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Review

Regulated oxygen sensing by protein hydroxylation in renal erythropoietin-producing cells

Roland H. Wenger and David Hoogewijs

Institute of Physiology and Zürich Center for Integrative Human Physiology ZIHP, University of Zürich, Zürich, Switzerland

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Wenger RH, Hoogewijs D. Regulated oxygen sensing by protein hydroxylation in renal erythropoietin-producing cells. *Am J Physiol Renal Physiol* 298: F1287–F1296, 2010. First published March 10, 2010; doi:10.1152/ajprenal.00736.2009.— The kidney is a major site of systemic oxygen sensing, regulating blood erythrocyte and hence oxygen content by hypoxia-inducible erythropoietin (Epo) expression. A constant ratio between blood perfusion and oxygen consumption, a stable corticomedullary oxygen gradient, and a relatively low tissue P_{O_2} are the prerequisites for the function of renal Epo-producing and oxygen-sensing (REPOS) cells, which are located in the juxtamedullary cortex. In kidney disease, renal oxygen consumption is decreased, leading to an increase in P_{O_2}, dysfunction of REPOS cells, and anemia. The molecular principles of cellular oxygen sensing have been elucidated in the last few years, and genetically altered mouse models as well as hereditary diseases causing erythrocytosis have clarified the oxygen-signaling cascade leading to increased Epo expression in REPOS cells. However, the consequences of a number of recently discovered factors for the regulation of oxygen signaling in REPOS cells are unclear, asking for novel cell culture models which might be hampered by the putative neuron-like nature of this enigmatic cell type.

hypoxia; anemia; erythrocytosis; gene expression; kidney disease; Krebs cycle; reactive oxygen species; von Hippel-Lindau

TWO MAJOR ORGANS IN THE ADULT body measure the systemic arterial oxygenation of the circulating blood: the carotid body and the kidney. The carotid body is located close to the lung circulation and obtains freshly oxygenated blood. Its type I chemoreceptor cells reside in a highly perfused tissue, and their oxygenation depends largely on the oxygen partial pressure (P_{O_2}) rather than the oxygen content of the blood. The carotid body thus serves as a feedback control system that acutely surveys the function of the lung. Every drop in P_{O_2} will immediately result in increased alveolar ventilation to normalize arterial P_{O_2}.

The kidney, on the other hand, features a unique oxygen gradient which is stabilized by the countercurrent exchange of oxygen along the corticomedullary axis, keeping tissue P_{O_2} at relatively low values (116). This oxygen gradient is independent of the relatively high renal perfusion (20% of cardiac output) since every change in perfusion will lead to a similar change in renal oxygen consumption. Contrary to most other organs, the relatively low renal oxygen consumption (only 7% of the oxygen is extracted from the blood) is a function of renal perfusion and not vice versa. The more blood flow, the higher the glomerular filtration rate, the more sodium reabsorption and hence oxygen requirement, but also the more oxygen the kidney obtains, leading to a stable relationship between oxygen supply and oxygen consumption.

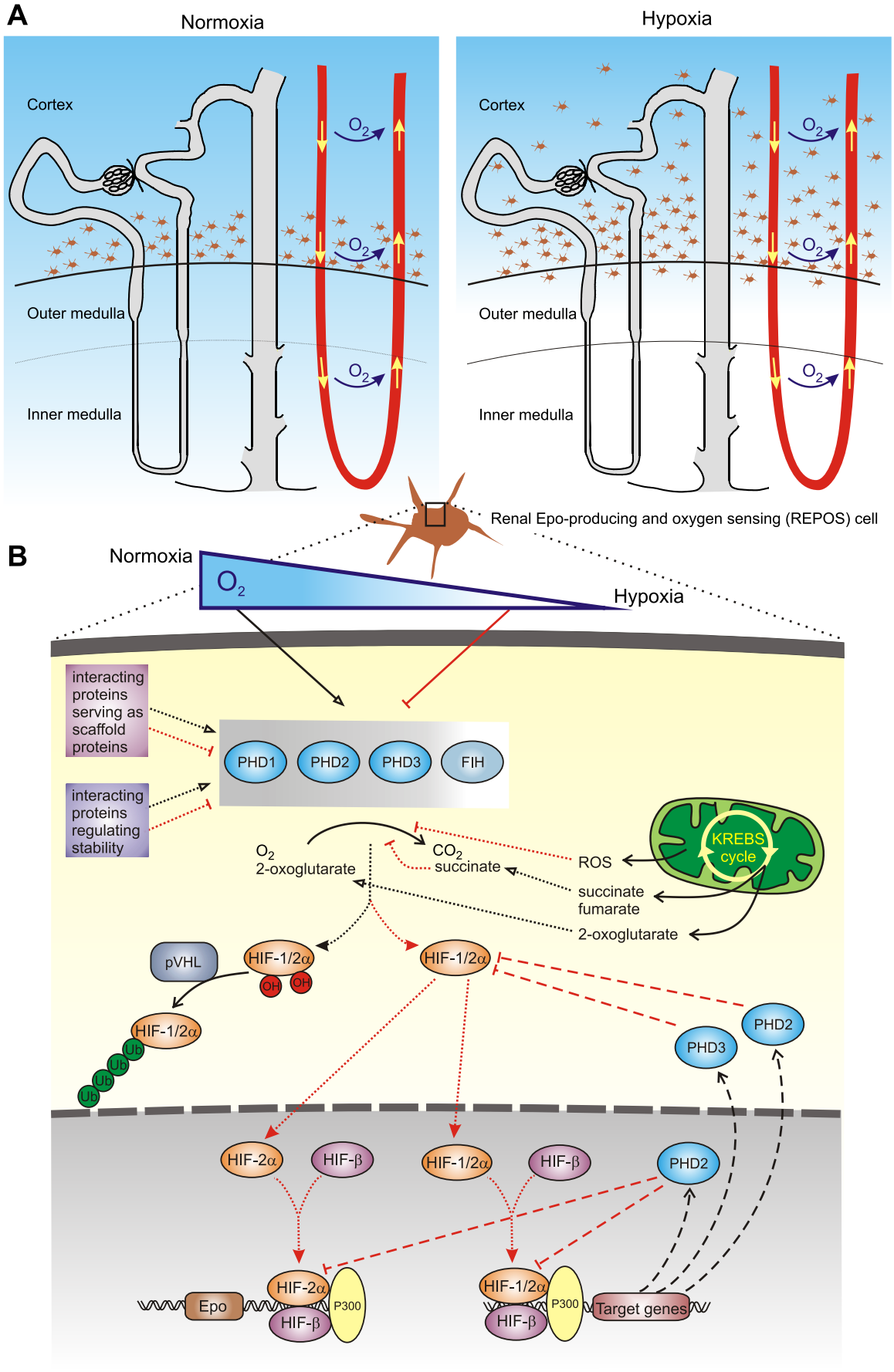
Address for reprint requests and other correspondence: R. H. Wenger, Institute of Physiology, Univ. of Zürich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland (e-mail: roland.wenger@access.uzh.ch).

The renal erythropoietin (Epo)-producing and oxygen-sensing cells, which we suggest to name "REPOS" cells, are peritubular, interstitial, fibroblast-like cells located along the corticomedullary oxygen gradient in the juxtamedullary cortex (7, 68, 70, 84, 126). Tissue P_{O_2} in this region depends on oxygen diffusion and consumption on its way from arterial blood to the REPOS cells. Because a constant fraction of oxygen is consumed already in the outer cortex, the dropping P_{O_2} is dependent on the oxygen loaded on hemoglobin according to the sigmoidal oxygen binding properties of hemoglobin. Therefore, oxygenation of REPOS cells depends largely on the regional blood oxygen content rather than the arterial P_{O_2}. As illustrated in Fig. 1*A*, a drop in oxygen content, maybe due to inspiratory hypoxia or anemia, will lead to an increase in the number of REPOS cells that detectably produce Epo, extending their recruitment to the border of the subcapsular tissue (30, 69). These REPOS cells account for ~90% of total Epo synthesis in the adult (67). Following endocrine secretion, Epo stimulates bone marrow erythropoiesis to counteract decreased oxygen content by increased oxygen transport capacity (58).

Whereas the molecular oxygen-sensing mechanisms in the carotid body remain enigmatic, the last decade brought a wealth of novel insights, but also novel questions and novel therapeutic opportunities, on the oxygen-sensing and -signaling pathways regulating renal Epo synthesis.

Ubiquitous Cellular Oxygen Sensing by Protein Hydroxylation

The molecular mechanisms originally identified in hepatocytes and REPOS cells apply to all cells of the body: prolyl-



4-hydroxylase domain (PHD) enzymes continuously "sense" the oxygen levels and covalently modify hypoxia-inducible transcription factor-α (HIF-α) subunits (62, 123, 141). In the presence of sufficient oxygen, two distinct HIF-α prolyl residues within an oxygen-dependent degradation domain are hydroxylated in a nonreversible reaction involving oxygen, 2-oxoglutarate, ferrous iron, and probably vitamin C (54, 56). One oxygen atom is used for prolyl-4-hydroxylation, the other for oxidative decarboxylation of 2-oxoglutarate to form succinate and CO_2. Vitamin C might be involved in keeping the central iron atom in its ferrous form, but other reducing agents apparently can substitute for vitamin C (unpublished observations). This "intrinsic" reduction-oxidation (redox) reaction might be sensitive to externally generated reactive oxygen species (ROS), e.g., by mitochondrial electron transport chain complexes or by NADPH oxidases (2), but the physiological relevance of these "extrinsic" pathways is currently unknown. However, the entire PHD-HIF-von Hippel-Lindau (VHL) system can be reconstituted in a cell-free system in vitro where it shows the same oxygen-dependent HIF-α hydroxylation characteristics in the absence of any ROS-generating enzymes (146), suggesting that the intrinsic redox reaction is both necessary and sufficient for cellular oxygen sensing.

Under normoxic conditions, hydroxylation of HIF-α increases the affinity for binding by the tumor suppressor protein VHL, which serves as a recognition interface for an ubiquitin E3 ligase complex (85). Polyubiquitylation and destruction in proteasomes is a very rapid process that can be reverted instantaneously if oxygen supply is ceased (59). Under hypoxic conditions, HIF-α remains stable, heterodimerizes with HIF-β, and transcriptionally activates a large number of genes involved in the adaptation to decreased oxygen supply, including the gene encoding Epo (144). Another oxygen-dependent hydroxylase, factor-inhibiting HIF (FIH), triggers the transcriptional activity of HIF by asparagine hydroxylation, which blocks the recruitment of transcriptional coactivators (72, 76).

Hereditary Erythrocytosis Reveals PHD2, VHL, and HIF-2α as Key Players in Renal Oxygen-Regulated Epo Gene Expression

In vertebrates, both the PHD oxygen sensors and the HIF-α transcription factor subunits are encoded by three distinct but related genes. The PHD family is encoded by the human *EGLN2*, *EGLN1*, and *EGLN3* genes and their respective gene products are called PHD1, PHD2, PHD3, or HIF prolyl hydroxylase (HPH) HPH3, HPH2, and HPH1 (20, 31). All three family members are expressed in the kidney (122). The physiological relevance of a fourth family member, called PH-4, is less clear: HIF-α seems to be regulated under PH-4 overexpression conditions only (66, 96). The human *HIF1A*, *HIF2A*, and *HIF3A* genes encode HIF-1α, HIF-2α, and HIF-3α, respectively. There exist two related genes even for HIF-β, also known as aryl hydrocarbon receptor nuclear translocator (ARNT)-1 and ARNT-2, but only HIF-β/ARNT seems to serve as an HIF-α heterodimerization partner. While HIF-1 and HIF-2 αβ heterodimers function as transcriptional activators of oxygen-regulated target genes, the role of HIF-3α is less clear, and a short splice variant of HIF-3α, termed inhibitory PAS protein (IPAS), functions as a hypoxia-inducible transcriptional repressor (77). The HIF family has been discovered based on the cloning of HIF-1α and HIF-β/ARNT by virtue of their binding to the 3′ hypoxia response element (HRE) of the Epo gene in hepatoma cell lines (139). Thus it came as a surprise that it is not HIF-1 which regulates renal Epo expression. Mouse gene targeting and human hereditary diseases provided genetic evidence for the relevance of each isoform of the HIF and PHD gene families for oxygen-dependent Epo gene expression.

Two independent inducible PHD2 knockout mouse models showed increased erythropoiesis and angiogenesis as well as evidence for dilated cardiomyopathy and premature death (90, 132, 133). These findings are in line with in vitro data demonstrating that PHD2 is the main oxygen sensor responsible for normoxic HIF-α turnover (16). Consistently, systemic PHD2 but not PHD1 or PHD3 knockout mice die during embryonic development (134). A further confirmation for the major role PHD2 is playing in oxygen-regulated Epo synthesis in vivo comes from the identification of gene mutations that cause familial erythrocytosis: the affected amino acids identified to date confer residues P317, R371, and H374 of PHD2 (71, 103, 106). Neither PHD1 nor PHD3 mutations in familial erythrocytosis have been reported so far.

Due to its nature as a tumor suppressor, a large number of mutations in the human *VHL* gene were known before its role as a HIF-α E3 ubiquitin ligase was discovered. In accordance with its essential and nonredundant role in normoxic HIF-α degradation, it did not come as a surprise that such mutations also provoke erythrocytosis (145). Interestingly, Chuvash polycythemia is caused by a VHL R200W mutation in the absence of cancer (3). A number of additional VHL mutations causing congenital erythrocytosis have been identified thereafter (12, 21, 101, 108). Finally, a genetically altered mouse model recapitulated the polycythemic phenotype (45).

The first evidence that HIF-2α rather than HIF-1α might regulate renal Epo synthesis came from descriptive studies by Rosenberg and coworkers (115), who convincingly demonstrated in situ overlapping of HIF-2α but not HIF-1α with the peritubular, interstitial REPOS cells. Similar results have recently been reported using chemical PHD inhibition (99). The critical role of HIF-2α was further confirmed by RNAi experiments in liver-derived hepatoma (140). Because it is not yet possible to target the REPOS cells in mice and since HIF-1α and HIF-2α knockout mice do not survive embryonic development (55, 135), knockout mouse models initially provided only circumstantial evidence for the role of each HIF-α isoform in Epo gene expression. Using liver-specific mouse gene targeting, VHL deficiency has been shown to cause polycythemia. This phenotype could be reverted by a VHL-HIF-β/

Fig. 1. Simplified schematic overview of the oxygen signaling cascade in renal Epo-producing and oxygen sensing (REPOS) cells. *A*: renal tubular epithelial oxygen consumption and countercurrent exchange of oxygen leads to a stable corticomedullary oxygen gradient (blue background). Under hypoxic conditions, this gradient is shifted to the cortex, leading to increased recruitment of REPOS cells and elevated Epo production. *B*: oxygen partial pressure as well as Krebs cycle intermediates, reactive oxygen species (ROS), and a large number of interacting proteins regulate the abundance and activity of the oxygen-sensing PHD/FIH protein hydroxylases as discussed in detail in the text.

ARNT but not by a VHL-HIF-1α double knockout, suggesting that HIF-2α is responsible for Epo induction, at least in the liver (113). These results were subsequently confirmed in a hepatic HIF-2α knockout mouse model (112). Quite surprisingly, an independent HIF-2α global knockout mouse strain turned out to be viable, and these mice were indeed anemic with decreased renal Epo expression (124). Finally, conditional global deletion of HIF-2α but not HIF-1α resulted in anemia in adult mice (43). The mouse data have recently been confirmed by the identification of HIF-2α P534L, M535V, M535I, G537W, G537R, and D539E mutations that cause human familial erythrocytosis (36, 37, 80, 102, 104, 107, 138). No HIF-1α or HIF-3α mutations have been reported so far to be involved in erythrocytosis.

Altogether, there is now compelling evidence that the oxygen-signaling pathway regulating renal Epo synthesis consists of the PHD2-VHL-HIF-2α axis. Recent RNAi-based studies confirmed the major role of PHD2 in Epo regulation in vitro as well as in vivo and showed that FIH and the other PHD family members can play a modulatory role in Epo gene expression (32, 98). However, to directly investigate the function of these proteins in the kidney, a transgenic mouse model would be required which targets specifically the REPOS cells.

Multimodal Regulation of Oxygen Sensing

Adding more complexity to renal oxygen sensing, the ratio between PHD and HIF-α levels is interconnected. If either one is upregulated, it overcomes the function of the other. As well as an increase in PHD synthesis that leads to HIF-α degradation, an increase in HIF-α synthesis leads to its own stabilization by saturating the degradation machinery. PHDs have a low oxygen affinity, with K_m values that correspond roughly to the P_{O_2} of room air, which is clearly higher than the highest renal tissue P_{O_2} (46). Thus even under hyperoxic conditions the PHDs are not fully active and a further decrease in the oxygenation as well as a decrease in the PHD levels allow for the stabilization of HIF-α (41, 64, 91, 131). This feature explains why regulation of the PHD levels likely is of physiological relevance: even the slightest up- or downregulation of PHD abundance and/or activity potentially affects HIF-2-dependent Epo expression. Indeed, PHDs are regulated on three levels: transcription, protein abundance/stability, and enzymatic activity.

PHD2 and PHD3, but not PHD1 or FIH, are HIF target genes induced under hypoxic conditions (5, 16, 23, 26, 27, 31, 81, 88, 109). To date, little is known about the tissue-specific and signal-related expression of the genes encoding PHDs: estradiol and LIF are known to induce PHD1 and PHD2 gene expression, respectively, and transforming growth factor (TGF)-β1 has been shown to inhibit PHD2 gene expression (4, 18, 87, 127).

In addition to transcriptional regulation, the members of the oxygen-signaling cascade can also be regulated at the posttranscriptional level. An iron response element has been identified in the 5′-untranslated region of HIF-2α mRNA. Iron-regulatory proteins bind to this element and inhibit HIF-2α translation, thereby suppressing renal Epo production when iron deficiency limits efficient hematopoiesis (120). The Epo mRNA is hypoxically stabilized, contributing to its enormous hypoxic inducibility (42). Epo mRNA binding proteins have been identified, and the binding site has been shown to be required for hypoxic inducibility (86, 114, 121). More recently, microRNAs (miRNAs) emerged as regulators of the oxygen-signaling cascade: the 3′-untranslated regions of FIH, VHL, and HIF-1α mRNA are targeted by miR-31, miR-92–1, and miR-20b/miR-199a, respectively (40, 73, 74, 111). However, no miRNAs targeting PHD2, HIF-2α, or Epo have been reported so far, and how miRNAs are involved in REPOS cell-specific oxygen sensing and Epo expression remains to be investigated.

A number of proteins have recently been identified to interact with PHDs (Table 1). The newly identified interaction partners are able to regulate various aspects of PHD function, including stability, folding, subcellular localization, and enzymatic activity (142). One group of interaction partners serves as a molecular scaffold and includes osteosarcoma amplified 9 (OS-9), A-kinase anchor proteins (AKAP 6 and 12), mitogen-activated protein kinase organizer 1 (Morg1), inhibitor of growth (ING4), and melanoma antigens (MAGE 9 and 11) (6, 8, 22, 24, 44, 48, 97, 147). Other interacting proteins regulate PHD proteins levels, including the RING finger E3 ubiquitin ligase seven in absentia homolog 2 (Siah2), the FK506-binding protein 38 (FKBP38), and the chaperonin TCP-1 ring complex (TRiC) (10, 11, 82, 92, 93, 136). Importin-α5 and CRM1 are involved in PHD1 nuclear import and PHD2 nuclear export, respectively (130). The functional importance of the nuclear-cytoplasmic distribution of PHD2 has recently been demonstrated for the growth of cancer cells (60).

The Siah1 family member additionally regulates FIH protein levels, suggesting that Siah proteins also play a role in fine-tuning the transcriptional activity of HIFs (34, 35). FIH activity is further suppressed by binding to neuronal munc18–1-interacting protein 3 (Mint3)/amyloid β (A4), precursor protein-

Table 1. *Putative functions of proteins binding to PHD and FIH oxygen sensors*

Interactor	Target	Putative Function	Reference(s)
Protein stability			
Siah1a/2	PHD1/3	PHD degradation	93
Siah1	FIH	FIH degradation	35
FKBP38	PHD2	PHD degradation	10, 11
TRiC	PHD3	Chaperonin	82
Scaffolding proteins			
mAKAP12	PHD2/3	Molecular scaffold	22, 147
ING4	PHD2	HIF-α inhibition	24, 97
OS-9	PHD2/3	Molecular scaffold	8
MAGE-9/11	PHD2	PHD inhibition	6
Morg1	PHD3	Molecular scaffold	44, 48
Importin-α5	PHD1	Nuclear import	130
CRM1	PHD2	Nuclear export	130
Mint3/ABPA3	FIH	FIH inhibition	119
Others			
VHL	FIH	HIF-α inhibition	76
Cdr2	PHD1	HIF-α inhibition	9
IOP1	PHD2	Induces HIF-1α mRNA	50

PHD, prolyl-4-hydroxylase domain; FIH, factor-inhibiting HIF; HIF-α, hypoxia-inducible transcription factor-α; Siah, seven in absentia homolog; FKBP38, FK506-binding protein 38; TRiC, TCP-1 ring complex; AKAP, A-kinase anchor protein; ING, inhibitor of growth; OS-9, osteosarcoma amplified 9; MAGE, melanoma antigen; Morg1, mitogen-activated protein kinase organizer 1; Mint3, munc18-1-interacting protein 3; Cdr2, cerebellar degeneration-related protein 2; IOP1, iron-only hydrogenase-like protein 1; VHL, von Hippel-Lindau.

binding family A member 3 (APBA3), leading to increased HIF activity (119). Intriguingly, FIH has been reported to recruit VHL to HIF, which inhibits HIF transcriptional activity (76). Of note, also PHD2 appears to directly inhibit HIF-1 transcriptional activity, independently of protein hydroxylation and without affecting HIF-1α protein levels (128, 137). Two other PHD interactors, the iron-only hydrogenase-like protein 1 (IOP1) and the onconeuronal cerebellar degeneration-related protein 2 (Cdr2) additionally affect HIF-α mRNA and protein, respectively, by unknown mechanisms (9, 50).

PHD-dependent protein hydroxylation is a multicomponent reaction, allowing for the integration of further signaling pathways (94). Indeed, it has been shown that small molecules, such as ascorbate, transition metals, and ROS, including nitric oxide, affect PHD activity, establishing molecular cross talk between oxygen homeostasis and redox-active substances (13, 39, 47, 65, 79, 89). Of major physiological interest is the finding that Krebs cycle intermediates interfere with PHD function (25, 75, 125). Germline mutations of the genes encoding fumarate hydratase (FH) or succinate dehydrogenase (SDH) result in the accumulation of fumarate and succinate, respectively, two potent inhibitors of PHD activity (53, 110). Moreover, mutations in the gene encoding isocitrate dehydrogenase-1 (IDH-1) lead to a decrease in the PHD cosubstrate 2-oxoglutarate and an increase in HIF-1α levels (148). Thus the PHDs link oxygen sensing with mitochondrial metabolism.

Intrinsic Feedback Mechanisms Trigger Hematocrit-Controlled Renal Epo Synthesis

Figure 1*B* schematically summarizes the cross talk between the oxygen-sensing and other signaling pathways as mentioned above. However, the physiological relevance of these findings for oxygen-regulated renal Epo expression is currently unknown and awaits appropriate kidney-derived cell culture and mouse models. Since the majority of hereditary erythrocytosis cases are idiopathic diseases (105), this overview might provide the basis for screening additional gene mutations in these patients. Conversely, the identification of such mutations would unequivocally demonstrate the physiological relevance of these genes in renal oxygen sensing.

Regarding the HIF-PHD regulatory loop, it is of interest that hypoxically induced serum Epo levels decrease clearly before the hematocrit is increased, excluding an early negative feedback loop via the subsequent increase in blood oxygen content (1). The drop in serum Epo is paralleled by a drop in renal Epo protein and mRNA levels and is independent of changes in extracellular Epo levels (29, 57). These data suggest one or several intrinsic negative feedback loop(s) that control renal Epo synthesis. A very likely mechanism, albeit unproven regarding Epo regulation, might consist of the HIF-dependent transcriptional increase in PHD2 and PHD3 levels, which in turn downregulate HIF-α protein levels (Fig. 1*B*). This model shows a biphasic response, with an early PHD3 and a late PHD2 induction, and it allows for ongoing oxygen sensing, suggesting that it could easily adapt to both acute and chronic changes in oxygenation (41, 64, 91, 131).

Neuronal Phenotype of REPOS Cells?

The lack of an appropriate cell culture model seriously hinders further investigation into the physiological relevance of the complex oxygen-signaling (sub)pathways, as outlined above, for regulation of renal Epo synthesis. Most of our knowledge is based on findings derived from the human hepatoma cell lines HepG2 and Hep3B. Obviously, caution is required when one is transferring these findings to REPOS cells. So far, it was not possible to establish a kidney-derived cell culture system capable of oxygen-regulated Epo synthesis in vitro. Extensive in situ analyses of the REPOS cells suggested a fibroblast-like phenotype (7, 84). However, fibroblasts would not be considered to be impossible to cultivate. Moreover, despite the many reports on extrarenal Epo expression, fibroblasts have not been shown to express Epo in any other organ than the kidney. Currently, the evidence for a fibroblast-like phenotype comes from renal coexpression of Epo and HIF-2α together with CD73/ecto-5′-nucleotidase that is considered to be a fibroblast marker (7, 84, 99). However, even under severely hypoxic conditions only a subset of the CD73/ecto-5′-nucleotidase-positive cells is also positive for HIF-2α/Epo, and CD73/ecto-5′-nucleotidase is not solely expressed by fibroblasts (according to the Human Protein Atlas, http://www.proteinatlas.org; and the GNF atlas, http://biogps.gnf.org), suggesting that REPOS cells could also be of a different nature.

Intriguingly, a recent study using genetically modified mice that carry a green fluorescent protein (GFP) reporter protein under the control of a 180-kb Epo transgene suggested a "neuron-like" phenotype (95). The GFP-positive cells have dendrite-like processes and express the neuronal markers microtubule-associated protein 2 (MAP2) and neurofilament light polypeptide. A neuron-like phenotype would explain the failure of in vitro cultivation of these cells and is in line with their sensory function. Remarkably, in the central nervous system neurons and astrocytes are known to express Epo (14, 78, 83).

There is also an interesting analogy between chemosensory cells of the kidney and carotid body. Both oxygen-signaling pathways need the more infrequently expressed HIF-2α isoform. The dopaminergic type I cells of the carotid body rely on HIF-2α for hypoxia-inducible expression of tyrosine hydroxylase, the rate-limiting enzyme in catecholamine synthesis: HIF-2α knockout mice die from bradycardia due to the lack of catecholamines (135); HIF-2α knockdown results in decreased catecholamine synthesis in adrenomedullary chromaffin cells (19); HIF-2α, but not HIF-1α, deficiency reverts the reduced apoptosis and increased cell numbers in the sympathoadrenal system, including the carotid body, of PHD3 knockout mice (17); and mutations in the *VHL* and *SDH* genes are associated with an increased risk of paraganglioma, including carotid body tumors (61). Considering this analogy, kidney-derived cell culture systems might profit from the recent finding that glia-like stem cells generate neurotropic factors that sustain carotid body neurogenesis (100). All in all, there might be new hope for a REPOS cell culture model.

Tissue-Specific Gene Expression in REPOS Cells

Another important question that is difficult to address due the lack of a REPOS cell culture model is the tissue specificity of oxygen-sensing and -signaling factors in REPOS cells. Whereas PHD2 and VHL are ubiquitously expressed, HIF-2α and Epo show highly REPOS cell-specific expression patterns on the mRNA level. As mentioned above, renal oxygen sensing

and signaling, mandatory to systemic red blood cell and oxygen homeostasis, depends on the correct spatial localization of the cells expressing Epo within the renal oxygen gradient. REPOS cell-restricted gene expression is equally important to proper oxygen sensing as hypoxia inducibility itself. While virtually nothing is known about the REPOS cell-specific gene expression of HIF-2α, a number of transcription factors have been implicated in the tissue-specific expression of the Epo gene (28). Whether similar mechanisms are involved in HIF-2α expression in REPOS cells remains to be investigated.

Under normoxic conditions, Epo gene expression is strikingly low, consistent with the idea that the conditional (i.e., hypoxic) HIF-2-mediated induction of the Epo gene plays a major role in its REPOS cell-specific expression. Epigenetic modifications by CpG DNA methylation are likely to be involved in the constitutive suppression of Epo expression in non-REPOS cell types. Of note, it has been shown that HIF binding to its consensus DNA recognition site in the Epo 3′-HRE is CpG DNA methylation sensitive (117, 143). At least for hepatocytes, HIF-2 cooperation with hepatic nuclear factor 4 (HNF-4) at the 3′ HRE might contribute to tissue-specific gene expression (38). However, how HNF-4 contributes to renal Epo expression is less clear, and it should be remembered that the importance of the Epo 3′ HRE for REPOS cell-specific gene expression awaits a formal proof. Very low basal Epo expression also suggests the involvement of transcriptional suppressors. Indeed, GATA factors have been shown to repress Epo gene promoter activity (51, 52). Using transgenic mice, it has been demonstrated that the GATA *cis*-regulatory element of the Epo promoter is important for repression of Epo gene expression in non-REPOS cells of the kidney (95).

Conclusions

Albeit not fully understood regarding renal Epo production, the wealth of novel insights into the basic mechanisms of oxygen sensing and signaling opened new routes to the treatment of diseases related to oxygen sensing. In kidney failure, oxygen consumption is decreased, resulting in increased tissue P_{O_2}, decreased renal Epo production, and anemia. Kidney dialysis patients hence need lifelong Epo treatments. Pharmaceutical interference with PHD/FIH enzymatic activity proved to be efficient in vitro and beneficial for the treatment of a number of diseases in animal models in vivo, including tissue ischemia, kidney injury, and transplantation (15, 33, 63, 99, 129). Protein hydroxylase inhibitors also stimulated Epo production in mouse and rhesus macaque models (49, 118). Clinical phase II studies are being conducted to test the safety and efficacy of protein hydroxylase inhibitors in the treatment of patients with renal anemia (ClinicalTrials.gov identifiers NCT00456053 and NCT00761657). Even if it is currently unclear to what extent Epo is derived from the liver and to what extent from the kidney following treatment with these protein hydroxylase inhibitors, these examples demonstrate the clinical relevance of basic research in renal oxygen sensing. Studies in the near future will answer the question as to whether the currently available inhibitors of the oxygen-sensing protein hydroxylases are sufficiently efficient and specific to serve as novel therapy strategies for the treatment of anemia caused by renal disease.

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DISCLOSURES

No conflicts of interest are declared by the authors.

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