ELUCIDATING THE MECHANISM OF HYPOXIA-INDUCIBLE FACTOR 1 (HIF-1) REGULATION IN KIDNEY

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Introduction and Aims: Tubulointerstitial hypoxia is a critical mediator in the pathogenesis of kidney disease. In light of accumulating knowledge on protective roles of HIF-1, we aimed to elucidate the mechanism of HIF-1 regulation in kidney.

Methods: An shRNA library was created against hypoxia-inducible genes screened from a microarray analysis of rat chronic hypoxia model, renal artery stenosis model. The impact of candidate genes on HIF-1 was evaluated in vitro, leading to identification of novel upregulators of HIF-1. Its regulation of HIF-1 and the underlying mechanisms were investigated in human proximal tubular cells (HK-2). Furthermore, we attempted to characterize the inflammatory nature of this gene and link inflammation to the HIF response.

Results: An shRNA library experiment identified 5 novel HIF-1 regulating genes including CCAAT/enhancer binding protein delta (CEBPD), a transcription factor, which we focused further. CEBPD was induced in kidneys subjected to systemic hypoxia, as well as in models of acute and chronic hypoxic kidney injuries, with predominant expression in the nuclei of proximal tubular cells, the most susceptible portion of kidney to hypoxia. CEBPD was expressed in tubular cells of the outer stripe of the inner medulla, a physiologically hypoxic area, too. In vitro, CEBPD siRNA knockdown and overexpression mediated down- and upregulation of HIF-1α as well as its target genes. Mechanistically, promoter and ChIP assay confirmed that CEBPD directly promoted the transcription of HIF-1α (-227). Notably, CEBPD was rapidly inducible by inflammatory cytokines, such as interleukin-1β, in an NF-κB-dependent manner, and was indispensable for the non-hypoxic induction of HIF-1α.

Conclusions: These results demonstrate CEBPD as a novel HIF-1 regulator in kidney. CEBPD was up-regulated in tubular cells via NF-κB-dependent pathway and regulated HIF-1 expression and its transcriptional activity. This pathway provides a novel insight into regulation of HIF-1 in ischemic kidney, characterized by co-existent hypoxia and inflammation.