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Hypoxia-inducible factor (HIF) and HIF-stabilizing agents in neonatal care

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Summary

Oxygen is essential for multicellular existence. Its reduction to water by the mitochondrial electron transport chain forms the cornerstone of aerobic metabolism. Conditions in which oxygen is limiting for electron transport result in bioenergetic collapse in metazoans. However, compared with postnatal existence, all of mammalian development occurs in a hypoxic environment in utero. Not just an epiphenomenon, this ‘physiological hypoxia’ is required for the activation of a transcriptional response mediated by the hypoxia-inducible factor (HIF) family of transcriptional regulators that coordinates the expression of hundreds of genes, many with developmentally critical functions. Oxygen tension, therefore, is a morphogen. Understanding the physiological significance of hypoxia responses during human development and the role of the HIF family of transcriptional regulators will have important consequences for the care of preterm neonates. Defining clinical care guidelines for the proper oxygenation of critically ill neonates that take account of these observations is therefore of paramount importance. The pharmacological stabilization of HIF family members may therefore have clinical utility in premature infants in whom this important morphogen has been inactivated by exposure to supraphysiological oxygen levels.

Keywords

Hypoxia; Hypoxia-inducible factor; Necrotizing enterocolitis; Neonate, retinopathy of prematurity; Prolyl hydroxylase inhibitor; Respiratory distress syndrome

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Conflict of interest statement

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Introduction

As photosynthesizing organisms increased ambient oxygen levels in the Earth's early atmosphere, life forms evolved that could utilize this newly oxygen-rich environment via aerobic metabolism.¹ The reduction of oxygen to water by the mitochondrial electron transport chain (ETC) is able to generate the ATP needed to meet the bioenergetic demands of increasingly complex multicellular existence. However, this process is not 100% efficient, and the incomplete reduction of oxygen results in the formation of toxic reactive oxygen species (ROS) that can cause oxidative damage to cellular macromolecules. This dual nature of oxygen as an essential but potentially harmful molecule required the evolution of oxygen-sensing mechanisms to respond to high as well as to low levels of oxygen, respectively known as hyperoxia and hypoxia. However, intermediate levels of these ROS appear to have been co-opted for signaling purposes, paradoxically to signal low oxygen levels.²

In mammals, one of the most important regulators of oxygen homeostasis is the hypoxia-inducible factor (HIF) complex. HIF coordinates the myriad responses to decreased oxygen tension by promoting compensatory mechanisms acting at the cellular as well as organismal level that include enhancing the oxygen-carrying capacity of blood, decreasing cellular oxygen demand, and increasing glycolysis, to name just a few.^{3,4} This response is critical for many facets of normal mammalian development.⁴

What is HIF?

HIF is a heterodimeric protein complex composed of an α and β subunit that forms a sequence-specific DNA-binding transcription factor.^{5,6} Both subunits contain basic helix-loop-helix (bHLH) as well as PAS (PER, AHR, ARNT, and SIM) domains that mediate dimerization and DNA binding at the hypoxia response elements (HREs) of target genes.^{7–10} The identification of an HRE in the enhancer region of the erythropoietin (*Epo*) gene led to the discovery of HIF-1, composed of HIF-1 α and ARNT. HIF-1 binding to the HRE either promotes or represses the transcription of a broad range of genes involved in maintaining biological homeostasis in response to changing oxygen levels. There are three structurally similar HIF- α proteins: HIF-1 α , HIF-2 α , and HIF-3 α these are strictly regulated in an oxygen-dependent manner and confer oxygen regulation to the complex overall.³ HIF- β subunits, also known as aryl hydrocarbon receptor nuclear translocator (ARNT 1, 2, 3) are constitutively expressed nuclear proteins.⁵ Although each of the three α -subunits is known to contribute in vivo to hypoxia responses, only two of the β -subunits are thought to participate.

Normoxic degradation of HIF- α subunits

HIF-1 α is constitutively transcribed and translated, but its stability and transcriptional activity are regulated by several post-translational modifications. For example, under normoxic conditions, it is hydroxylated on critical proline residues.^{11–13} Post-translational hydroxylation within the oxygen-dependent degradation domain (ODDD) located at the N-terminal transactivation domain (NTAD) occurs at one or both of two highly conserved proline residues. The molecular basis for this oxygen-dependent hydroxylation has been the subject of intense study over the past decade and is now known to be accomplished by members of the prolyl hydroxylase domain (PHD) family of proteins. PHD enzymes belong to a non-heme, Fe²⁺ and 2-oxoglutarate-dependent (a Krebs cycle intermediate) dioxygenase superfamily whose activity is dependent on oxygen availability. PHD uses both oxygen and α -ketoglutarate as substrates for the dioxygenase reaction that splits oxygen.¹⁴ One oxygen atom is transferred to hydroxylate the proline residue and the other reacts with 2-oxoglutarate to form succinate and carbon dioxide. The enzymes therefore require molecular

oxygen for their activity, but whether they represent the elusive cellular oxygen sensors is not clear, as oxygen concentrations within the physiologic range are unlikely to directly affect their activities.

Prolyl-4-hydroxylation of the ODDD creates a binding site for the von Hippel–Lindau tumor suppressor protein (pVHL), which is a component of an E3 ubiquitin ligase complex.¹⁵ As a result, HIF-1 α is polyubiquitinated at several sites and rapidly targeted for proteosomal degradation. Mutation of both proline residues disrupts interaction of HIF-1 α with pVHL and increases its stability in normoxic conditions whereas mutating one proline residue only partially stabilizes HIF-1 α .¹⁶ Mutations or deficiencies of PHD or pVHL lead to accumulation of high levels of HIF- α proteins during normoxia. 2-Oxoglutarate analogs, which inactivate PHDs, also increase the half-life of HIF-1 α ^{11–13} which is normally <5 min in normoxic conditions.

Additionally, factor inhibiting HIF (FIH) hydroxylates an asparaginyl moiety at the C-terminus of the HIF-1 α and HIF-2 α subunits. Hydroxylation of Asp803 blocks interaction of the C-terminal transactivation domain (CTAD) with the coactivator p300 and its paralogue CREB-binding protein (CBP).^{17,18} FIH-1, like PHD family members, is an Fe²⁺- and 2-oxoglutarate-dependent dioxygenase. Upon availability of oxygen and Fe²⁺, FIH-1 hydroxylates the conserved asparaginyl residue within the CTAD and prevents interaction with coactivator p300 and CBP due to steric interference.¹⁹

PHD isoforms

The PHD family consists of three members, PHD1, PHD2 and PHD3 (also known as HIF prolyl hydroxylase (HPH) or Egg-laying Nine (EGLN), HPH3/EGLN2, HPH2/EGLN1 and HPH1/EGLN3, respectively). The various PHD isoforms differ with regard to their tissue distribution, protein structure, cellular localization, protein interactions and hydroxylation of HIF- α isoforms.²⁰ PHDs are regulated by the presence of oxygen, ferrous iron, and 2-oxoglutarate. Ferrous iron (Fe²⁺) is found in the active site of the enzyme's catalytic triad comprised of two histidines and one aspartate residue. When diatomic molecular oxygen is cleaved, one oxygen atom is transferred to the proline residue. At the same time, the second oxygen atom interacts with 2-oxoglutarate which decarboxylates to form succinate and carbon dioxide.¹⁴ These enzymes can therefore be inhibited via iron chelation. Structural analogs of 2-oxoglutarate such as dimethyl-oxalyl-glycine (DMOG) have also been shown to inhibit PHD activity.

PHD1 is a 43.6 kDa enzyme highly expressed in the testis, with lower levels reported in the kidney, heart, and liver.²¹ Unlike PHD2 or PHD3, PHD1 is constitutively expressed and is not induced by hypoxia or other hypoxia mimetics (DFO and CoCl₂) but is responsive towards estrogen. PHD1 hydroxylates HIF-1 α at both the N-terminal ODDD at Pro564 and C-terminal ODDD at Pro402. PHD2 is known as the dominant prolyl-4-hydroxylase.²⁰ It is ubiquitously expressed but shows highest expression in the heart with moderate expression in the brain. A majority of PHD2 is localized in the cytoplasm with a smaller population found in the nucleus. The 46 kDa enzyme preferentially regulates HIF-1 α over HIF-2 α and hydroxylates both the N- and C-terminus ODDD. Additionally, the HRE that is recognized by HIF-1 is also found in gene regulatory regions of *Phd2* and *Phd3*, thereby conferring oxygen sensitivity to their expression. In-vivo studies confirm that PHD2 is the most important PHD family member during development. PHD2^{-/-} embryos all died between E12.5 and E14 whereas *Phd1*^{-/-} and *Phd3*^{-/-} embryos were viable.²² Somatic *Phd2*^{-/-} mouse models reveal elevated EPO levels in the kidney due to increased HIF- α levels.²³ *Phd1*^{-/-}/*Phd3*^{-/-} double knock-out mice do not exhibit erythropoiesis phenotypes.

By contrast with PHD2, PHD3 is distributed uniformly between the nucleus and cytoplasm. It is upregulated by hypoxia and other hypoxia mimetics, but still shows considerable hydroxylase activity under hypoxic conditions for an oxygen-dependent enzyme.²⁴ Similar to PHD1, the 27.3 kDa enzyme displays greater activity towards HIF-2 α over HIF-1 α . PHD2 preferentially hydroxylates the C-terminal ODDD at Pro564 in a sequence-specific manner, whereas PHD1 and PHD3 recognize the ODDD based on their spatial conformations.

HIF- α family members

There are three main HIF- α subunits. HIF-1 α is ubiquitously expressed whereas the related HIF-2 α exhibits a much more tissue-restricted expression pattern.²⁵ HIF-2 α , also known as endothelial PAS domain protein 1 (EPAS1), is structurally similar and can regulate many of the same target genes as HIF-1 α , although HIF- α family members can also act in different ways.²⁶ In cells that express both HIF-1 α and HIF-2 α , each appears to regulate overlapping as well as distinct functions. In endothelial cells, for example, HIF-1 α controls proliferation, metabolism, and survival whereas HIF-2 α controls cell migration, adhesion, and vessel integrity.²⁷

In murine embryos, HIF-2 α , but not HIF-1 α , induces Oct-4, a stem cell factor important in the self-renewal of undifferentiated embryonic stem cells.²⁸ These discovery of specific HIF-2 α target genes that promote growth and repress differentiation is consistent with possible HIF-2 α involvement in tumorigenesis. pVHL-defective renal carcinomas overproduce HIF-1 α and HIF-2 α or just HIF-2 α alone. Inhibition of HIF-2 α , but not HIF-1 α , can override the tumor suppressor activity of pVHL.²⁹ HIF-1 α and HIF-2 α can also perform opposite roles. For instance, HIF-1 α inhibits c-Myc activity by interacting through its PAS B domain and displaces Myc from regulatory sequences.³⁰ As a result, the c-Myc-repressed cyclin-dependent kinase inhibitors p21 and p27 are upregulated, and c-Myc-activated cyclin D2 and E2F are downregulated. HIF-2 α , therefore, potentiates c-Myc activity to enhance cell growth. Not much is known about the HIF-3 α subunit other than that it can act in a dominant negative fashion as an inhibitory PAS domain protein by repressing HIF-1 α transcriptional activity.³¹

Oxygen-sensing mechanisms

A large body of evidence suggests that reactive oxygen species can modulate HIF activity. Many intracellular sources of ROS such as the endoplasmic reticulum, peroxisomes, and plasma membrane NADPH oxidases have been cited, but the most significant contribution comes from the mitochondrial electron transfer chain (ETC). The ETC consists of five multiprotein complexes that are embedded within the inner mitochondrial membrane.² Complexes I and II oxidize the energy-rich molecules NADH and FADH₂, respectively, and transfer the resultant electrons to ubiquinol that then shuttles them to complex III. Complex III ferries these electrons across the inner mitochondrial membrane to cytochrome *c*, which carries them on to complex IV. Complex IV then uses these electrons to reduce oxygen to water. Each of these steps is associated with the pumping of protons into the intermembrane space, generating a proton gradient, the dissipation of which is coupled to complex V to drive the energy-costly phosphorylation of ADP to ATP. Early studies with ETC inhibitors, or cells depleted of mitochondrial DNA and hence critical electron transport chain components, laid the groundwork for more recent studies supporting a role for mitochondrial electron transport in hypoxic stabilization of HIF-1 α .³² These experiments showed that hypoxia could paradoxically trigger an increase in mitochondrial ROS production, and that antioxidant treatment could inhibit hypoxic HIF activation. Subsequently, it was shown that exogenous hydrogen peroxide could stabilize HIF-1 α during normoxia³³ and cells depleted

of mitochondrial DNA or critical ETC components failed to produce ROS during hypoxia.^{34–36}

Though there are many possible mechanisms by which mitochondrial ROS could regulate HIF- α subunit stability, direct inhibition of PHD activity would be quite attractive. In fact, one report showed that elevated ROS levels led to enzyme inactivation of PHD2 by catalyzing a ferrous oxidation in its active domain.³⁷ It has been proposed that ROS alter PHD2 at important disulfide bonds to inhibit its activity, a regulatory mechanism similarly seen in protein tyrosine phosphatases.^{38,39} Alternatively, ROS might initiate a signal transduction cascade that post-translationally modifies and inhibits PHD2 via the p38 mitogen-activated protein kinase family. In support of this, mouse embryonic fibroblasts lacking p38 or its upstream effector MKK3/6 failed to stabilize HIF-1 α in response to hypoxia-generated ROS.⁴⁰

HIF target genes and clinical implications of HIF activity in neonates

Hypoxia is defined by oxygen tensions below normal physiological levels that trigger a compensatory cellular response. Hypoxia, therefore, is context dependent. At term, following the first breath of a newborn infant, oxygen transport mechanisms in the lung enable a rapid increase in blood oxygen content. Prior to this point, from conception through birth, all of mammalian development occurs under oxygen tensions that are significantly more hypoxic than postnatal existence.⁴¹ Partial pressures of oxygen within the fetal circulation, as well as within the uterus at the time of implantation, rarely exceed 20 mmHg. This reflects a ‘physiological hypoxia’ that is normoxic for the developing human. And although the elevated hematocrit and enhanced oxygen affinity of fetal hemoglobin enable similar total oxygen contents between the maternal and fetal circulations, the partial pressure of oxygen within fetal tissues – the freely diffusible component able to interact with the mitochondrial ETC within developing tissues – is usually in the teens. Thus, following delivery of a preterm neonate and the institution of pulmonary oxygenation strategies, a profound change in the cellular oxygen environment of infants ensues. Understanding the consequences of this environmental insult is of paramount importance for improving the care of preterm infants in the intensive care nursery.

Under hypoxic conditions, oxygen-dependent PHDs and FIHs are inactivated, HIF-1 α is no longer hydroxylated and can translocate to the nucleus, where it heterodimerizes with ARNT to activate the transcription of many target genes.⁵ HIF-1 can activate the transcription of many genes that regulate diverse functions including erythropoiesis and iron transport, angiogenesis, and glucose metabolism, to name just a few.³ Following the transition to postnatal existence and the associated exposure to increased oxygen levels, many of these pathways are prematurely inactivated in preterm infants.

Erythropoiesis, iron transport and anemia

Low oxygen conditions increase blood oxygen-carrying capacity by upregulating the expression of genes involved in erythropoiesis and iron metabolism. EPO is a glycoprotein hormone initially produced by the fetal liver and then by specialized kidney cells that is necessary for red blood cell survival.⁴² Hypoxia orchestrates the transcription of *Epo* and *Epo* receptor (*EpoR*) genes⁴³ as well as genes involved in hemoglobin synthesis such as transferrin (*Tf*) and transferrin receptor (*Tfr*).^{44,45} *Tfr* is a cell-membrane-associated glycoprotein that regulates cellular uptake of iron from transferrin. *Tfr* transports ferric iron (Fe^{3+}) bound to transferrin into cells that can then be incorporated into the heme group of hemoglobin in erythrocytes. Hypoxic induction of these pathways is one of the main mechanisms contributing to elevated fetal hematocrits in utero.¹ The developmental requirement for HIF activity in regulating erythropoiesis was demonstrated in *Arnt*-null

embryos that showed erythropoietic defects as well as abnormal expression of iron metabolism proteins. HIF deficiency affected erythropoiesis during embryo and yolk sac development.⁴⁶ Interestingly, postnatal deletion of HIF-2 α showed that it is the key HIF- α subunit responsible for regulating adult erythropoiesis, especially under stress conditions.⁴⁷ Therefore, in addition to the clinically indicated blood sampling that results in significant anemia requiring blood transfusions in preterm neonates, exogenous oxygen administration, by suppressing HIF activity, likely also plays a contributory role in promoting clinically significant anemia in the ICN.

Angiogenesis and retinopathy of prematurity

In addition to residing in a globally hypoxic environment, proliferating tissues in utero also experience localized gradients of oxygen tension due to an increase in cell mass and the resultant cell distances from nearby vasculature. The globally hypoxic nature of the in-utero environment ensures that small changes in cellular oxygen tension are met with rapid and compensatory activation of HIF-dependent gene expression. This is because HIF- α stability begins to increase at approximately 6% O₂, representing the high end of in-utero O₂ tensions. Between 0.5% and 6%, small decreases in O₂ tension result in immediate and at times exponential increases in HIF- α subunit stability⁶, ensuring rapid and well-coordinated hypoxia responses. Local tissue responses to decreased oxygenation induce HIF-1 activity and trigger expression of angiogenic factors such as vascular endothelial growth factor (VEGF). VEGF promotes migration of endothelial cells in a process called angiogenesis to promote blood vessel growth to increase oxygen delivery. Importantly, *Hif-1 α* ^{-/-} or *Arnt*^{-/-} embryos exhibit failed vascularization and embryonic lethality due to impaired VEGF production.^{48–51}

The eye develops rapidly during gestation with blood supply to the optic nerve starting at about 16 weeks and blood vessel growth towards the retina continuing until birth. In premature neonates, this normal blood vessel growth is disrupted and ultimately results in dysregulated compensatory retinal neovascularization, which contributes to retinopathy of prematurity (ROP). ROP is a disease that occurs in premature babies and is the leading cause of blindness among children in the USA. In the retina, expression of HIF- α proteins and Vegf mRNA patterns are temporally and spatially correlated.⁵² Both HIF-1 α and -2 α appear to play contributory roles. In addition to VEGF, the VEGF receptor FLT-1 is also induced when endothelial cells are exposed to hypoxic conditions.⁵³ *Hif-2 α* ^{+/+} mice were indistinguishable from *Hif-2 α* ^{+/-} mice under normoxia. Interestingly, on a particular genetic background, a subset of *Hif-2 α* ^{-/-} embryos were viable and resulted only in minor vascular phenotypic changes, implying that HIF-2 α does not play a major role in developmental vascularization. However, *Hif-2 α* ^{+/+} and *Hif-2 α* ^{-/-} mice⁵⁴ had reduced retinal neovascularization and absent or diminished levels of angiogenic factors when subjected to an oxygen-induced retinopathy protocol. Conditional knockdown of *Hif-2 α* expression in the retina suggested that *Epo* was the critical target gene responsible for the effect of HIF-2 α , not *Vegf*.⁵⁵

Glucose metabolism and diabetes

Metabolic adaptations are induced by hypoxia as individual cells reprogram their glucose metabolism from oxidative to glycolytic pathways. The oxygen-dependent tricarboxylic acid (TCA) or Krebs cycle produces 38 ATP molecules per glucose molecule whereas anaerobic metabolism provides only two ATP molecules. As a result, hypoxic cells increase their glucose uptake and reduce oxygen consumption in an attempt to maintain energy homeostasis. HIF-1 plays a key role by up-regulating the expression of glycolytic enzymes and glucose transporters such as GLUT1 and GLUT3.⁵⁶ HIF-1 mediates transcription of the mitochondrial enzyme pyruvate dehydrogenase kinase 1 (PDK-1) to shunt pyruvate away

from mitochondria and reducing cellular oxygen consumption. ARNT activity in the pancreas has been shown to be important for the maintenance of euglycemia,⁵⁷ as has hepatic ARNT activity.⁵⁸ Therefore ARNT and the ARNT-like BMAL1 have been proposed as genetic loci associated with diabetes in adults.^{59–61} Whether dysregulated HIF activity contributes to the frequently observed glucose instability in premature neonates is currently unclear.

Lung development and respiratory distress syndrome

Lung disease is another common problem facing neonatologists due to arrested pulmonary vascular development and angiogenesis. Affecting about 1% of all births and 60% of premature infants born at <32 weeks of gestation and weighing <1000 g, respiratory distress syndrome (RDS) is a major cause of neonatal morbidity and mortality.⁶² In addition to its role in tissue angiogenesis, the three VEGF also stimulate vascularization, branching morphogenesis, and alveolar development in the lung.⁶³ VEGF is a major factor regulating fetal lung maturation and is tightly regulated by HIF activity. HIF-1 α expression levels decreased by almost 80% and HIF-2 α levels decreased by 55% in the lungs of RDS lambs in high oxygen concentrations following premature birth along with concomitant decreases in VEGF mRNA. The inhibition of PHDs using pharmacological inhibitors DMOG and FG-4095 show parallel increases of HIF-1 α , -2 α and VEGF.⁶⁴ Gene deletion studies show that HIF-1 α and HIF-2 α are critical for normal pulmonary development; however, similar to what was found in retinal development, HIF-1 α seems to act as the primary player in fetal lung development whereas HIF-2 α functions in the fine-tuning of vascularization. Surprisingly, HIF-2 α -deficient mice had reduced VEGF levels in alveolar cells that caused fatal RDS in neonatal mice due to insufficient surfactant production,⁶⁵ suggesting that HIF-2 α has a greater role than just promoting lung vascularization. In these studies, mice that were delivered prematurely exhibited improved alveolar maturation following intratracheal VEGF administration. Inhibition of VEGF also impairs normal pulmonary alveolar and vascular development in a rat model.⁶⁶

Inflammation and necrotizing enterocolitis

Necrotizing enterocolitis (NEC) is a disease of prematurity wherein the lining of the intestinal wall undergoes necrosis and results in tissue death.⁶⁷ This serious life-threatening gastrointestinal disorder has no definitive etiology but it is generally believed that decreased blood flow due to unknown causes damages the bowels and increases intestinal permeability and susceptibility to bacterial invasion. However, changes in the oxygen environment of the developing intestinal epithelium may also be playing contributory roles. For example, in mouse models of experimental colitis, HIF activity has been shown to be protective.⁶⁸ HIF-deficient intestines exhibited more severe features of intestinal colitis compared with their wild-type counterparts and HIF-overexpressing animals were protected. The intestinal epithelial barrier-protecting agents Mucin 3 and Trefoil Factor are induced by hypoxia in an HIF-dependent manner,^{69,70} suggesting a direct link between HIF activity and intestinal barrier function. Additionally, HIF family members have been shown to play important roles in innate immunity.⁷¹ Conditional gene targeting in myeloid lineages has revealed important roles for HIF activity in monocytes/macrophages as well as neutrophils. HIF promotes the bactericidal properties of phagocytic cells and enhances the innate immune functions of dendritic cells, mast cells and epithelial cells. Dynamic changes in HIF levels are seen in the context of various infectious states. This effect appears at least in part to be mediated via cross-talk between HIF and nuclear factor (NF)- κ B-dependent transcriptional pathways.⁷² NF- κ B, critical regulator of the innate immune response, has been shown to be critical for hypoxic HIF activity in the innate immune system, formerly linking ancient immune responses with ancient oxygen-sensing pathways. Taken together, these studies suggest that the in-utero hypoxic environment may be protective for intestinal epithelial integrity and

barrier function and that premature exposure to elevated oxygen tensions in the setting of preterm birth may predispose these infants to NEC.

PHD inhibitors in the ICN

Inhibition of developmentally critical HIF activity due to exogenous oxygen administration in preterm neonates contributes to their various comorbidities. It is therefore reasonable to speculate that, in addition to decreasing oxygen use, pharmacological stabilization of HIF family members should produce significant benefits for this at-risk population. In an oxygen-induced retinopathy mouse model, for example, PHD inhibition significantly attenuated the degree of disease.⁷³ In fact, a single dose of PHD inhibitor produced a more significant protective effect than multiple rounds of EPO or VEGF administration, highlighting the pleiotropic effects of oxygen exposure and HIF activity in developing neonates. PHD inhibition also enhances lung angiogenesis in a primate model of bronchopulmonary dysplasia, likely due to increased VEGF production.^{74,75} Development of lung microvasculature is critical for distal airway formation and therefore PHD inhibitors may play a protective role in promoting normal lung development in premature neonates. Therefore, the time to seriously consider PHD inhibitors in the intensive care nursery is here. While decreasing the oxygen exposure of premature neonates should still be the primary goal, PHD inhibition could have a significant impact on outcomes for premature infants.

Practice points

- Oxygen is a morphogen.
- Human development occurs under a physiological hypoxia and preterm delivery results in premature exposure to supraphysiological oxygen levels.
- Inactivation of HIF by exogenous oxygen administration impairs retinal vascularization, lung development and hematopoiesis and possibly contributes to the etiology of NEC.

Research directions

- Test the utility of PHD inhibitors in the neonatal intensive care setting to activate HIF-dependent gene expression in premature neonates.
- Develop biomarkers of lung disease and NEC using genomics as well as proteomics and mass spectrometry-based approaches to track disease severity, progression and response to PHD inhibition.

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Figure 1.

Schematic diagram of hypoxia-inducible factor (HIF)- α subunit molecular structure and interacting regulatory hydroxylases. HIF-1 α belongs to the basic helix-loop-helix (bHLH) and PAS (PER-ARNT-SIM) protein family. All HIF- α subunits contain an N-terminal transactivation domain (NTAD) within the oxygen-dependent degradation domain (ODDD) that mediates oxygen stability through the hydroxylation of proline residues via prolyl hydroxylase domains (PHD). The C-terminal transactivation domain (CTAD) region found only in HIF-1 α and HIF-2 α contains an asparagine residue that is hydroxylated by factor inhibiting HIF (FIH) and prevents recruitment of p300/CBP transcriptional activators.

Figure 2.

Outline of hypoxia-inducible factor (HIF)- α regulation. During normoxia, prolyl hydroxylase domains (PHD) hydroxylate HIF- α on proline residues in the presence of Fe²⁺ and 2-oxoglutarate. Hydroxylated HIF- α is recognized by the E3 ubiquitin ligase von Hippel-Lindau tumor suppressor protein (pVHL) and is ubiquitinated and targeted for proteasomal degradation. During hypoxia, mitochondria-generated reactive oxygen species (ROS) can lead to the inhibition of PHD activity via unknown mechanisms, allowing HIF-1 α to translocate to the nucleus, dimerize with HIF-1 β , bind to the HRE, and induce expression of target genes such as *Vegf*, *Glut-1* and *Pdk-1*. Hypoxia mimetics such as dimethyl-oxalyl-glycine (DMOG) display similar effects of blocking PHD hydroxylation.