Got glycogen? An energy resource in HIF-mediated prevention of ischemic kidney injury

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Hypoxia-inducible factor activation reprograms glucose metabolism and leads to glycogen accumulation in multiple cell types. In this issue of *Kidney International*, Ito and colleagues demonstrate that pharmacologic inhibition of hypoxia-inducible factor–prolyl hydroxylase domain oxygen sensors in renal epithelial cells enhances glycogen synthesis and protects from subsequent hypoxia and glucose deprivation. *In vivo* studies advance the concept that renal glycogen metabolism contributes to cytoprotection afforded by pre-ischemic hypoxia-inducible factor–prolyl hydroxylase domain inhibition.

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Over the past 25 years, the field of oxygen sensing and hypoxia-inducible factor (HIF) biology has made tremendous progress, culminating in the discovery of 3 HIF-prolyl hydroxylase domain (PHD) oxygen sensors and the development of compounds that reversibly inhibit PHDs and activate HIF signaling for the treatment of anemia.[1] The central role of the HIF/PHD pathway in cellular adaptation to hypoxia and its innovative potential for clinical translation were recently recognized by the 2019 Nobel Prize in Physiology and Medicine awarded to Professors Gregg L. Semenza, Sir Peter J. Ratcliffe, and William G. Kaelin, Jr. for their groundbreaking work on deciphering the molecular underpinnings of oxygen-dependent gene regulation. Although current clinical development has focused largely on HIF-activating compounds in renal anemia therapy, strong preclinical data suggest that reversible HIF activation may be therapeutically useful in other areas of clinical medicine. One of those areas is the prevention of ischemic organ injuries, as HIF activation has been shown to mimic the beneficial effects of ischemic preconditioning.[2]

HIF is a heterodimeric transcription factor that is central to cellular adaptation to hypoxia. It consists of an oxygen-sensitive α-subunit (either HIF-1α, HIF-2α, or HIF-3α) and a constitutively expressed β-subunit, HIF-β. Although HIF-α subunits are continuously synthesized, they are rapidly degraded under normoxia; that is, HIF-α is usually not detectable in normoxic cells, and HIF transcription factors are not formed.[2] HIF-α degradation is initiated by HIF-PHDs, which function as cellular oxygen sensors and utilize molecular oxygen and Kreb's cycle metabolite 2-oxoglutarate (2OG) for the hydroxylation of specific proline residues within HIF-α (Figure 1).[3] Of the 3 HIF-PHDs, PHD2 is the main HIF-PHD responsible for HIF-α degradation under normoxia in most cells. HIF-PHDs can be reversibly inhibited with structural analogs of 2OG, resulting in cellular HIF-α accumulation, the formation of HIF transcription factors, and increased transcription of HIF-regulated genes.[2]

Pre-ischemic HIF activation by genetic or pharmacologic means has been shown to protect the kidney from ischemia-reperfusion injury (IRI) involving several cell types and different mechanisms.[4] Ito and colleagues now propose that increased glycogen synthesis in renal epithelium induced by pre-ischemic pharmacologic HIF activation significantly contributes to the prevention of renal IRI.[3] The authors' work is mostly based on use of an oxygen-glucose deprivation (OGD) cell culture model (0.1% O_2 and glucose-free medium), in which pretreatment of renal epithelial cells with pan-HIF-PHD inhibitor (HIF-PHI) enarodustat (JTZ-951) resulted in protection from oxidative stress and cell death—that is, led to a reduction in reactive oxygen species (ROS) production and increased cell viability in an autophagy-independent manner. Using small interfering RNA (siRNA), the authors established that inhibition of PHD2 was sufficient to achieve cytoprotection and that enarodustat stimulated glycogen synthesis *in vitro* via a HIF-1–dependent increase in the expression of *phosphoglucomutase* (*PGM*) *1*, *glycogen synthase* (*GYS*) *1*, and *1,4-alpha glucan branching enzyme (GBE) 1* (Figure 1). Although Ito and colleagues did not generate direct support for their cell culture–based observations *in vivo*, for example, by use of a specific GYS1 inhibitor or a genetic model, administration of glycogenolysis inhibitor MOR-14 partially abrogated the

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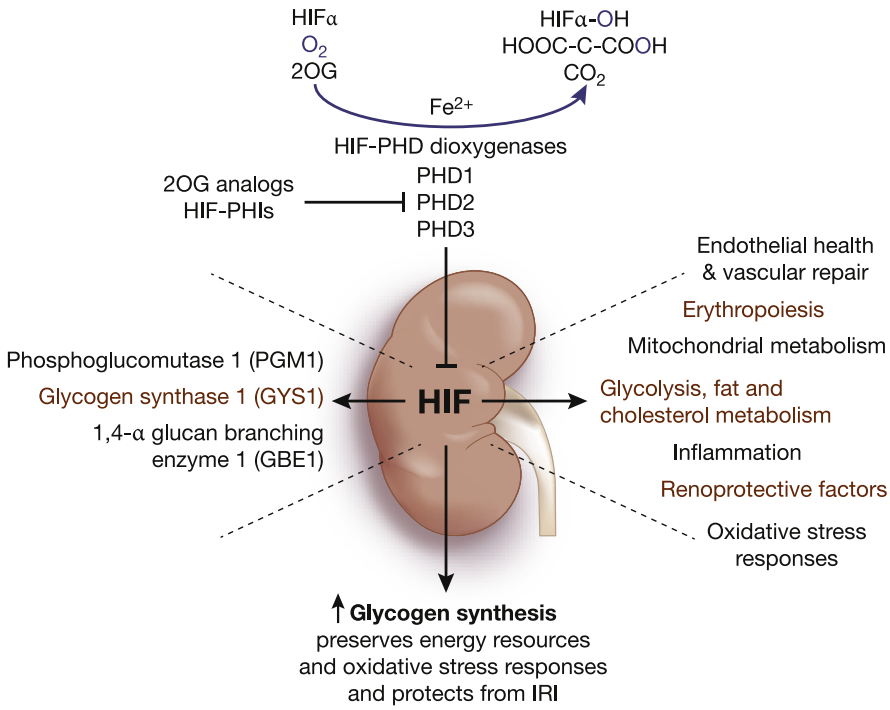

Figure 1 | Overview of hypoxia-inducible factor (HIF)–regulated mechanisms involved in renoprotection. HIF-α is constitutively synthesized but rapidly degraded in the presence of molecular O_2. Degradation of HIF-α is initiated by prolyl hydroxylation. Three 2-oxoglutarate (2OG)–dependent prolyl hydroxylase domain (PHD) dioxygenases (PHD1, PHD2, and PHD3) utilize molecular O_2 and 2OG for the hydroxylation of specific proline residues within HIF-α; this reaction requires ferrous iron (Fe^{2+}) and also results in the formation of succinate (HOOC-C-C-COOH) and CO_2. Hypoxia reduces HIF-PHD catalytic activity resulting in HIF-α stabilization, formation of the HIF transcription factor (HIF-α/β heterodimer), and activation of cellular HIF signaling. HIF-PHDs are reversibly inhibited by structural 2OG analogs (HIF-prolyl hydroxylase inhibitors, HIF-PHIs), which results in the activation of HIF signaling irrespective of O_2 levels. In many cell types, the inhibition of PHD2 alone is sufficient for the activation of HIF responses. HIF regulates multiple processes that facilitate oxygen delivery and oxygen utilization and mediate cytoprotection and tissue repair. Ito and colleagues demonstrated that HIF-1 activation stimulated glycogen synthesis *in vitro*, which was associated with the increased expression of *GYS1*, *PGM1,* and *GBE1*, whereas *in vivo*, only the expression of *GYS1* was increased following treatment with HIF-PHI enarodustat.[3] IRI, ischemia-reperfusion injury.

protective effects of HIF-PHI pretreatment in a rat model of renal IRI. These findings provide support for a role of glycogen metabolism in HIF-PHI–mediated protection from renal IRI *in vivo*.

The inducing role of hypoxia and HIF-1 in the regulation of glycogen storage has been previously established in neoplastic and non-neoplastic cells and occurs at multiple levels of glycogen metabolism.[5] When glucose is taken up by cells, it is phosphorylated and converted from glucose-6-phosphate to glucose-1-phosphate (PGM1), and then via the action of UTP:glucose-1-phosphate uridylyltransferase (UGP) 2 is conjugated to UDP at carbon position 1 (UDP-glucose). Activated UDP-glucose is then incorporated into glycogen particles through α1-4 linkage (GYS1). Once a certain chain length has been reached, the outer α1,4-linked glucosyl unit is transferred to form an α1-6 glycosidic bond on the same or an adjacent chain (GBE1), thereby enhancing glycogen solubility. Whereas hypoxia and/or HIF stimulate the expression of genes encoding enzymes directly involved in glycogen synthesis (*PGM1*, *UGP2*, *GYS1*, and *GBE1*), HIF-regulated protein phosphatase 1 regulatory subunit 3C (PPP1RC3) increases glycogen accumulation, presumably by inhibiting the catalytic activity of glycogen phosphorylase, which is the rate-limiting step in glycogenolysis.[5] Taken together, these findings provide support for the concept that hypoxia via HIF-1, in addition to promoting glucose uptake and shifting metabolism toward increased glucose consumption and glycolysis, promotes glucose and energy storage in the form of glycogen. This response may appear paradoxical at first, as cells are less likely to divert energy resources to biochemical storage reactions when challenged energetically under acute hypoxic conditions. However, once adaptation to prolonged hypoxia and metabolic reprogramming has occurred, cells will need to rebuild glycogen stores. Increased glycogen reserves would allow cells to better cope with subsequent hypoxic and/or glucose-depleted conditions by increasing adenosine triphosphate availability. In the setting of cancer, this has been proposed to help neoplastic cells survive and metastasize in *harsh* microenvironments.[5]

In contrast to liver and skeletal muscle, kidneys are not a major storage site for glycogen, as renal epithelial glycogen levels are usually negligible under physiological conditions. However, abnormal glycogen accumulation in the kidney can result from genetic defects in glucose transport or in glucose and glycogen metabolism, and is found, for example, in Fanconi-Bickel syndrome and von Gierke's glycogen storage disease. Furthermore, glycogen accumulation is a well-described metabolic feature of clear cell renal cell carcinomas,[5] which are characterized by chronic HIF activation due to mutations in the von Hippel-Lindau tumor suppressor. It can also occur under diabetic conditions.[6] In the latter case, epithelial glycogen accumulation can be transient, or in the worst-case scenario, lead to periodic acid–Schiff–positive and diastase-sensitive Armanni-Ebstein lesions.[7] In diabetes, glycogen is commonly found in the distal nephron but can also accumulate in proximal tubules depending on the degree of hyperglycemia and diabetes duration.[6]

Whereas the proximal nephron produces glucose from glutamine and preferentially utilizes free fatty acids for adenosine triphosphate generation, adenosine triphosphate generation in distal nephron segments is more glucose-dependent, which explains the distal glycogen accumulation in diabetic nephropathy. In the context of HIF-PHI therapy for the prevention of renal IRI, it is important to determine which nephron segments are stimulated to synthesize glycogen. Susceptibility to ischemia differs along the nephron, and it is not clear to what degree sensitivity to ischemia correlates with a reduced ability to synthesize glycogen. Currently, the dynamics of glycogen metabolism and regional distribution of glycogen synthesis in the hypoxic kidney are not well understood and warrant further exploration.

Strong preclinical data support the use of systemically administered HIF-PHIs for the prevention of acute kidney injury and its transition to chronic kidney disease. The molecular mechanisms that underlie HIF-induced renoprotection in acute kidney injury are complex and involve multiple processes and factors including renal angiogenic, anti-inflammatory, and vasomotor responses and probably also remote effects in other organs (Figure 1).[4] A major mechanism, however, which is likely to play a key role in the immediate response to ischemic injury, is HIF-induced reprogramming of renal metabolism.[8] This, depending on cell type, occurs at multiple levels and involves glucose, fatty acid, amino acid, mitochondrial metabolism, and other metabolic processes. A HIF-1–induced shift toward increased glucose uptake and glycolysis, which occurs in hypoxia and is known as the Pasteur effect, permits cells to generate adenosine triphosphate when the ability to perform oxidative phosphorylation is restricted, provided sufficient glucose is available. Given this, a direct correlation would be predicted between glycogen store size and cell survival, that is, clinical outcome of acute kidney injury, which is not investigated in the study by Ito and colleagues. To what degree enarodustat, which is a daily administered HIF-PHI currently in clinical development for renal anemia,[1] increased renal glycogen content in rats prior to IRI is unclear. Therefore, the optimal level of renal glycogen content, as glycogen can be toxic to cells, and the duration and timing of HIF-PHI administration to achieve maximal protection from ischemia will have to be established in future studies.

Several HIF-PHIs are currently in advanced clinical development for renal anemia, with one compound already being licensed in China and Japan. Given that multiple biochemical processes and factors are involved in HIF-mediated ischemic preconditioning, induction of a short-term broad transcriptional HIF response is desirable. This is unlikely to be accomplished with current HIF-PHI dosing strategies, which are designed for long-term treatment of renal anemia, where the activation of broad HIF responses is not desirable.[1] Furthermore, the development of compounds that preferentially activate HIF-1 responses may be clinically advantageous in this context, as erythropoietic responses are mostly mediated by HIF-2.[9]

In summary, work by Ito and colleagues highlights the importance of glycogen as a cellular energy source that can be mobilized when renal epithelial cells are faced with hypoxia and glucose deprivation and identifies a metabolic mechanism that is likely to contribute to HIF-mediated protection from renal IRI. Furthermore, their work adds to previously published studies, which taken altogether provide a strong preclinical basis and rationale for performing clinical trials with HIF-PHIs for the prevention of IRI in native or transplanted kidneys.

DISCLOSURE

The author declared no competing interests.

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