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A nest in renal fibrosis?

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Sakairi and collaborators show that some tubular cells as well as some interstitial myofibroblasts express the intermediate filament protein nestin. These findings evoke questions about the origin and role of these nestin-positive cells in the development of tubulointerstitial fibrosis.

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Renal tubulointerstitial fibrosis is, regardless of the initial insult, a histological hallmark of most forms of chronic kidney disease, and its presence has been strongly correlated to the progressive loss of renal function. The development of tubulointerstitial fibrosis in chronic kidney disease has therefore been associated with a poor long-term prognosis.[1] Even though the currently used therapeutic approaches slow down the progression of tubulointerstitial fibrosis, they do not specifically target tubulointerstitial fibrosis. A number of groups have recently focused their research toward better understanding of the development of tubulointerstitial fibrosis, which is steadily increasing our knowledge of this important event in the progression of chronic kidney disease.

Sakairi and collaborators[2] (this issue) identify the intermediate filament protein nestin as a new marker of the progression of tubulointerstitial fibrosis. Nestin was originally discovered in rat neuroepithelial stem cells[3] and, in further studies, was found to be expressed in rat, mouse, and human embryonic and fetal tissues. It was therefore initially thought that nestin was a typical marker of multilineage progenitors, but more recent studies showed that nestin is also expressed in adult tissues, mainly in areas of regeneration.[4] In addition, nestin was also shown to be induced in a variety of injured tissues, including the central nervous system, the liver, skeletal muscle, the pancreas, and odontoblasts.[4] In kidney development, nestin-positive progenitor cells have been detected in the mouse metanephric embryonic kidney,[5] and in the adult kidney nestin is selectively expressed in differentiated podocytes (Figure 1). As part of the cytoskeleton, nestin is probably involved in the stabilization of podocyte architecture.[5] More recently, it has been shown that nestin is upregulated in podocytes in an experimental rat model of podocyte injury.[6]

Now Sakairi *et al.*[2] present evidence of induction of nestin in an adult rat model of unilateral ureteral obstruction (UUO)-induced tubulointerstitial fibrosis. Notably, even though a complete obstruction of the ureter is rarely found in humans, the widely used UUO animal model has the advantage that it mimics, in an accelerated manner, the different stages leading to tubulointerstitial fibrosis: tubular atrophy, monocyte/macrophage infiltrate, tubular proliferation, apoptosis, epithelial-to-mesenchymal transition, and extracellular matrix accumulation.[7] In UUO-induced tubulointerstitial fibrosis, nestin expression was induced in both renal tubular cells and interstitial myofibroblasts. These nestin-positive tubular and interstitial cells mainly appeared in areas with severe tubular damage. This location is consistent with areas where a process called epithelial-to-mesenchymal transition (EMT), which produces myofibroblasts, is described as occurring.[8]

Although myofibroblasts are thought to be the main cell type responsible for the production of tubulointerstitial fibrosis,[8] their origins and activation are still a matter of debate. Current knowledge allows us to presume that interstitial myofibroblasts can have three origins: (1) local activation of resident fibroblasts, (2) infiltration from the circulation, and (3) EMT, a process that has been shown, in many animal models, to occur in an advanced stage of the development of tubulointerstitial fibrosis.[9] The most often used marker to show the presence of myofibroblasts is α-smooth muscle actin (α-SMA). It has also been shown that this cell is positive for other cytoskeletal proteins such as vimentin or desmin.[8]

Sakairi *et al.*[2] show that all nestin-positive cells that accumulated in the interstitium, 7 days after UUO, co-stained with α-SMA. Nestin- and α-SMA-positive cells continued to accumulate until day 13, the end point of the study. Together with the spindle-shaped morphological appearance of these cells, the data strongly suggest that these nestin-positive interstitial cells were myofibroblasts. Interestingly, not all α-SMA-positive interstitial cells stained positively for nestin, which suggests that nestin might be a marker of tubulointerstitial injury distinct from conventional markers such as vimentin, HSP47, and α-SMA. This indicates the existence of two populations of interstitial myofibroblasts: nestin positive and nestin negative. So why do some of these myofibroblasts express nestin? Does this mean that these cells are of a different origin than those that do not express nestin, and/or have a different function? It is possible that during chronic injury both nestin-positive and nestin-negative cells contribute to the development of interstitial fibrosis whereas in situations in which the injury is removed these cells respond differently. For example, because nestin is also a marker of progenitor cells, one can speculate that nestin-positive cells are more easily engaged in a possible reversion process. Further studies are necessary to elucidate this point.

Tubular nestin expression is concomitant with interstitial nestin expression. Furthermore, all nestin-positive tubular cells co-stained with the mesenchymal marker vimentin, whereas none stained positively for E-cadherin; this suggests that these 'tubular' cells are closer to the mesenchymal than the epithelial phenotype.

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These data are consistent with previous work on other tissues showing an upregulation of nestin when loss of intercellular contacts occurs.[4] However, not all vimentin-positive cells express nestin, which raises the same question as with nestin-positive and -negative myofibroblasts: do they differ in origin and/or function? For example, difference in nestin expression in tubular cells might change their destiny to death by apoptosis, as it has been shown, *in vitro*, that these transformed cells might be more susceptible than normal epithelial cells to apoptosis.[8]

The molecular mechanism of nestin upregulation during injury is not well understood.[4] Sakairi *et al.*[2] show (1) that hypoxic tubules during UUO express nestin, and (2) that, *in vitro*, hypoxia induces nestin expression in renal epithelial cells. However, it seems that hypoxia is not directly inducing nestin expression, but that nestin is induced secondary to the phenotypic changes induced by hypoxia. Sakairi *et al.*[2] also show that the well-known profibrotic transforming growth factor-β also stimulated *in vitro* nestin expression. Transforming growth factor-β-induced expression of nestin was observed together with increased α-SMA expression in cultured fibroblasts. Thus two events, important in the genesis of renal tubulointerstitial fibrosis, induce nestin: hypoxia and transforming growth factor-β expression. The detailed molecular mechanism of nestin induction seems more complex, though. Whereas nestin protein levels were found to be overexpressed in the obstructed kidney, no increase in nestin mRNA was observed. Data from the study by Sakairi *et al.*[2] argue for a decrease in proteasome activity concomitant with increased nestin protein expression that can explain this discrepancy. Finally, in contrast to what is found in malignant tumors, where upregulation of nestin is highly correlated with the elevated proliferation and migratory activity of malignant cells,[4] Sakairi *et al.*[2] mention that the nestin-positive cells in the obstructed kidney were not in a proliferating state, which suggests that nestin is expressed once the cell is in a more static state. Independently of the necessity to determine the precise molecular mechanism of nestin induction in tubulointerstitial fibrosis, it is important to note that, because advanced tubulointerstitial fibrosis leads to progressive hypoxia of renal tubules due to the accumulation of extracellular matrix, and thus to enlargement of the tubulointerstitial space, the upregulation of nestin in such fibrotic areas links nestin expression to advanced stages of the fibrotic process.

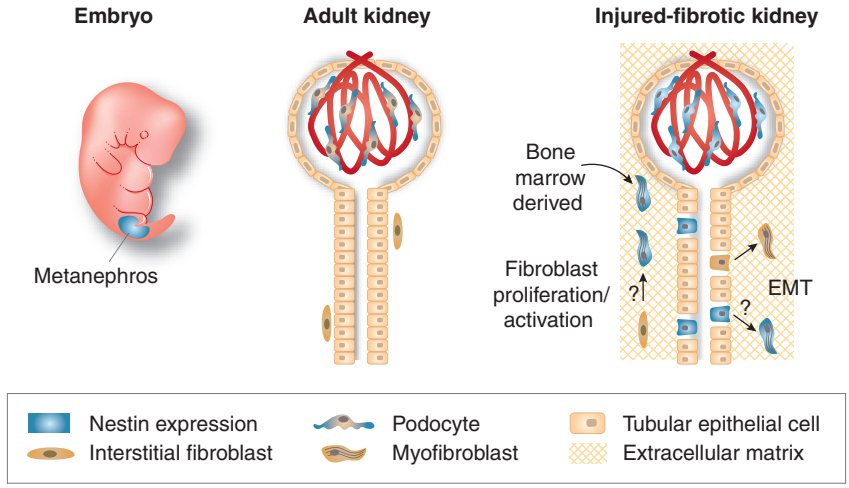

Figure 1 | Renal nestin expression under normal and pathological conditions. During embryogenesis, nestin progenitor cells are expressed in the metanephric mesenchyme mainly in the vascular cleft of the S-shaped body and in the vascular tuft of immature glomeruli of the capillary loop stage. In the newborn, transient nestin expression is localized in proximal tubular cells. In the adult kidney, nestin is expressed only in glomeruli — more precisely, in glomerular podocytes. Under pathological conditions, depending on the injury type, renal nestin upregulation can be observed in podocytes, and *de novo* nestin expression was observed in some tubular cells and myofibroblasts in areas with severe tubular damage and interstitial fibrosis.[2,6] Question marks indicate that the origin of the nestin-positive myofibroblasts remains to be clearly established. EMT, epithelial-to-mesenchymal transition.

In conclusion, Sakairi *et al.*[2] identify the intermediate filament protein nestin as a new marker of myofibroblasts and dedifferentiated tubular cells in renal tubulointerstitial fibrosis. The concomitant expression of nestin in both dedifferentiated tubular cells and myofibroblasts makes it tempting to speculate that nestin-positive myofibroblasts accumulate by EMT. However, this remains to be confirmed before nestin can be considered a new temporal and possibly functional marker for EMT in the pathological context of renal tubulointerstitial injury. The paper by Sakairi *et al.*[2] brings to mind the old, but nonetheless recurrent, questions of the reversion of renal fibrosis and the existence of a 'point of no return'[10] in progressive renal disease. Indeed, as nestin expression has been associated with multilineage progenitor cells,[4] which suggests potential regenerative abilities of this type of nestin-positive cell, nestin-positive mesenchymal cells might be possible targets for reversion of interstitial fibrosis once the (chronic) injury has been removed. In this context it would be interesting to verify if there is a difference in the ability of nestin-positive and -negative myofibroblasts to undergo mesenchymal-to-epithelial transition. On the other hand, as nestin is involved in the maintenance of the cytoskeletal structure and therefore could participate in the stabilization of the myofibroblasts' structure, nestin expression could be a sign of an engagement in the scarring process, which has often been suggested as the point of no return in chronic renal disease.

Further studies will thus be necessary to determine whether nestin is a marker of the establishment of a 'cozy nest' in which renal interstitial fibrosis progresses, or whether nestin, on the contrary, is a marker of cells that still have the potential to revert to their maternal phenotype.

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