Prolyl hydroxylase domain inhibitors as a novel therapeutic approach against anemia in chronic kidney disease

Mai Sugahara[1], Tetsuhiro Tanaka[1] and Masaomi Nangaku[1]

[1]Division of Nephrology and Endocrinology, The University of Tokyo Graduate School of Medicine, Tokyo, Japan

Anemia is a common complication of chronic kidney disease and is mainly caused by the inability of injured kidneys to produce adequate amounts of erythropoietin. Studies elucidating the regulation of erythropoietin production led to the identification of hypoxia-inducible factor (HIF), which activates the transcription of genes that mediate adaptive responses to hypoxia. HIF is a heterodimer that consists of an α and β subunit. While HIF-β is constitutively expressed, HIF-α is subjected to ubiquitination and proteasomal degradation under normoxic conditions. This process is mediated by prolyl hydroxylase domain proteins, the inhibition of which results in an increased expression of hypoxia-induced genes, including erythropoietin. These findings led to the development of prolyl hydroxylase domain inhibitors as novel therapeutic agents against anemia in chronic kidney disease. Prolyl hydroxylase domain inhibition improves iron metabolism, which also contributes to erythropoiesis. To date, at least 6 small-molecule inhibitors of the prolyl hydroxylase domain have been tested in humans, and clinical trials have shown that they are effective without causing serious adverse events. However, there is a theoretical concern that the systemic activation of HIF could also induce deleterious effects such as tumorigenesis and severe pulmonary hypertension, which demands careful assessments in future clinical studies.

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Correspondence: *Masaomi Nangaku, Division of Nephrology and Endocrinology, The University of Tokyo Graduate School of Medicine, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan. E-mail: mnangaku-tky@umin.ac.jp*

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Anemia is a common manifestation of chronic kidney disease (CKD) and is a significant determinant of the overall prognosis and quality of life in patients with kidney failure, making its careful management throughout the CKD continuum essential. The use of erythropoiesis-stimulating agents (ESAs) and iron supplementation are currently the 2 major therapeutic strategies for anemia in CKD. However, although the administration of ESAs is a well-established and highly effective treatment, an important safety issue must not be overlooked; several randomized controlled studies have demonstrated that the use of ESAs with a target hemoglobin level of >13 g/dl has been associated with an increased rate of cardiovascular events and mortality compared with the use of a lower target hemoglobin level.[1–3] Whether this occurs because of the high hemoglobin level itself or the high ESA dosage is unclear; however, this safety concern is one of the reasons for the considerable interest in developing an alternative therapeutic strategy against anemia in CKD.

One promising candidate is small-molecule inhibitors of prolyl hydroxylase domain (PHD) enzymes. PHD inhibition results in the stabilization of hypoxia-inducible factor (HIF), which is a transcription factor that regulates the expression of a number of molecules, including erythropoietin (EPO). At least 6 PHD inhibitors are currently being tested in humans, some of which are showing promising results. In this mini review, we describe the mechanism by which PHD inhibitors alleviate anemia in CKD and summarize the current clinical experiences with these drugs. Possible consequences of PHD inhibition other than increases in EPO are also discussed in detail.

The PHD-HIF pathway regulates EPO production

Erythropoiesis mainly occurs in the bone marrow, where red blood cell production is regulated by the 30.4-kilo Dalton glycoprotein hormone EPO. EPO is largely synthesized by the kidneys, and the inability of injured kidneys to secrete adequate amounts of EPO is considered to be the main cause of anemia in CKD, although other factors such as chronic inflammation, impaired iron metabolism, uremic toxins, and a shortened half-life of erythrocytes also contribute to the pathogenesis. Because oxygen delivery is the main function of erythrocytes, hypoxia serves as the major stimulus of EPO production. The exploration of the molecular mechanism of this response led to the identification of HIF transcription factors.

The PHD-HIF oxygen-sensing pathway. HIF is a heterodimer that consists of 1 of 3 α subunits (HIF-1α, HIF-2α, or HIF-3α) bound to the aryl hydrocarbon receptor nuclear translocator, also known as HIF-β. HIF-α comprises basic helix-loop-helix and Per-aryl hydrocarbon receptor nuclear translocator-Sim homology domains, in which the helix-loop-helix/Per-aryl hydrocarbon receptor nuclear translocator-Sim domain mediates subunit dimerization, whereas the basic domain mediates binding to the hypoxia response element of target genes. HIF-mediated gene transcription is activated following DNA binding and the recruitment of transcriptional coactivators.

Cells continuously synthesize HIF-α subunits, and their transcriptional activity is primarily controlled by their degradation rate. Under normoxic conditions, oxygen-dependent HIF-α degradation is initiated by three 2-oxoglutarate-dependent oxygenases, namely PHD1, PHD2, and PHD3. These enzymes use molecular oxygen to hydroxylate HIF-α at specific proline residues (Pro402 and Pro564 in human HIF-1α, Pro405 and Pro531 in human HIF-2α). Proline-hydroxylated HIF-α is recognized by the von Hippel-Lindau (VHL)-E3 ubiquitin ligase complex, resulting in the ubiquitination of HIF-α. Ubiquitinated HIF-α is then destroyed by the proteasome. In contrast, under hypoxic conditions, hydroxylation of HIF-α is inhibited, allowing it to be translocated to the nucleus where it dimerizes with HIF-β and binds to the hypoxia response element (Figure 1).[4,5]

EPO production in the healthy kidney. Peritubular interstitial fibroblasts are the principal source of EPO synthesis in the kidney and are considered to be either "on" or "off" depending on the tissue oxygen tension. Under normoxic conditions, only a small number of fibroblasts in the corticomedullary region produce EPO. In contrast, under hypoxia, other fibroblasts also start to synthesize the hormone through HIF-mediated EPO gene transcription, as described above, resulting in a more widespread distribution of EPO-producing cells throughout the entire cortex and outer medulla. This increase in the number of EPO-producing cells results in an increase in the total level of renal EPO transcription and subsequently, the total amount of EPO in the systemic circulation.[4]

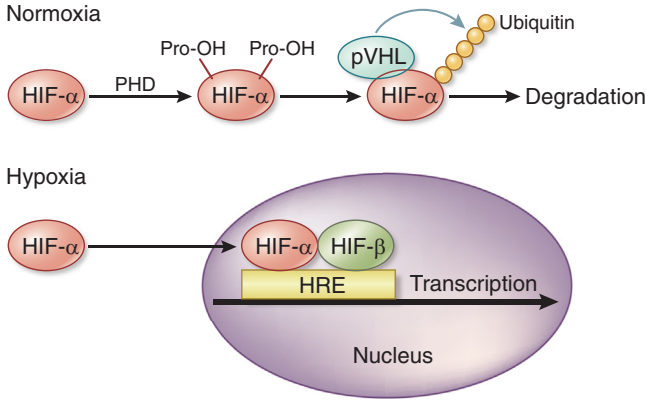

Figure 1 | Prolyl hydroxylase domain–hypoxia-inducible factor (PHD-HIF) oxygen-sensing system. Under normoxic conditions, HIF-α is hydroxylated at specific proline residues by PHD proteins. Proline-hydroxylated (Pro-OH) HIF-α is recognized by the von Hippel-Lindau E3 ubiquitin ligase, resulting in HIF-α ubiquitination. Ubiquitinated HIF-α is then destroyed by the proteasome. Under hypoxic conditions, HIF-α hydroxylation is inhibited, allowing it to be translocated to the nucleus where it dimerizes with HIF-β, binds to the hypoxia response element (HRE), and induces the transcription of target genes. pVHL, von Hippel-Lindau protein.

HIF-1α and HIF-2α are both expressed in the kidney, but with different expression patterns: HIF-1α is mainly induced in tubular and glomerular epithelial cells and in papillary interstitial cells, whereas HIF-2α is expressed in endothelial cells and fibroblasts upon hypoxic stimulation, suggesting that HIF-2α, not HIF-1α, is the main regulator of EPO production.[6] This hypothesis is supported by the systemic postnatal knockout experiments of HIF-2α in which mice deprived of HIF-2α developed anemia[7] and the observation that mice deficient in HIF-2α in platelet-derived growth factor receptor β–positive cells fail to respond to PHD inhibition.[8] Furthermore, mutations that activate HIF-2α can result in erythrocytosis in humans.[9]

Among the 3 PHD isoforms, PHD2 predominantly regulates HIF-2α stability upstream of EPO production. Silencing of PHD2 by small, interfering RNA is sufficient to upregulate HIF *in vitro*,[10] and the inactivation of PHD2 in mice induces a striking increase in serum EPO and hematocrit levels.[11] Furthermore, the genetic investigation of familial erythrocytosis in humans revealed a missense mutation in *PHD2* that results in a marked decrease in its enzymatic activity.[12]

EPO production in the injured kidney. Kidney fibrosis is considered to be the final common pathway that leads from CKD to end-stage kidney disease. Myofibroblasts are the source of excessively accumulated extracellular matrix, and recent cell-fate mapping studies have shown that the majority of these myofibroblasts are derived from resident fibroblasts with EPO-producing ability.[13,14] When unilateral ureteral obstruction was applied to inherited super anemic mice, it was found that myofibroblast-transformed fibroblasts lose their EPO-producing potential and actively contribute to renal fibrosis in CKD.[14] However, the genetic inactivation of PHD in EPO-producing cells can restore EPO gene transcription in injured kidneys,[15] suggesting a promising potential for the use of PHD inhibitors as a novel therapeutic strategy against anemia in CKD.

PHD inhibitors and iron metabolism

Along with EPO deficiency, abnormal iron metabolism contributes to the pathogenesis of anemia in CKD, and the upregulation of HIF may improve both the absolute and functional iron deficiencies that are characteristic of renal failure. The most intensely studied molecule of iron metabolism in CKD is hepcidin, a hormone responsible for maintaining systemic iron homeostasis. Hepcidin is predominantly synthesized in the liver, and its main function is to internalize and degrade the iron efflux transporter ferroportin on