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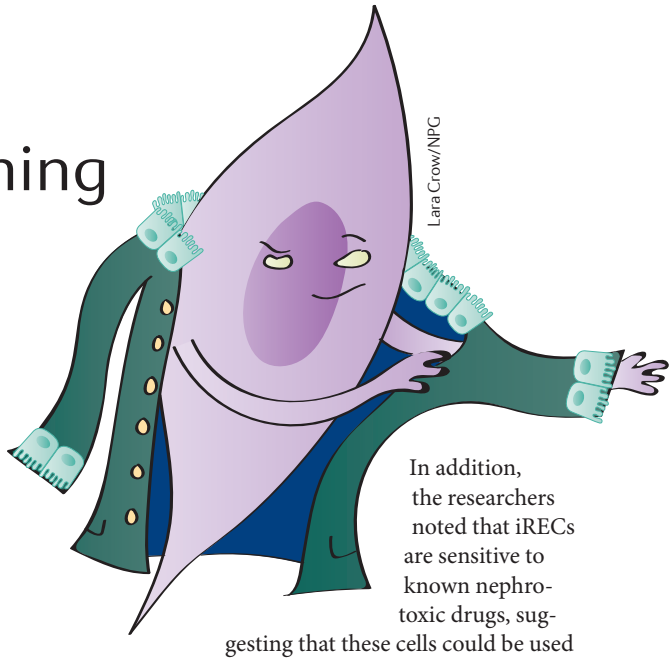
DEVELOPMENTAL BIOLOGY

Transcriptional reprogramming of kidney tubules



The direct programming of differentiated cells into organ-specific cell types bypasses pluripotency and might enable the development of models with which to test disease pathogenesis and regeneration. Using specific combinations of transcription factors, fibroblasts have been directly reprogrammed into various cell types, including neurons and cardiomyocytes, but not into renal cell types. A new study now demonstrates that the expression of four specific transcription factors is sufficient to convert mouse and human fibroblasts into induced renal tubular epithelial cells (iRECs), which exhibit morphological, transcriptional and functional properties of differentiated renal tubule cells. "Our approach of using four transcription factors to convert fibroblasts into iRECs bypasses any pluripotency, previously needed to generate kidney tissue from differentiated cells," explain researchers Soeren Lienkamp and Sebastian Arnold. "We think that direct reprogramming may be useful in many research areas, in particular in developing novel models of genetic tubular diseases *in vitro* and for testing novel drugs for potential side effects, which in principle could be done using patient-derived iRECs," adds researcher Michael Kaminski.

To identify transcription factors that could induce the differentiation of fibroblasts into renal cells, the researchers selected candidate factors on the basis of their high expression in renal tissue, their evolutionarily conserved expression during early nephrogenesis in *Xenopus* and mouse, and their essential function in renal development, as assessed by their association with congenital renal diseases in humans or renal loss-of-function phenotypes in mice. This selection process narrowed the number of potential reprogramming factors to 13, which were then tested for their ability to induce a renal cell fate. By eliminating one factor at a time, the researchers found four transcription factors — *Emx2*, *Hnf1b*, *Hnf4a* and *Pax8* — that converted mouse and human fibroblasts into cells that closely resembled renal tubule cells, which they termed iRECs.

Examination of the morphology of cultured iRECs revealed characteristics of epithelial cells, with formation of tight epithelial layers and expression of epithelial cell-adhesion transcripts. Using transcriptional profiling, the researchers demonstrated that primary renal tubular epithelial cells more closely resembled iRECs than mouse embryonic fibroblasts, and that iRECs more closely resembled kidney tissue than any other tissue type. They also identified elevated expression of transcripts for tubular ion channels and transporters, and protein expression of the α1 subunit of the Na^+/K^+-ATPase (ATP1A1) and aquaporin 1 in iRECs.

To assess the morphological and functional properties of iRECs, the researchers characterized them in 3D culture, where iRECs formed spheres with polarized cuboidal cells linked by tight junctions and extended luminal microvilli from their apical surface. The cells also exhibited correct localization of the tight junction protein, ZO-1, as well as β-catenin, ATP1A1 and megalin, and possessed evidence of endocytic activity.

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In addition, the researchers noted that iRECs are sensitive to known nephrotoxic drugs, suggesting that these cells could be used to screen nephrotoxic agents.

The researchers finally assessed the ability of iRECs to aggregate and self-organize into renal organoids. They found that iRECs integrated into the tubule epithelial structures of organoids generated from embryonic kidneys, forming a continuous basement membrane layer with neighbouring epithelial cells. In the absence of other cells, iRECs seeded onto decellularized kidney scaffolds grew into long and partially convoluted tubules, demonstrating the potential of iRECs to repopulate along preformed guidance structures.

The researchers say their study is the first to convert fibroblasts directly into tubule cells. "In addition to providing a model to study hereditary tubular diseases and assess drug toxicity, we think that the selection criteria used to find the reprogramming factors for the kidney could be used to find reprogramming factors for other cell types, including those of other organs," says Lienkamp.

Susan J. Allison

ORIGINAL ARTICLE Kaminski, M.M. *et al.* Direct reprogramming of fibroblasts into renal tubular epithelial cells by defined transcription factors. *Nat. Cell Biol.* http://dx.doi.org/10.1038/ncb3437 (2016).