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PERSPECTIVES

The role of hypoxia-inducible factors in renal fibrosis

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Chronic kidney disease (CKD), defined by loss of renal function, is highly prevalent, and imposes a large economic burden worldwide. Renal fibrosis is the final common pathway leading to CKD regardless of the initial insult. The extent of renal fibrosis is highly correlated with the severity of CKD. Understanding the pathophysiology of renal fibrosis is of paramount importance.

Microscopically, four major pathological features are seen in renal fibrosis: interstitial extracellular matrix (ECM) deposition, cellular infiltration, tubular atrophy, and capillary rarefaction.[1,2] ECM deposition may be the most prominent feature. The major components identified in fibrotic ECM are collagen type I, collagen type III, and fibronectin. In our previous study, we showed that renal pericytes/perivascular fibroblasts are the major source of scar-producing myofibroblasts in the progressively fibrotic kidney.[1] Many argue that damaged tubular epithelial cells undergo epithelial-to-mesenchymal transition (EMT) and are responsible for renal fibrosis. However, the phenomenon of EMT was not observed *in vivo* in a robust model of the genetic fate-tracing technique.[1] In line with our findings, pericytes have been identified as the major progenitors of the scar tissue in many organs including the central nervous system, colon, liver, and skin.[1]

Hypoxia-inducible factor (HIF) is a DNA-binding transcription factor activated under hypoxic conditions. It is composed of a constitutively expressing β-subunit and an oxygen labile α-subunit. Three types of α-subunit have been discovered, namely HIF-1α, HIF-2α, and HIF-3α. The function of HIF-1α and HIF-2α is currently being intensively investigated, while the role of HIF-3α is less well known. Under normoxic conditions, oxygen stimulates hydroxylation of HIF-α by prolyl-hydroxylase domain (PHD) containing enzymes. The hydroxylated HIF-α is recognized by von Hippel-Lindau tumor suppressor protein (pVHL) and subsequently ubiquitylated by the Elongin BC/Cul2/pVHL ubiquitin–ligase complex. The ubiquitylated HIF-α is then sent to the proteasome for degradation. Under hypoxic conditions, the PHD-mediated hydroxylation of HIF-α is inhibited. The stabilized HIF-α binds to HIF-β and together they translocate into the nucleus, where the dimer binds to hypoxia-responsive elements and induces enhanced transcription of the target genes. Many target genes have been implicated, including erythropoietin, vascular endothelial cell growth factor (VEGF), heme oxygenase 1, tissue inhibitor of metalloproteinase, connective tissue growth factor, and nitric oxide synthase.

The role of HIFs in CKD is obscure. Although some animal studies showed beneficial effects of HIF activation in CKD, others have yielded conflicting results. Possible explanations for this discrepancy include the nature and duration of the animal models as well as the methods for manipulating HIF activity. Tanaka et al demonstrated that global activation of HIFs by cobalt chloride can preserve peritubular capillaries and reduce tubulointerstitial injury in rats with renal mass reduction.[3] However, Norman et al demonstrated that hypoxia can increase the production of

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collagen along with upregulation of HIF-1α in human renal fibroblasts, and Higgins et al found that conditional knockout of HIF-1α in proximal tubular epithelial cells of transgenic mice receiving unilateral ureteral obstruction (UUO) attenuates renal fibrosis.[4,5] Schietke et al also found that constitutional transgenic overexpression of HIF-2α in distal tubular cells in mice results in renal fibrosis.[6] However, these studies should be interpreted with caution. HIF-1α and HIF-2α may possibly have different functions and the result of activation of HIF may be cell type and context dependent. As demonstrated by Rosenberger et al,[7] upon insult, HIF-1α is mainly expressed in renal cortical tubular cells, medullary interstitial cells, and endothelial cells. The expression of HIF-2α is discernible in renal cortical interstitial cells and endothelial cells, with a distribution nearly complementary to that of HIF-1α. Since pericytes represent the majority of cortical interstitial fibroblasts which express HIF-2α rather than HIF-1α and pericyte—myofibroblast transition is a novel target to attenuate renal fibrosis,[1] we propose that HIF-2α may be a better candidate than HIF-1α for studying renal fibrosis and targeting pericyte—myofibroblast transition.

In normal kidney, pericytes maintain microvascular stability by secreting angiogenic factors for endothelial cells, whereas pericyte-derived myofibroblasts lose their ability to support microvascular stability by detaching from the normal peri-endothelial position and even by secreting dysangiogenic factors.[1,2] In healthy kidney, VEGF164 is the abundant isoform that binds to the VEGF receptor on endothelial cells, but during fibrotic injury pericyte-derived myofibroblasts switch to synthesis of VEGF120 and VEGF188, isoforms known to lead to dysangiogenesis in tumors and developing organs.[2] It is noteworthy that mice with genetic ablation of HIF-2α in endothelial cells have shown impaired angiogenesis and blood flow recovery after femoral artery ligation, as demonstrated by Skuli et al.[8] Given the close contact of pericytes and endothelial cells and the crosstalk observed in previous studies,[1,2] we propose that HIF-2α plays an important role in stabilizing the microvasculature by pericytes through alteration of HIF-targeted angiogenic factors.

Since HIF serves as a master regulator that controls adaptation to hypoxia by regulating the expression of genes involved in angiogenesis, cell proliferation, immunity, and ECM production, specific manipulation of HIFs in kidney pericytes is needed to elucidate their detailed role in healthy and diseased kidneys. It would be of great interest to determine the role of HIF-2α, which is specifically expressed in pericytes, during the progression of renal fibrosis. Taking advantage of the current advanced state of mouse genetics, future study should be directed at the conditional manipulation of HIF-2α in pericytes to clarify its role in microvascular stability, inflammation, and ECM production. As the mechanism of renal fibrosis is better elucidated, it may be possible to halt or even reverse the progression of renal fibrosis and CKD.

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