Epigenetic Changes Induced by Hypoxia-Inducible Factor: a Long Way Still To Go as a Target for Therapy?

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MicroRNA (miR) are small noncoding RNA molecules that regulate gene expression and play important roles during kidney development, homeostasis, and disease. Novel contributions to the field of cell death, cell cycle, and miR regulation in the kidney have come from the Laboratory of Dong, and in the present issue of JASN, a study from this laboratory clarifies a role of miR-687 as a key regulator and therapeutic target in acute ischemic injury to the kidney.1 AKI is associated with high morbidity and mortality and is closely intertwined with CKD, with each disease serving as a risk factor for developing the other and sharing causes and other risk factors in common. Furthermore, ischemia is a major cause of AKI, and hypoxia serves as a final common pathway to end stage kidney failure. Therefore, their choice of a model is appropriate.

The authors used microarrays and profiled miR expression in kidney tissues rendered to ischemia reperfusion. Among the miR, miR-687 showed a highest up-regulation at 12 hours of reperfusion. Their beautiful in situ hybridization study localized miR-687 induction predominantly in the cells of renal cortical tubules.

Then the authors studied the upstream of miR-687 induction and found that miR-687 induction was mediated by a transcriptional factor, hypoxia-inducible factor-1 (HIF-1), that plays an integral role in the body’s response to low oxygen concentrations.2,3 The amount of HIF-1 is regulated mainly at the degradation of HIF-1α via hydroxylation of the proline residues and subsequent polyubiquitylation by the von Hippel–Lindau protein that acts as its E3 ligase. This proline hydroxylation is regulated by oxygen-dependent activity prolyl hydroxylases (PHD) that belong to the family of iron- and 2-oxoglutarate-dependent dioxygenase enzyme, and hypoxia inactivates PHD, allowing HIF-1α to escape degradation. Then the α subunit translocates to the nucleus where it forms a heterodimer with a constitutively expressed β-subunit and transactivates 100–200 target genes. The representative HIF target genes include those involved in erythropoiesis (e.g., erythropoietin) and those involved in angiogenesis (e.g., vascular endothelial growth factor) to increase oxygen delivery to tissues. The authors used HIF-1α-null mouse embryonic fibroblasts and proximal tubule-specific HIF-1α knockout mice, confirming HIF-1-dependent miR-687 induction. This demonstration of specificity is important because HIF has two active isoforms, HIF-1 and HIF-2. HIF-1α and HIF-2α have 48% amino acid sequence identity, but they are often nonredundant and have distinct target genes. This isoform specificity can explain tubular specific localization of miR-687 because HIF-1α is expressed in tubular cells and HIF-2α is expressed in endothelial cells and interstitial cells in the kidney.4

To investigate the downstream pathway of miR-687, the authors predicted targets of miR-687 using several databases and confirmed phosphatase and tensin homolog (PTEN), a modulator of cell cycle and cell death, as the downstream gene target. As previously described, HIF-1 is a master regulator of adaptive responses against hypoxia, and their functional studies using cultured tubular cells suggested that miR-687-mediated downregulation of PTEN facilitated cell cycle progression for tubular cell proliferation and kidney repair during hypoxia. Previous studies of experimental animals demonstrated protective effects of pharmacologic HIF activation against ischemia reperfusion injury of the kidney, and miR-687-mediated downregulation of PTEN may be one of the mechanisms of renoprotection by HIF activation.5,6

However, the authors’ functional studies of mice showed paradoxical results. The authors used locked-nucleic-acid oligonucleotides to neutralize the effects of miR-687 in vivo. Mice treated with anti-miR-687 developed significantly less kidney injury as evidenced by the examination of physiologic parameters, histology, and renal apoptosis. Therefore, blockade of miR-687 and preservation of PTEN expression resulted in protection against kidney injury in vivo. Although HIF-1 is the most potent defensive mechanism against hypoxia, it regulates a number of pathways. It is possible that miR-687 induced by HIF-1 may provoke proapoptotic responses in a PTEN-independent manner. The presence of other types of cells beside tubular cells may explain the discrepancy from the in vitro experimental system composed of exclusively tubular cells. In addition, various stresses, such as oxidative stress and endoplasmic reticulum stress interact...
together, complicating the disease manifestations after ischemia reperfusion injury in animals. Currently, a number of PHD inhibitors to active HIF and upregulate erythropoietin, a representative target of HIF, are currently undergoing clinical trials of anemia in CKD. This study suggests context-dependent effects of miR-687 induced by HIF-1. Context-dependent outcomes of HIF activation therapy were also reported in the remnant kidney model, a representative model of chronic kidney failure. These findings emphasize the importance of understanding the complicated downstream pathways for estimation of efficacy and safety of HIF activation therapy.

Demonstration of epigenetic changes regulated by HIF-1-dependent miR expression is pathophysiologically important. Epigenetic regulation includes changes in DNA methylation, histone modifications, chromosomal conformational changes, and alteration in miR expression. Although the study clearly demonstrated a critical role of miR induced by HIF-1, recent studies also showed other epigenetic regulation mechanisms by HIF-1, such as histone modification and chromosomal conformational change. HIF-1 target genes include histone lysine demethylases (lysine (K)-specific demethylases or Jumonji C lysine demethylases). Lysine (K)-specific demethylases belong to the family of iron- and 2-oxoglutarate-dependent dioxygenase enzyme like PHD, suggesting an intricate link between oxygen tensions and histone modifications. Oxygen-dependent epigenetic regulation is a focus of intensive research today.

There are some unanswered questions. A previous study showed that loss of PTEN increases the transcriptional activity of HIF-1 through the inactivation of Forkhead transcription factors, and it remains to be determined whether the miR-687-mediated downregulation of PTEN by HIF-1 makes up a positive feedback loop. Our previous study showed a protective role of miR-205 against hypoxia reoxygenation via the suppression of PHD1. As previously described, PHD controls the amount of HIF-1, suggesting a role of miR-205 in regulation of HIF-1. Furthermore, HIF-1 also regulates various miR, and miR-21 is one of the most intensively studied miR regulated by HIF-1. The complex network of HIF-1 and miR is a critical subject for future studies.

PTEN is also known to be involved in a network of interactions with p53, which transcriptionally activates genes involved in cell cycle control and apoptosis. PTEN regulates p53 protein levels and transcriptional activity through both phosphatase dependent and independent mechanisms. A crucial role of p53 in tubules damaged by ischemia reperfusion has been reported by the authors’ group and others, and a role of p53 in the HIF-1/miR-687/PTEN signaling pathway is an important issue to be pursued.

Finally, hypoxia is a condition of energy depletion caused by disturbance of aerobic respiration of mitochondria. HIF-1 plays a critical role in energy metabolism, reprogramming cellular metabolism toward enhanced glycolysis and reduced oxidative phosphorylation (i.e., Warburg effect). Recent studies clarified the functional role of HIF-1-induced large intergenic ncRNA-p21 (lincRNA-p21) in this metabolic reprogramming. LincRNA-p21 is rapidly induced by HIF-1α, and lincRNA-p21 then disrupts the HIF-1α von Hippel–Lindau interaction, stabilizes HIF-1α, and increases expression of HIF-1α-responsive genes (e.g., glycolytic enzymes Glut1 and LDHA), thereby promoting glycolysis and reprogramming cellular metabolism. Regulation of energy metabolism by the HIF-1/miR-687/PTEN signaling pathway should be investigated in the future.

This study is well-conducted with sophisticated techniques, validating the observations using independent methodologies from various aspects. Thomas Edison gave us the aphorism “Everything comes to him who hustles while he waits” that emphasizes the importance of the energetic and ongoing pursuit of a given goal. The work of Bhatt and colleagues is a wonderful example of how the vigorous and unremitting exploration of mechanisms underlying acute ischemic injury can lead to rewarding insights, including those, as shown in this study, regarding miR-687 and HIF delineation of the novel HIF-1/miR-687/PTEN signaling pathway providing new insights into the understanding of the pathogenesis of AKI to develop novel therapeutic approaches.

DISCLOSURES
None.

REFERENCES


