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FIBROSIS

Targeting EMT to reverse renal fibrosis

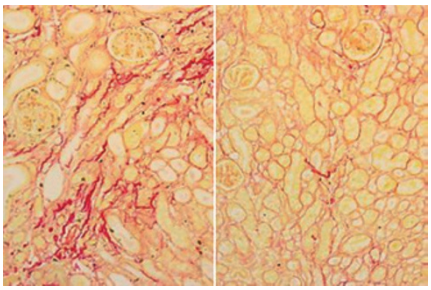
Epithelial-to-mesenchymal transition (EMT) of tubular epithelial cells is a feature of renal fibrosis, but the contribution of EMT to the myofibroblast population and the importance of EMT to the fibrotic process are unclear. Two studies published in *Nature Medicine* now show that partial EMT is required for the development of renal fibrosis. These studies demonstrate that the partial EMT response does not directly generate interstitial myofibroblasts but rather induces signals that stimulate myofibroblast differentiation and inflammation, suggesting that EMT could be targeted for the treatment of renal fibrosis. "In contrast to the situation in embryonic and cancer cells where the EMT endows cells with invasive properties, renal epithelial cells respond to the activation of EMT inducers by undergoing epithelial dedifferentiation but do not engage in delamination or invasion programmes," says Angela Nieto. "Thus our results help to reconcile previous apparently conflicting data with regard to the role of EMT in fibrosis."

The contribution of EMT to renal fibrosis is a matter of debate, with some studies indicating that renal epithelial cells contribute substantially to the myofibroblast population and others indicating only a marginal contribution. Previous work by Raghu Kalluri and colleagues showed that renal epithelial cells contribute only around 5% of the myofibroblast population. To assess the functional role of EMT in renal fibrosis, Kalluri and colleagues generated mice in which *Twist1* and *Snai1*—two transcriptional regulators of EMT—were deleted specifically in proximal tubular epithelial cells. They found that mice lacking these transcription factors had reduced EMT and were protected against multiple renal insults, including unilateral ureteral obstruction (UUO), with less renal fibrosis and better renal function compared to similarly treated wild-type mice. "We demonstrate that EMT programme acquisition by tubular epithelial cells is a form of cell injury and is associated with the loss of transporters," says Kalluri. "These events lead to a host repair response known as fibrosis."

As previous research had demonstrated a link between chronic kidney disease and inadequate epithelial cell cycle progression, Kalluri and colleagues used their conditional knockout mice to investigate the potential contribution of EMT to G2/M arrest of tubular epithelial cells. Using flow cytometry and immunohistochemical analyses, they found that deletion of *Twist1* and *Snai1* in tubular epithelial cells attenuated the cell-cycle arrest induced by UUO. In a mouse proximal tubular epithelial cell line, overexpression of *Twist1* and *Snai1* or treatment with TGF-β1 induced G2 arrest together with *Cdkn1a* (p21) expression, whereas silencing of *Cdkn1a* by RNA interference prevented TGF-β1-induced G2 arrest. "Once the EMT programme is initiated many things happen, including the loss of functioning epithelial cells needed for organ function and the arrest of cell proliferation that is important for cell and tissue regeneration," explains Kalluri.

Kalluri and colleagues also looked at the effects of *Twist1* and *Snai1* knockout on renal injury-induced inflammation and demonstrated that decreased EMT was associated with suppressed immune infiltration. In a separate study, Nieto and colleagues used mice in which activation of *Snai1* was inhibited in renal epithelial cells to similarly demonstrate an attenuated inflammatory response and protection from UUO and folic-acid-induced fibrosis in the absence of *Snai1* signalling.

Using fate-mapping techniques, Nieto *et al.* found that tubular epithelial cells do not delaminate from tubules to contribute to the myofibroblast population, even in the presence of *Snai1*, demonstrating that tubular epithelial cells undergo only a partial EMT in response to renal injury. Nieto says these findings show that reactivation of *Snai1* in tubular epithelial cells induces their dedifferentiation; these damaged tubular epithelial cells then relay crucial signals to the interstitium to promote myofibroblast differentiation and enhance inflammation, together promoting and sustaining renal fibrosis.



Inactivation of Snail1 (right) reverses UUO-induced renal fibrosis (left) in mice as assessed by Sirius red staining. Permission obtained from Nature Publishing Group © Grande, M. T. *et al. Nat. Med.* doi:10.1038/nm.3901.

To investigate whether fibrosis could be reversed, Nieto and colleagues targeted *Snai1* expression in mice following UUO by injecting antisense oligonucleotides. This approach ameliorated renal fibrosis, with kidneys from treated mice showing improved morphology with reduced inflammation and expression of *Tgfb1*. "Our data show that fibrosis can be reversed and support the use of anti-Snail1 and anti-EMT therapeutic strategies for the treatment of fibrosis. Further studies need to determine the point at which fibrosis can no longer be reversed by Snail inhibition," says Nieto. "These two studies refute the misconception that EMT is of no functional consequence to kidney dysfunction," adds Kalluri.

Susan J. Allison

Original articles Grande, M. T. *et al.* Snail1-induced partial epithelial-to-mesenchymal transition drives renal fibrosis in mice and can be targeted to reverse established disease. *Nat. Med.* doi:10.1038/nm.3901 | Lovisa, S. *et al.* Epithelial-to-mesenchymal transition induces cell cycle arrest and parenchymal damage in renal fibrosis. *Nat. Med.* doi:10.1038/nm.3902