and interstitial cells, in addition to podocytes, in the kidney at day 13 after UUO. Nestin-positive tubular cells and tubulointerstitial cells were localized mainly at the outer medulla, rather than cortex and inner medulla (Figure 1c and d). Dilated tubules tended to show nestin expression and its distribution within cells was abundant on the basolateral side and scant at the apical pole of these cells (Figure 1c–f). Nestin-positive cells were also detected in the tubulointerstitial area and most were spindle-shaped (Figure 1e and f).

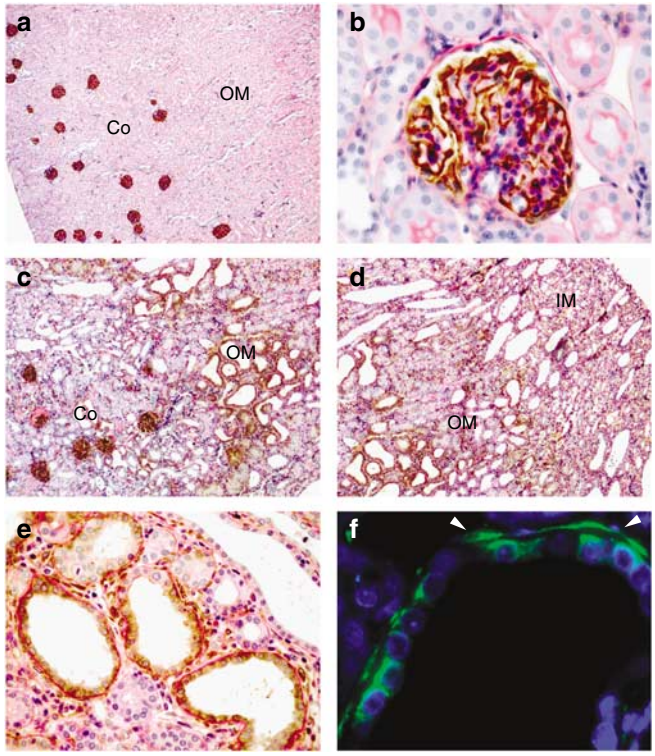

Figure 1 | Expression of nestin in the normal and UUO kidney. Nestin expression was detected by (**a**–**e**) immunohistochemical staining and (**f**) immunofluorescent staining. (**a** and **b**) Nestin expression was predominantly in glomeruli in normal adult rat kidney. (**c**–**f**) In UUO kidney at day 13, nestin expression was observed at tubular cells and tubulointerstitial cells mainly at outer medulla (OM), rather than cortex (Co) and inner medulla (IM). Nestin expression tends to be observed at the (**c**–**e**) dilated tubules and (**f**: arrowheads) spindle-shaped interstitial cells. (**f**: arrows) Nestin expression is more abundant at the basolateral plasma membrane and scant at apical pole of the tubular cells. Immunohistochemistry used counterstaining with periodic acid-Schiff. Immunofluorescence was counterstained in blue with DAPI. Original magnifications: (**a**) ×100, (**b** and **e**) ×400, (**c** and **d**) ×200, and (**f**) ×1000.

Time-course study of nestin expression and interstitial fibrosis after UUO

We then examined the temporal expression of nestin after UUO. At day 3 after UUO operation, only a small number of interstitial cells expressed nestin and no tubular cells were positive for nestin (Figure 2a–d). At day 7, nestin-positive interstitial cells were markedly increased and some nestin-positive tubular cells were detected. At day 13, the number of nestin-positive tubular cells increased as well as that of interstitial cells. As shown in Figure 2e and f, the numbers of nestin-positive interstitial cells and tubular cells were significantly correlated with the tubulointerstitial fibrosis score. The changes of nestin expression were further determined by Western blotting using whole kidney lysate after the UUO procedure (Figure 2g). Nestin expression was faint at day 0. Then it increased gradually in the UUO kidney, whereas no increase was noted in the contralateral kidney. As podocytes express nestin in the normal kidney[18,19] and their nestin expression is reported to be increased during puromycin aminonucleoside nephropathy,[19] we used kidney lysates without glomeruli, and performed Western blot analysis. Increased nestin expression was clearly confirmed in kidney lysates in which glomeruli were eliminated by sieving (Figure 2h).

Distribution of nestin-positive tubular cells in UUO

To determine which segment of tubular cells express nestin in UUO, we performed double immunostaining using several markers of nephron segments (Figure 3). Many nestin-