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See related article, "Nephrin Contributes to Insulin Secretion and Affects Mammalian Target of Rapamycin Signaling Independently of Insulin Receptor," on pages 1029–1041.

## The Dawning of a New Day in CKD Anemia Care?

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Oxygen first appeared in the Earth's atmosphere around 2–2.5 billion years ago, and with it, eukaryotic (mitochondria-containing) organisms emerged. As oxygen levels rose further, the relative efficiency of aerobic metabolism permitted the development of increasingly complex organisms. In humans, cellular hypoxia is a key component of many, if not most, disease processes. The last 25 years have seen a huge increase in our understanding of how cells react and adapt to changes in oxygen availability. The key elements of cellular oxygen sensing machinery are the hypoxia-inducible factor (HIF) transcription factors, of which there are two well understood isoforms (HIF1 $\alpha$  and HIF2 $\alpha$ ), the prolyl hydroxylase (PHD) enzymes, of which there are three isoforms (PHD1–PHD3), and the asparaginyl hydroxylase, factor inhibiting hypoxia-inducible factor1 $\alpha$  (FIH).<sup>1,2</sup>

The oxygen sensitivity of the HIF pathway is conferred by the PHD and FIH enzymes, which require oxygen to function and effectively switch off in response to decreases in cellular pO<sub>2</sub>. HIF1 $\alpha$  mRNA and protein are continually produced in all cells. However, in the presence of cellular oxygen, HIF1 $\alpha$  protein is immediately hydroxylated by the active PHDs,

bound by the E3 ubiquitin ligase Von Hippel–Lindau protein (VHL), and degraded in the proteasome. The efficiency of this process is such that intact HIF1 $\alpha$  protein cannot be identified in cells cultured in normoxic conditions using Western blot. HIF1 $\alpha$  hydroxylation by active FIH acts as further insurance against *HIF* gene transcription in normoxia by inhibiting HIF-cofactor interaction. However, when oxygen levels fall, the hydroxylases become inactive, allowing HIF1 $\alpha$  protein to accumulate in the cell. HIF1 $\alpha$  then translocates to the nucleus, where along with cofactors ARNT and CBP/p300, it binds to hypoxia response elements in the promoter regions of its target genes.<sup>2</sup> Over 500 HIF binding sites have been identified in the human genome.<sup>3</sup>

In simple terms, *HIF* target genes act to buffer against hypoxic cell stress by better matching oxygen supply with demand through (1) improving cellular oxygenation and (2) decreasing cellular oxygen utilization. The former is mediated through enhanced red blood cell production (erythropoietin and transferrin), vasodilation (nitric oxide synthase), and angiogenesis (vascular endothelial growth factor [VEGF]); the latter is achieved through shifting cell metabolism toward and enhancing the capacity for anaerobic glycolysis (pyruvate dehydrogenase kinase 1 and glucose transporter 1) and streamlining cells through autophagy (BCL2/adenovirus E1B 19-kD protein-interacting protein 3).<sup>2,4</sup>

HIF1 $\alpha$  and HIF2 $\alpha$  are regulated in a broadly similar manner but differ in terms of their cofactor interaction, expression patterns, and target genes. HIF1 $\alpha$  is expressed in all cell types. HIF2 $\alpha$  is much more selectively expressed, with functions including the coordinated control of erythropoiesis and iron metabolism. HIF1 $\alpha$  and HIF2 $\alpha$  have opposing effects on cell cycle regulation: inducing cell cycle arrest and proliferation, respectively. The three PHD isoforms have overlapping but nonredundant functions as evidenced by their different tissue expression patterns and distinct animal knockout phenotypes.<sup>5</sup> Important roles for the PHD enzymes outside of the HIF pathway are increasing recognized.<sup>6</sup>

In addition to oxygen, PHD and FIH enzymatic function is dependent on the presence of iron (Fe<sup>++</sup>), 2-oxoglutarate, and ascorbate. Inhibitors of the hydroxylases include 2-oxoglutarate analogs, such as dimethylxalylglycine, iron chelators, such as desferoxamine, and cobalt which acts by depleting cellular levels of ascorbate. Putative processes for which therapeutic hydroxylase inhibition may have a role include the prophylaxis of ischemic injury, inflammatory bowel disease, and the anemia associated with kidney disease. The phenomenon of ischemic preconditioning, where resistance to subsequent ischemic injury ensues after a prior ischemic event, is at least partly mediated through HIF. Animal studies have shown that pharmacologic hydroxylase inhibition resulting in HIF stabilization before organ ischemia diminishes subsequent injury across a range of organs.<sup>7</sup> Therapeutic hydroxylase inhibition has also shown great promise in animal models of inflammatory bowel disease, a condition where hypoxia and inflammation coexist.<sup>8</sup>

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This current issue of the *Journal of the American Society of Nephrology* reports the results from two small, short duration trials of novel oral PHD inhibitors for the treatment of the anemia associated with renal disease.<sup>9,10</sup> A potential advantage of the PHD inhibitors over recombinant human erythropoietin and its analogs is the beneficial effect of HIF activation on iron metabolism. HIF2 $\alpha$  activation results in the induction of not only erythropoietin, but also a number of genes involved in iron uptake, mobilization, and transport, including the divalent metal transporter 1, transferrin, and ferroportin, with the net effect of maximizing iron availability for erythrocytosis.<sup>11</sup> Furthermore, hepcidin is downregulated (probably indirectly) by HIF2 $\alpha$  activation. Hepcidin, which is induced in CKD and other inflammatory states, inhibits the transmembrane iron exporter ferroportin, resulting in the sequestration of iron within enterocytes, hepatocytes, and reticuloendothelial cells and as a consequence, hypoferrremia.<sup>12</sup> Indeed, several compounds that inhibit PDH are currently in various stages of clinical development. Holdstock *et al.*<sup>9</sup> report that GSK1278863 increases hemoglobin in predialysis patients with CKD in a dose-dependent manner and when dosed at 5 mg daily, maintains hemoglobin in a manner similar to recombinant human erythropoietin in patients on hemodialysis (HD). A reduction in hepcidin associated with GSK1278863 treatment was evident in predialysis but not dialysis-treated subjects. Plasma VEGF did not differ between groups. Besarab *et al.*<sup>10</sup> studied the effects of roxadustat in four groups of erythropoietin-stimulating agent-naïve patients with ESRD: patients on HD not treated with iron, patients on HD treated with oral iron, patients on HD treated with intravenous iron, and patients on peritoneal dialysis treated with oral iron. Hemoglobin rose from baseline in all four groups but was significantly lower at 12 weeks in the noniron-treated HD group. Hepcidin was lower than baseline at 12 weeks in all but the intravenous iron-treated group. The improvement in hemoglobin seen with roxadustat treatment without concomitant intravenous iron therapy undoubtedly represents a clinical and economic selling point, although it remains to be seen whether this effect will be sustained in the longer term and a less selected patient population.

Although there is considerable excitement about or at least anticipation of the availability of a new and fundamentally different class of anemia treatment agents, the nephrology community has been put on high alert by a number of safety issues with other anemia treatments in the past decade and a half. Serious safety signals have triggered multiple label revisions for erythropoietin-stimulating agents, an outbreak of pure red cell aplasia associated with Eprex caused panic and was finally attributed to a rubber syringe stopper, and the erythropoiesis-stimulating agent peginesatide was withdrawn postmarketing after a number of serious hypersensitivity reactions. Concerns regarding these new PHD inhibitor agents are likely to center on potential off-target effects from chronic HIF stabilization, especially the potential for promoting neoplasia. Rapidly growing and disorganized tumors frequently outstrip their blood supply, resulting in intratumoral hypoxia.

Unsurprisingly, hypoxia *per se* and positive HIF immunostaining are both markers of poor prognosis. The role of the HIF in cancer development and progression is complex and likely differs by tumor type; however, HIF certainly has an important role in the pathogenesis of many cancers through, among other things, its promotion of angiogenesis and cell survival.<sup>13</sup> Indeed, VEGF inhibitors are in use, and HIF inhibitors are currently under development as cancer therapeutics.

Additional insights into the effects of chronic HIF stabilization are gained by studying two rare genetic diseases: VHL disease and Chuvash polycythemia. VHL disease is an autosomal-dominant condition characterized by the development CNS, retinal, renal, and endocrine tumors.<sup>14</sup> In VHL disease, an inherited inactivating mutation of one VHL allele is followed by a spontaneous second hit that inactivates the remaining functioning protein. Loss of both functional VHL alleles results in chronic stabilization of HIF1 $\alpha$  and HIF2 $\alpha$  proteins and transcription of their target genes. In VHL disease-associated clear cell renal cell carcinoma, experimental evidence suggests that HIF2 $\alpha$  stabilization is the key promoter of tumorigenesis.<sup>15</sup> Chuvash polycythemia is a rare autosomal-recessive VHL loss of function defect that is associated with chronic HIF stabilization.<sup>16</sup> Affected individuals have increased expression of HIF gene products, including erythropoietin, transferrin, glucose transporter 1, and VEGF. Clinical features of the condition include polycythemia, lower limb varicosities, low BP, and hemangiomas. The malignancies associated with the VHL disease are not seen in Chuvash polycythemia.

Interestingly, all-cause mortality is actually lower in patients on dialysis residing at high (versus low) altitude, perhaps suggesting that chronic physiologic HIF activation may actually be beneficial.<sup>17</sup>

There are concerns beyond malignancy as well. Chronic HIF stabilization, especially in a population enriched in diabetes, could conceivably stimulate proliferative diabetic retinopathy through enhanced VEGF expression. In addition, an earlier FibroGen hydroxylase inhibitor compound, FG-2216, was withdrawn after a patient had fatal hepatic necrosis in a phase 2 clinical trial, a stark reminder of the risks of developing and trialing novel agents.

This all leaves the question of whether the nephrology community is ready to embrace a new generation of anemia therapeutics that present a whole new set of uncertainties. Luckily, the times seem to have passed when regulatory agencies were willing to approve new drugs or classes of drugs solely on the basis of efficacy trials on intermediate or biochemical end points (*e.g.*, hemoglobin correction or maintenance in the case of anemia drugs). A query on Clinicaltrials.gov indicates that FibroGen and their partners have embarked on a robust phase 3 trials program for roxadustat, including placebo and active treatment comparisons, in patients with CKD not requiring dialysis and patients with ESRD requiring it, and it has sufficient (pooled) power to identify potential mortality and cardiovascular risks of even reasonably small magnitude. However, the safety of these drugs with regard to

much rarer events, such as cancer incidence and progression (or anaphylaxis as the peginesatide example woefully high-lights), will remain unaddressed even in such ambitious phase 3 programs and will require additional efforts (and regulator mandates) for top quality and prospective postmarketing surveillance on potential approval.

## DISCLOSURES

C.R.L. has nothing to disclose. In the past year, W.C.W. has served as a scientific advisor to Amgen and Astra-Zeneca and on an event adjudication committee for Medtronic.

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## Dialysis Vascular Access Intervention and the Search for Biomarkers

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Hemodialysis vascular accesses are often referred to by any one of a triad of tropes: *lifeline* because of their indispensability for hemodialysis; *Achilles' heel* since access complications contribute substantially to the morbidity and mortality in this patient population; and *Cinderella* as the field of vascular access has never received its merited attention. Understanding the basis for access complications and failure and developing strategies that effectively mitigate both sets of adverse outcomes are necessary objectives in improving the wellbeing of patients maintained on chronic hemodialysis.<sup>1</sup>

Of the available vascular accesses, the least desired is the central venous catheter, and the most favored is the arteriovenous fistula (AVF). Even for the AVF, the outcomes are dismal, with 30%–60% of the created AVFs never usable for hemodialysis<sup>2,3</sup>; recent analyses indicate that the median cumulative patency for AVFs (placed as the first access) is 7.4 months, which increases to 61.9 months when primary AVF failures are excluded.<sup>4</sup> Moreover, a significant subset of functioning AVFs requires endovascular or surgical procedures to achieve and/or maintain such functionality; AVFs that require intervention to achieve maturation have decreased cumulative survival compared with AVFs that successfully mature on their own.<sup>5</sup> Arteriovenous grafts (AVGs) are generally placed when available veins are inadequate for AVFs, and, in a quite limited number of specific settings, there is a gathering sense that an

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