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Interrelationships Among Hypoxia-Inducible Factor Biology and Acid-Base Equilibrium

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In this article, we try to summarize the most important novel biological information on the complex interrelationships between acid-base alterations and hypoxia-inducible factor (HIF) signaling. Extracellular and intracellular acid-base alterations affect HIF signaling in part independently of hypoxia, and involve, among others, effects on cytoprotection and apoptosis. Conversely, HIF signaling may affect systemic and local acid production rates and has been implicated in the mechanism of the acute hyperventilatory response (ie, respiratory alkalosis) in response to hypoxia as well as for hypoxia-induced pulmonary artery hypertension (PAH), although the latter data are quite preliminary and can be explained by alternative mechanisms. Thus, this review calls attention to these relationships for renal physiologists and nephrologists to stimulate focused clinical observations and specific investigative efforts as proposed in this overview.
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KEYWORDS cancer, iron deficiency, HIF, hypoxia, acidosis, acid-base, respiratory alkalosis, pulmonary artery pressure, endogenous acid production

Hypoxia-inducible factor (HIF) is a heterodimeric transcription factor family comprising oxygen-sensitive HIF-α subunits that function only after heterodimerizing with an oxygen-insensitive HIF-1β subunit (Fig 1). Thus, HIF-1β is stably present intracellularly whereas HIF-α (chiefly HIF-1α and HIF-2α isoforms) is produced constitutively but fails to accumulate under normoxic conditions owing to recruitment and binding by the tumor-suppressor protein, von Hippel-Lindau (pVHL), and targeted degradation through the ubiquitin-proteasome pathway.[1] pVHL exists in the cytoplasm as a multimeric protein complex (VCB-Cul2 in which VHL is bound to elongin C, elongin B, Cul-2, and Rbx1) and shows numerous other functions besides HIF binding. The molecular mechanism for oxygen-dependent degradation of HIF-α is based on the hydroxylation of defined proline residues on HIF-α, catalyzed by a family of HIF prolyl hydroxylases (dioxygenases) that require molecular oxygen as a substrate, with iron and 2-oxoglutarate as cofactors. Thus, HIF prolyl hydroxylases (HIF-PHDs) act as the body's main oxygen sensor, and under hypoxic conditions become inactivated to allow for HIF-α stabilization and initiation of the HIF-responsive transcriptional cascade.[2,3] As counterpoint, the PHD isoform PHD2 has been reported to undergo robust hypoxic induction. This may represent a feedback control mechanism that dampens hypoxic gene responses or prevents overstimulation in the posthypoxic period.[4] The PHD isoforms also appear to have low substrate specificity, raising the possibility that non-HIF substrates may be regulated by this family of dioxygenases.[5]

On stabilization, the HIF-α/HIF-β heterodimer rapidly translocates into the nucleus, where it binds to a conserved DNA motif found within the promoters of all HIF target genes, termed the *hypoxia response element* (HRE), and, in cooperation with other coactivators such as p300, initiates de novo transcription.[6,7] Numerous genes have been shown to be under HRE control including those with erythropoietic, cytoprotective, and metabolic functions (eg, erythropoietin [EPO], heme oxygenase-1, glucose transporter-1, vascular endothelial growth factor A [VEGF-A]).

Role of Acidosis or Intracellular pH in Regulating HIF: Possible Role in Cytoprotection

Acidification of media in vitro provides cytoprotection during hypoxic injury in myocardial,[8] renal cortical,[9] and corti-

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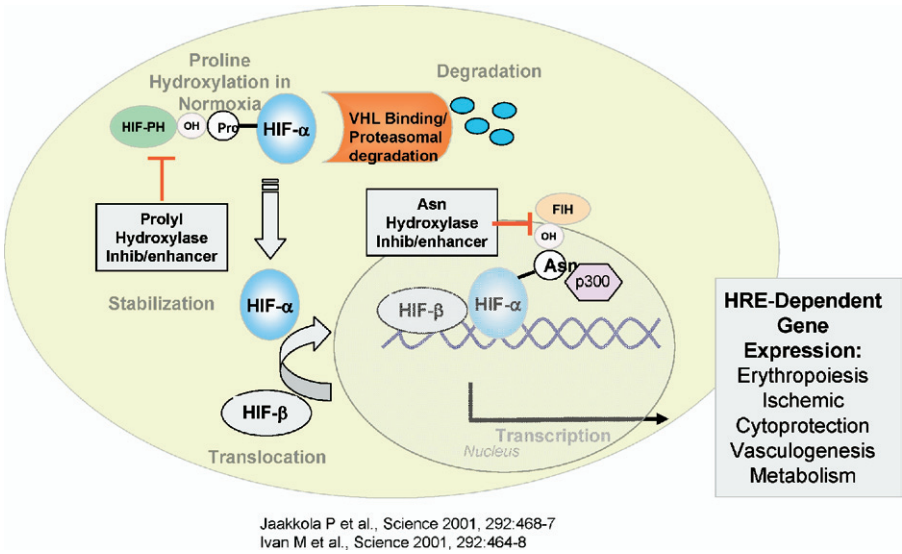

Figure 1 HIF regulation of gene transcription. PH, prolyl hydroxylase; Asn, asparaginyl; FIH, factor inhibiting HIF. (Color version of figure is available online.)

cal neuronal[10] cells, as well as in isolated perfused liver,[11] raising the possibility that cell acidification also might regulate HRE gene responses or share the HIF pathway with hypoxic stimuli. After 6 hours of normoxic exposure to hypercapnic media (pH 7.0, in vitro respiratory acidosis), HIF-1α protein was increased by more than 3-fold in Hep3B liver cells and this effect also was found after 20 hours of isocapnic pH reduction (14 mmol/L HCO_3), albeit to a lesser extent than that achieved by severe hypoxia.[12] Moreover, in hypoxic HeLa cells, both 6 and 20 hours of superimposed hypercapnic acidification resulted in significant additional HIF-1α protein accumulation beyond that produced by hypoxia alone. Isocapnic acidification (a model of in vitro metabolic acidosis) of hypoxic HeLa cells also produced significant additional HIF-1α accumulation beyond that afforded by hypoxia alone.[12] Importantly, using a luciferase reporter gene construct in HeLa cells, isocapnic medium acidification increased HRE-mediated gene transcription rates under both normoxic and hypoxic conditions, which in the case of hypoxic cells resulted in an additional doubling of reporter gene expression. Normocapnic acidification induced selective amplification of specific hypoxia-induced gene responses. As analyzed by quantitation of messenger RNA (mRNA) levels of carbonic anhydrase IX, a HRE-modulated gene, a significant amplification by normocapnic acidification was shown, although such amplification was not found for glucose transporter-1.[12]

The inducible isoform of heme oxygenase (HO-1), another HRE-responsive gene, also has been reported recently to be up-regulated by extracellular acidification, via both transcriptional and posttranscriptional mechanisms in primary cultures of both aortic and pulmonary artery smooth muscle cells.[13] Because heme oxygenase-1 provides a classic cytoprotective, anti-inflammatory, and antiproliferative response to hypoxia as mediated by HIF stabilization, it will be interesting to evaluate the relative roles of acidosis and hypoxia in the overall response to organ injury (eg, in response to ischemia) and acute renal failure in particular.

Although overall understanding of the mechanism of acidification-mediated HRE gene transcription is at a very early stage, there are a number of possible control mechanisms to consider. For example, the relative roles and possible interplay of HIF-1α and HIF-2α on acidosis-induced HRE gene expression has not been explored thoroughly and will be important to consider because some HIF target genes are relatively isoform-specific (eg, EPO is under predominant control by HIF-2α in hypoxia). In HeLa cells, hypoxia increased PHD3 mRNA whereas superimposed isocapnic acidification significantly further amplified PHD3 mRNA abundance without an effect on either PHD1 or PHD2.[12]

Thus, it will be important to assess the specific question of whether acidosis might provide a homeostatic signal to brake or counter-regulate the exuberant HIF stabilization engendered by hypoxia. For example, if PHD1 and PHD2 protein expression are ordinarily rate limiting for HIF-α stabilization as a hypoxic event occurs, then is it possible that a variable concomitant local or systemic acidosis provides additional PHD activity to extinguish HIF-mediated HRE activity in a necessary and timely manner?

Possible Role of VHL Transport/Sequestration in Determining Acidosis-Induced HRE Gene Expression Responses

Recently reported studies in cultured myoblast cells, neurons, and renal cell cancer cells have shown that isocapnic extracellular fluid (ECF) acidification from pH 7.2 to 6.3 (HCO_3 reduction) results in VHL protein undergoing rapid transport from its normally diffuse cytoplasmic/nuclear localization into a highly sequestered nucleolar locale under

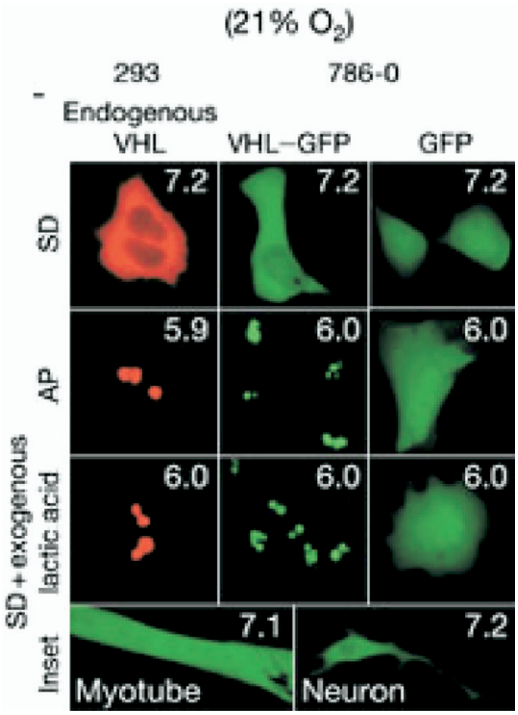

Figure 2 Extracellular acidification by metabolically active cells triggers the redistribution of VHL to a subnuclear (nucleolar) locus in normoxia. Human embryonic kidney (HEK) 293 cells with endogenous VHL or VHL-deficient 786 to 0 renal cancer cells expressing adenovirus-introduced VHL-green fluorescence protein (GFP) or GFP are shown. Cells were incubated in standard (SD, pH stable at 7.2) or acidic (AP, low HCO_3, pH 5.8-6.0) media. Alternatively, exogenous lactic acid was added to cells in standard media (SD, pH stable at 6.0). Staining for endogenous VHL or detection of GFP signal identified protein localization. Inset shows C2C12 mouse fibroblast myotubes and PC12 neurons, which failed to acidify AP media under normoxia and showed no change in the localization of adenovirus-introduced VHL-GFP. Adapted by permission from Nat Cell Biol,[14] 2004. (Color version of figure is available online.)

either hypoxic or normoxic conditions (Fig 2).[14] In these studies, normohydric hypoxia stabilized HIF as expected by preventing PHD activity owing to the unavailability of O_2 for HIF hydroxylation. In contrast, under acidic conditions, under either basal normoxia or after re-oxygenation after hypoxia, HIF also was stabilized. Thus, under all conditions in which nucleolar sequestration of pVHL (exclusively acidic conditions) is obtained, pVHL was inactive or physically denied access to its HIF target for subsequent HIF degradation.

The mechanism for extracellular acidification to sequester pVHL in the nucleolus has been elucidated further. Ordinarily, under normohydric conditions, the VCB-Cul2 complex is quite mobile within the cell. A protein surface region of the VHL β sheet domain has been identified as a discrete pH-responsive nucleolar detention signal that targets the entire multimeric VCB-Cul2 ubiquitin ligase complex to nucleoli.[15] To date there are no reports relating changes in intracellular pH (pHi) to nucleolar detention. Interestingly, nucleolar pH measurements using intracellular fluorophores and confocal microscopy have found the nucleolus to be significantly more acidic than surrounding nucleus and cytoplasm.[16]

Because acidosis is observed in ischemic tissues and is a function of hypoxic stress, Mekhail et al[17] have proposed that acid-induced nucleolar sequestration of pVHL provides a mechanism for prolongation of HIF-induced cytoprotection by virtue of making pVHL refractory/unavailable to degrade HIF even when tissue O_2 tension subsequently is normalized as noted in Figure 3.

Can HIF Stabilization Control Systemic Metabolic Acid Production Via its Effects on Glycolysis?

Not only is acidosis reported to be a mediator of HIF stabilization and downstream gene expression, but there is evidence that the generation of metabolic acid itself can be dependent on the presence of HIF. In mouse embryonic fibroblast (MEF) cells, the ability to shift during hypoxia from oxidative to glycolytic metabolism with increased acid generation, the *Pasteur effect*, has been shown to be dependent on HIF. When glucose-replete MEF cells are exposed to 72 hours of hypoxia, cells with deleted HIF-1α show a significant defect in lactate and acid generation of sufficient magnitude to fully prevent the medium pH reduction observed in wild-type cells and is associated with a significant decrease in free adenosine triphosphate (ATP) levels.[18]

Further evidence for a critical role of HIF in glycolytic capacity with metabolic acid generation and its effects on cell survival was reported using A549 lung adenocarcinoma cells. Knockdown of HIF-1α expression with specific small interfering RNA (siRNA) inhibited lactate production and increased medium pH while protecting the cells from hypoxia-induced apoptosis.[19] On the other hand, overexpression of HIF-1α resulted in increased glycolysis, acidification of the medium, and increased hypoxia-induced apoptosis. Whether HIF-induced glycolysis-driven acidification per se explains some of the reported tumor-protective effects of HIF stabilization[20,21] remains to be determined.

Thus, although existing evidence suggests that systemic HIF stabilization might result in increased organic acid production owing to increased expression of glycolytic enzymes, it is still not clear, in vivo, how HIF stabilization might affect lactate and pyruvate oxidation rates that are critical determinants of the net organic acid production rate. For example, for glycolysis to manifest as an increased in vivo acid production rate, mitochondrial pyruvate disposal must be inhibited. Normally pyruvate dehydrogenase converts pyruvate to acetyl-CoA. Pyruvate dehydrogenase activity is ordinarily under the inhibitory influence of pyruvate dehydrogenase kinase-1, activation of which increases lactic acid production. Recently, it has been reported that HIF-1α has the potential to suppress lactate oxidation via transactivation of pyruvate dehydrogenase kinase-1 in mouse embryo fibroblasts.[22] HIF effects on in vitro and in vivo organic acid production rates have not been reported. Nevertheless, organic acidosis has not been

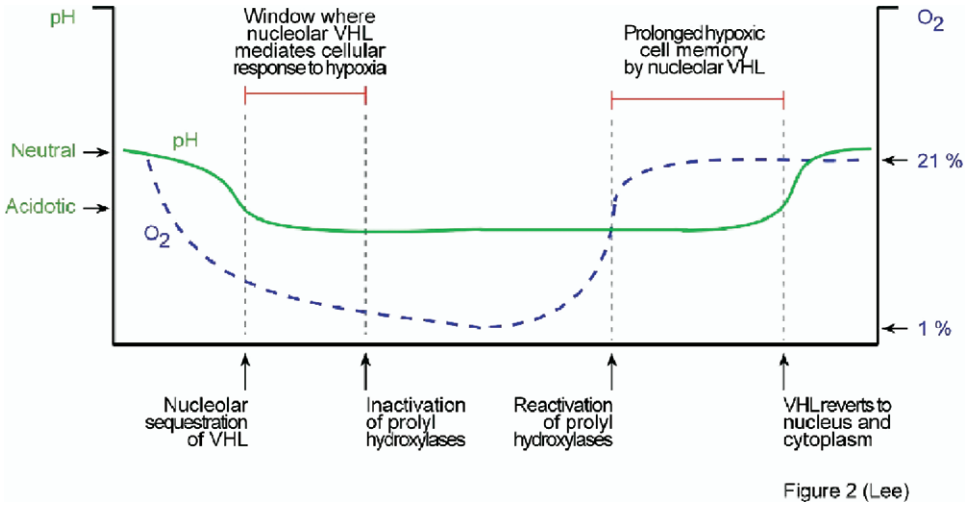

Figure 3 Oxygen sensing by H^+ and hypoxic cell memory. A moderate decrease in oxygen concentration is accommodated by a shift to glycolysis, leading to acidification of the ECF via excess lactic acid production. ECF acidosis leads to redistribution of VHL to nucleoli and stabilization of HIF-α before inactivation of PHDs. Further decreases in oxygen prevents hydroxylation of HIF-α. Reoxygenation of acidotic cells reactivates PHDs without affecting nucleolar VHL. This prolongs HIF stability and hypoxic cell memory until neutral conditions are restored. From Mekhail et al.[17] (Color version of figure is available online.)

reported to be present in states with demonstrable HIF-α overexpression (eg, Chuvash polycythemia, see later).

Recent Studies Have Broadly Implicated Extracellular and Intracellular Acidification in HIF-Mediated Effects on Apoptosis and Cell-Cycle Arrest

Recently, several reports have appeared regarding the specific effects of intracellular pH reductions on HIF stabilization or HRE-responsive gene transcription. By using 8 different human cancer cell lines, the effect of bafilomycin (a V-type H-ATPase inhibitor), an agent expected to cause a reduction in cell pH by its ability to block proton extrusion, was examined for HIF stabilization.[23] This study showed the large effect that bafilomycin had in increasing HIF-1α half-life with no change in its mRNA abundance. Bafilomycin also resulted in a significant G1 cell-cycle arrest (antitumor growth) that was abrogated in HIF-1α null cells. Intracellular pH was not measured, but the results are consistent with a role for intracellular acidification. Although extracellular alkalinization did not affect bafilomycin-induced HIF-1α accumulation in this report and this particular finding has been editorialized[24] as evidence against a role for reduced cytosolic pH in stabilizing HIF-1α, extracellular alkalization per se may have no effect on pHi or even endosomal pH under these conditions. Abundant V-type H-ATPase protein has been localized to the plasma membrane of cancer cells with high metastatic activity by immunocytochemistry, whereas H-ATPase was not found in the limiting membrane in tumors with low metastatic activity.[25] In isolated plasma membranes, V-type H-ATPase enzymatic activity displayed a similar pattern and was sensitive to bafilomycin inhibition. Importantly, highly metastatic cells examined using pHi measurements, were shown to selectively and quantitatively use H-ATPase activity over Na/H exchange activity to regulate cytosolic pH. Bafilomycin's ability to decrease pHi in highly malignant cancer cells correlated with an acute decrease in cell migratory ability.[25-27] A recent report using glioblastoma cell lines showed that normoxic extracellular acidosis resulted in increased carbonic anhydrase IX (a known HRE gene, a transmembrane protein with extracellular enzymatic activity) mRNA and protein accompanied by HIF-1α accumulation.[28] Taken together, these results provide further support for acidification-induced HIF stabilization that, in cancer cells, fosters apoptosis and cell-cycle arrest and that the cell-acidifying Pasteur effect itself may be HIF-dependent. The selective presence of V-type H-ATPase in the plasma membrane may contribute significantly to ECF acidification within invasive tumors while permitting cell viability by prevention of undue cytosolic acidity. Acidic ECF pH benefits tumor cells because it promotes invasiveness, whereas an alkaline pHi provides a growth advantage.[25] Importantly, V-type ATPase is antiapoptotic in tumor cells,[25] possibly providing a critical intersection for tumor control/amelioration by HIF-1α.

Role of HIF Stabilization, Per Se, in Oncogenesis or Tumor Progression: Evidence That HIF Stabilization by Acidosis or Hypoxia is Insufficient to Produce Cancer

In typically acidic cancer cells, for example, where excess protons, pyruvate, and lactate are produced even in the pres-

ence of only mild hypoxia or even normoxia (Warburg effect), preferential reliance on glycolysis versus oxidative phosphorylation is correlated with disease progression. Although oncogenes such as *ras*, *src*, and *myc* have been found to enhance aerobic glycolysis by increasing the expression of glucose transporters and glycolytic enzymes, the relevance of the Warburg effect to cancer cell biology has remained obscure.[29] An important question, therefore, is whether extra/intracellular acidification/acidosis-induced HIF stabilization and/or down-stream gene expression plays a role in tumorigenesis or tumor progression.

Because certain, but not all, inactivating mutations within the VHL gene are highly associated with VHL syndrome (autosomal-dominant pattern with inheritance of a single mutated allele followed by somatic inactivation/mutation of the other allele), a cancer syndrome predisposing individuals to several tumors, including renal cell carcinoma and pheochromocytoma, it is difficult to argue that permanent loss of pVHL's HIF regulatory function is responsible/supportive for such tumors. Polycythemia is uncommon in VHL syndrome, but has been reported in VHL syndrome with hemangioblastoma.[30] Nevertheless, it is not surprising that nucleolar pVHL sequestration by acidosis has received speculation as another conceivable way to generate or foster tumor growth.[17]

Further understanding of the prospects for acidosis to modulate oncogenesis or tumor progression via VHL effects can be achieved from an understanding of VHL pathophysiology in general. Apart from the VHL syndrome, additional heterozygous and homozygous VHL mutations in human beings have been characterized for polycythemia-causing or associated phenotypic classes that are associated with increased plasma EPO levels but are not associated with tumor predisposition, consistent with VHL functioning to interact with multiple proteins and signaling pathways.[31] Among the reported polycythemia-causing/associated mutations beyond the common germline homozygous 598 mutation in the Chuvash polycythemia (CP) syndrome (homozygous mutation of VHL at C598T) are the combination of the C598T with another VHL mutation at either 562 or 574 and a homozygous 571 germline mutation.[31,32] The CP syndrome (polycythemia with increased plasma EPO and VEGF-A levels) is still not well understood in molecular terms. Studies in 786-O, VHL-null, renal cell carcinoma cells monitoring HIF-1α–dependent HRE reporter activity using a vector containing either the human wild-type or CP C598T VHL mutant, showed only a very small increase in transcription rate with mutant C598T VHL, and the ability of C598T pVHL to capture HIF-1α was only modestly impaired.[33] Moreover, when this mutation was tested for HIF-2α regulation in murine embryonic stem cells, no impairment was found.[34] When numerous tumor-associated VHL syndrome mutants (from both α and β VHL structural domains) were tested in murine embryonic stem cells, none affected hypoxic/normoxic HIF-1α regulation.[34,35] Although testing of HIF-2α protein regulation by VHL syndrome mutants revealed no defects in nearly all tested mutants, 1 mutant (Y112H found in a type 2A pheochromocytoma-associated phenotype) showed abnormally increased HIF-2α during normoxia.[34] Thus, there is no consistent evidence reported to date linking abnormal stabilization of either HIF-1α or -2α to tumor-associated VHL mutations. Importantly, numerous reports using mice injected with tumors have shown that experimental overexpression of HIF-1α or -2α, per se, in injected cells is insufficient to produce a tumor phenotype as long as a functionally intact VHL protein also is expressed.[36,37]

pVHL targets numerous other cell proteins besides the HIF isoforms, including fibronectin, such that VHL null cells are unable to properly assemble fibronectin matrix. In each of 6 tested VHL tumor-associated mutants, there was defective binding of pVHL to fibronectin.[34] It thus has been proposed that a tissue-specific effect on fibronectin-pVHL interaction could produce tissue-specific phenotypes independent of HIF isoform stabilization, and that a fibronectin-related defect could relate to tumor effects.[31,34] Additional molecular targets for pVHL have been implicated in tumorigenesis. Among them, protein kinase Cλ (PKCλ) has important roles in proliferation, cell survival, and cell polarity.[38] PKCδ is another pVHL target and a key regulator of cell growth and metabolism via its binding to IGF-IR. A human renal cell carcinoma cell tumor invasion assay showed that transfection with either wild-type VHL, dominant-negative PKCδ, or anti-insulin-like growth factor-I receptor antibody resulted in a similar loss of invasiveness.[39] pVHL recently has been reported to directly bind and stabilize the tumor-suppressor gene, p53, and to thereby enhance p53 transcriptional control activity including cell-cycle arrest in renal cell carcinoma cells and the induction of apoptosis after DNA damage, providing another HIF-independent mechanism whereby a defective VHL might induce tumors.[37,40] Recently, a HIF-independent protein with function related to apoptosis induction, clusterin, has been linked to VHL syndrome pheochromocytomas and renal cell carcinomas by the finding that in pVHL-defective tumors, clusterin secretion and expression were greatly reduced.[41,42] Thus, future research is needed to understand the links between VHL mutations and tumorigenesis. There is currently no clear relation among the structural mutation sites and the tumor and nontumor phenotypes. Importantly, neither pVHL inactivation nor HIFα isoform stabilization in mice has resulted in solid tumors,[43-45] although introduction into liver and skin of dual HIF-1α and HIF-2α mutants incapable of hydroxylation by PHDs produced increased tissue vascularity.[45] Consistent with the thesis that HIF stabilization per se does not confer tumor risk, an additional HIF-stabilizing mutation in human beings has been reported recently. A family with a loss of function PHD2 mutation (the mutated PHD2 is defective in vitro in inhibiting HRE reporter gene activity) with autosomal-dominant secondary polycythemia has been reported and has no apparent tumor predilection.[46] Regarding the specific experimentally induced effects of the HIF isoforms in cancer, a large and growing body of literature has documented, both for HIF-1α and HIF-2α, that their underexpression in tumor cell lines fosters tumor growth and that overexpression reduces growth.[20,21]

A direct test of the potential for HIF-α stabilization to modulate tumor progression has been reported recently us-

Table 1 Comparison of Phlebotomized CP Subjects, Normal Controls, and Phlebotomized Control Polycythemic Subjects

Analysis	CP (N = 3)	*P* value	NI Controls (N = 6)	Polycythemic Controls (N = 3)	*P* value (versus CP)
[HCO_3]a, mmol/L	21.1		23.0	23.7	
$Paco_2$, mm Hg	34.2	<.05	40.3	41.5	<.01
pHa, units	7.41	<.05	7.38	7.38	
PaO_2, mm Hg	102.1		98.9	–	
Hct, vol %	48		42	45	
MCV, fL	64	<.001	89	79	
Serum Fe, μmol/L	3.5	<.001	17.0	12.4	
Serum ferritin, μg/L	2.2		48.5	8.4	
Serum transferrin, g/L	3.8	<.02	2.6	3.3	
TSAT,%	3.6		26	15	

TSAT was computed as follows: [S Fe (μg/dL) × 70.9] ÷ S transferrin (mg/dL). To convert S Fe from μmol/L to μg/dL, multiply by 5.56. To convert S transferrin from g/L to mg/dL, multiply by 100. Statistical significance for TSAT and bicarbonate were not reported. Adapted from Smith et al.[65]

Abbreviations: NI, normal; Hct, hematocrit; MCV, mean corpuscular volume; fL, femtoliter; TSAT, transferrin iron saturation.

ing systemic administration of a small molecule PHD inhibitor, FG-2216. By using numerous xenograft mouse models of lung and colon cancer showing the anemia of chronic disease, chronic oral FG-2216 increased blood hemoglobin concentration and serum erythropoietin concentration. Tumor progression was not increased versus control animals as assessed by tumor weight, volume, and metastasis, with a trend observed toward tumor retardation.[47]

CP and HIF Stabilization: A Recently Proposed Cause of Chronic Repiratory Alkalosis

In subjects in the CP population, a homozygous mutation in the VHL gene leads to permanent, low-level, normoxic HIF stabilization with a persistent increase of plasma VEGF-A and EPO concentrations and with no increase in tumor incidence or retinopathy.[31,33,48] Lymphocyte HRE gene product VEGF-A and aldolase C mRNA have been reported to be increased in CP subjects as well.[65] Although it is highly likely that erythropoiesis is indeed stimulated as a result of HIF-α stabilization induced by homozygous mutant VHL, with HIF-α acting to up-regulate EPO and other erythron-regulating genes, the magnitude of increases in HRE-responsive gene products also may be explained by co-existing iron deficiency from the subjects' acute and/or chronic phlebotomy history (see later and Table 1). A retrospective mortality study using matched controls in Chuvashia in which affected CP homozygotes were paired with unaffected spouses and also with unaffected community members showed that the mean age at death in CP subjects was age 44 versus 46 in spouses and 44 in community members, with a higher incidence of stroke deaths in the CP population, consistent with their polycythemia.[48]

Smith et al[65] recently reported a study of basal ventilatory status and pulmonary artery hemodynamics and their responses to acute hypoxia in 12 subjects: 3 subjects with CP, 3 age- and sex-matched polycythemic subjects with idiopathic erythrocytosis or polycythemia vera, and 6 age- and sex-matched control subjects. Both CP and polycythemic controls were iron deficient as a result of prior phlebotomies (Table 1). As shown in Table 1, resting data in CP subjects revealed a significant chronic respiratory alkalosis (CRA) with an increased mean arterial pH versus the control subjects by 0.03 pH units (~3 neq/L reduction in blood H^+ concentration) and arterial carbon dioxide tension ($Paco_2$) reduced by approximately 6 mm Hg. This degree of decrease in blood [H^+] is consistent with normative data provided by Krapf et al[49] who defined the characteristics of this acid-base disorder by inducing experimental hypobaric hypoxemic CRA in normal subjects on a fixed dietary intake. Based on these data, a

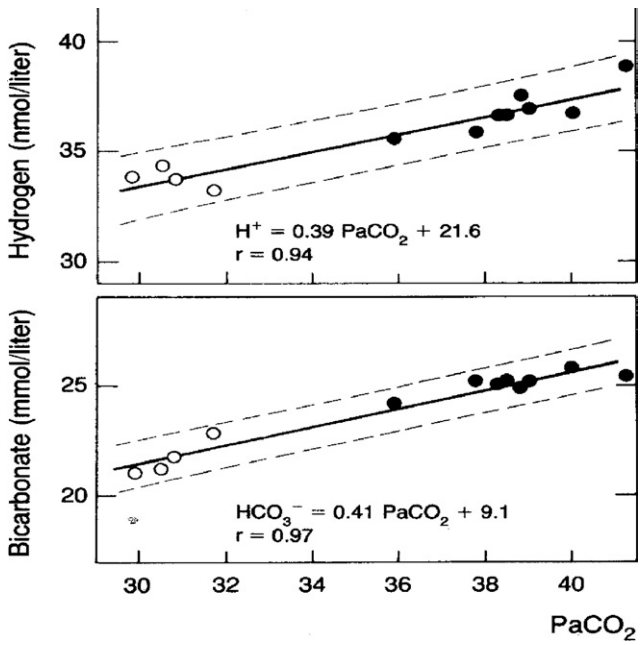

Figure 4 Relations of blood hydrogen ion concentration and plasma bicarbonate concentration to $Paco_2$ in normal subjects prior to and following induction of CRA. These data define the 95% confidence interval for the acid-base disorder, chronic respiratory alkalosis in human beings. Solid symbols represent control and recovery while open symbols reflect steady-state values for CRA. From Krapf et al.[49]

decrease in blood [H^+] of 0.41 neq/L for every mm Hg decrease in $Paco_2$ is expected, with the expected tolerance for variation as reported in the 95% confidence band for CRA in those subjects (Fig 4). Consistent with the presence of respiratory alkalosis, the minute ventilation measured with a mouthpiece tended to be higher in CP subjects than in control subjects. In response to 10-minute periods of experimentally induced isocapnic hypoxia, targeting end-tidal partial pressure of oxygen (PO_2) values of 70 and 50 mm Hg, the markedly iron-depleted CP subjects showed significantly greater increments in minute ventilation versus iron-replete control subjects with the more moderately iron-deficient polycythemic controls, showing an intermediate degree of hypoxic ventilatory hyper-response. Thus, the CP subjects showed both a reduced $Paco_2$ set point for basal control of ventilation and an enhanced acute hypoxic ventilatory (AHV) sensitivity.

It is well known that surgical bilateral loss of the carotid bodies in human beings results in complete abrogation of the AHV response and significant blunting of the hypercapnic response.[50,51] Other than for its location in the carotid bodies, the mechanistic basis for AHV response remains somewhat of a mystery as recently reviewed by Lahiri et al.[52] As shown in Figure 5, the instantaneous AHV is independent of protein synthesis and largely is attributable to depolarization of carotid body glomus cells by a sudden hypoxia-induced decrease in cell membrane K^+ conductance, resulting in membrane depolarization leading to a secondary increase in intracellular [Ca] and a subsequent increase in the discharge rate and release of dopamine and other neurotransmitters. Dopamine is recognized as the most abundant and potent neuromodulator influencing the nerve-ending response to hypoxemia. Dopamine release from glomus cells in response to hypoxia acts predominately via low-affinity excitatory postsynaptic receptors but also on high-affinity inhibitory D_2 receptors.[53] Dopamine signaling thus can either increase or inhibit the AHV response. The importance of dopamine is shown by the high glomus cell content of the dopamine synthetic enzyme, tyrosine hydroxylase (TH). Although many studies have found pro-ventilatory effects of dopamine release, a recent report showed that the administration of a dopamine D_2 antagonist resulted in acute hyperventilation in rats and an accentuation of the AHV response.[53] Both sustained and intermittent hypoxia result in increased levels of carotid body TH protein and dopamine and norepinephrine content.[54] The relative roles of each of many proposed neurotransmitters remains to be determined.

There also is evidence that more prolonged hypoxia can induce structural changes requiring protein synthesis in carotid body that include increased vascularity, hypertrophy, and hyperplasia.[52] Although there is currently no convincing evidence for classic HIF stabilization to play a role in the de novo AHV response, HIF-1α protein has been shown to accumulate in the nucleus of rat glomus cells as early as 1 hour after initiating hypoxia.[55] Accordingly, recent evidence has emerged for the role of HIF in chronic intermittent hypoxia. Although prior chronic intermittent hypoxia wild-type mice showed the expected accentuated AHV response to hypoxia, mice heterozygous for a null mutation for HIF-1α showed a complete loss of chronic intermittent hypoxia AHV accentu-

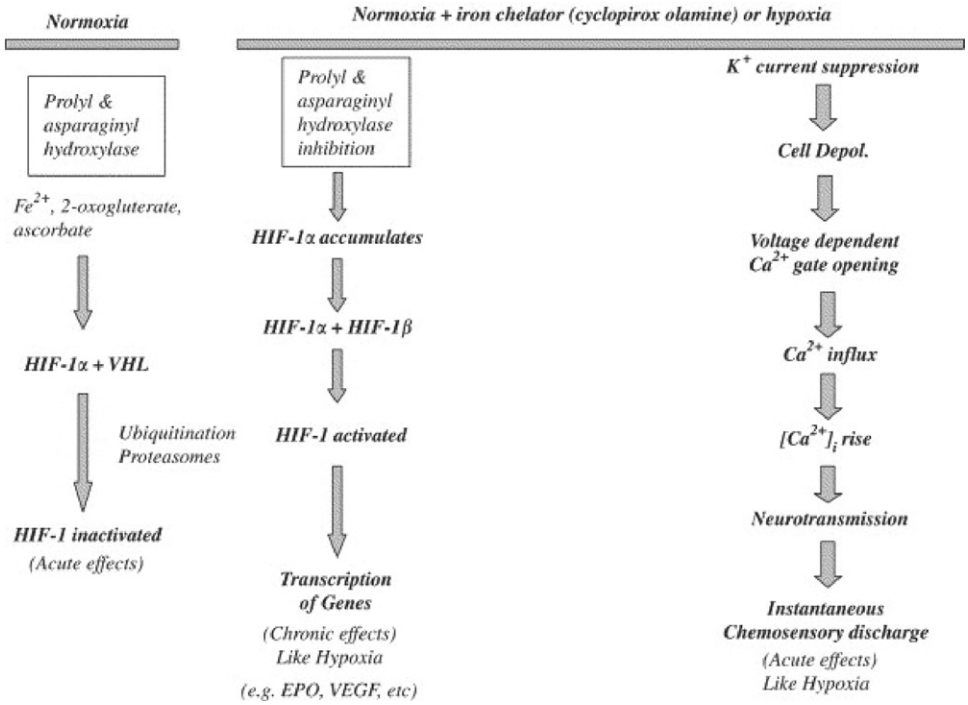

Figure 5 Schematic understanding of potential for HIF-α to become activated in carotid body glomus cells by acute Fe depletion. Effect of acute/instantaneous Fe depletion with an Fe chelator to potentially stabilize HIF-α in carotid body glomus cells despite the presence of normoxia. Parallel observations are depicted for the observed effects of acute hypoxia to suppress K+ current leading to chemosensory discharge. From Lahiri et al.[52]

ation.[56] Heterozygotes showed no impairment in their hypercapnic acute ventilatory response.

Thus, although evidence is limited at this stage, it seems unlikely that HIF stabilization at any level will modify or participate in the acute hypoxic or hypercapnic ventilatory response. The effects of physiologic or pharmacologically achievable HIF stabilization on chronic ventilatory responses to hypoxia and hypercapnia have not been reported in any species. Based on the small effects observed in heterozygous HIF null mice, it is unlikely that significant physiologically meaningful effects will be observed in wild-type animals. The overall effects of systemic HIF-α stabilization on renal and systemic acid-base equilibrium could be tested under conditions of constant dietary intake with $CoCl_2$ administration or by the use of small organic molecule inhibitors of PHDs.

Evidence That Iron Deficiency Can Result in an Accentuated AHV Response: An Alternative Iatrogenic Explanation for CRA in CP

Iron deficiency, possibly by its effect to inhibit PHD activity, induced by a 1-hour exposure of glomus cells to superfusion with an iron chelator resulted in a significant increase in cellular HIF-1α protein to levels equivalent to those of 1 hour of hypoxia.[55] Thus, it is conceivable that more chronic iron deficiency could result in sufficient HIF stabilization to result in an accentuated AHV response of the magnitude observed in chronic intermittent hypoxic conditioning.

Importantly, iron chelator exposure of rat glomus cells for less than 1 minute was reported to significantly decrease outward K^+ current and the effect was rapidly reversible with Fe addition.[57] Cytosolic [Ca] and dopamine release followed by chemosensory discharge responses were all reversibly and essentially immediately increased in parallel with the effects on K^+ current. Thus, iron sequestration or depletion can increase the sudden (eg, instantaneous) dopaminergic signaling responsible for the AHV response.

Carotid body and adrenal cells share many similarities regarding dopamine metabolism. In both locations in rats, TH protein and enzymatic activity increase in tandem during chronic hypoxia.[58] The finding that short-term dietary-induced iron deficiency resulted in a significant increase in both adrenal TH protein and enzymatic activity in rats (carotid bodies were not examined), provides a third line of evidence that iron deficiency theoretically is capable of increasing the AHV response.[59]

Pathophysiology of CP and PAH

Hypoxic pulmonary artery vasoconstriction is a normal human physiologic response to hypoxia in which increase pulmonary artery (PA) pressure directs blood from poorly ventilated lung regions to better-ventilated segments, thereby improving ventilation-perfusion mismatch. The mechanism is unknown (see later).[60]

The CP subjects studied for ventilatory function by Smith et al[65] also were reported to have PAH, in the absence of hypoxia, as assessed by echocardiography. Other than the authors' proposed lifelong stabilization of HIF-α by a defect in VHL signaling, there are additional possible explanations for the finding of increased pulmonary artery pressure (PAP) in subjects with CP. First, these subjects have been exposed to presumed lifelong polycythemia, a background not shared by the control populations selected for comparison. The major medical complication of polycythemia in this population is an increased thrombotic risk starting in the third or fourth decade of life. Thrombosis is known to be associated with sustained polycythemia of other causes, with polycythemia vera (PV) being the most common. In long-term follow-up studies the proportion of deaths attributed to cerebrovascular or other thrombotic events was reported at 47% in CP[48] and 30% in PV subjects,[61] with the lower proportion of thrombotic deaths in PV likely a result of the high competing malignancy death rate in that condition. Over half of venous thromboembolism deaths in the largest PV follow-up study were attributed to pulmonary embolism.[61] Given the difficulty of diagnosis of pulmonary thromboembolism in life, it is likely that clinically relevant chronic or intermittent pulmonary embolism affects the vast majority of polycythemic subjects. Importantly, subjects with PV and related myeloproliferative diseases showing polycythemia or thrombocytosis are reported to have a high prevalence PAH attributable in large part to pulmonary thromboemboli.[62-64] Thus, as pointed out by Smith et al,[65] prior pulmonary emboli are a reasonable explanation for PAH in CP as an alternative to lifelong HIF-α stabilization.

A second possible mechanism for the finding of increased PAP values in CP subjects is that CP patients were demonstrably iron depleted as a result of either study protocol requirements for normal hematocrits/phlebotomy or of lifelong clinically administered phlebotomies.[48,65,66] Iron, similar to O_2, is a substrate/cofactor for HIF-α hydroxylation by PHDs and HIF-α is thereby demonstrably stabilized by the presence of desferrioxamine (DFO) and other Fe chelators. Fe-dependent regulation of HIF-α, using DFO, has been shown to occur at proline residues at both N-terminal and C-terminal loci within HIF's oxygen-dependent degradation domain, whereas the C-terminal site alone is reported to participate in O_2-dependent regulation.[67] As noted in Table 1, Fe deficiency was demonstrably present in the CP subjects in whom basal increased PAP and increased hypoxia-induced PAP has been reported. Mean serum Fe, transferrin iron saturation, and ferritin levels were greatly reduced in CP subjects compared with nonphlebotomized normal controls and the values in the phlebotomized non-CP polycythemic controls also were reduced to values intermediate between CP and normals.[65] The sensitive role for mild Fe depletion in up-regulating HRE-responsive genes in human beings has been reported previously from the same laboratory. In those studies, a DFO-induced dose-responsive increase in serum EPO in normal subjects was observed as early as 8 hours after the

start of DFO infusion.[68] Subjects with the lowest ferritin values at baseline had the greatest increases in serum EPO and those with the highest ferritin values provided the least increase in EPO levels. Moreover, in a separate study in normal subjects, acute cellular iron depletion with DFO resulted in significant increases in PAP.[69] Accordingly, Fe deficiency is a reasonable explanation for the observation of PAP increases in phlebotomized CP subjects.[65]

The finding of Smith et al[65] that phlebotomized, iron-deficient CP subjects displayed an accentuated PAP response to experimentally induced acute hypoxia in comparison with both normal controls and phlebotomized iron-deficient idiopathic polycythemics, was interpreted as most likely owing to the VHL mutation inducing lifelong HIF-α stabilization, despite their prior DFO-induced EPO data. Thus, because the CP subjects in the study by Smith et al[65] had both a baseline VHL mutation–driven HIF-α stabilization (that caused the CP form of polycythemia) and a likely iron deficiency–driven HIF-α stabilization, the experiment lacks sufficient controls to attribute any cause for PAP changes after experimental hypoxia because hypoxia represents a third, and thereby confounding, mechanism for HIF-α stabilization and up-regulation of HRE-responsive genes. That is, when hypoxia is applied in the presence of imposed iron depletion–confounded HRE status in CP subjects but not in normals, it is difficult to interpret the results for physiologic responses (eg, PAP) to the additional HRE stimulus (ie, hypoxia) as well as to possible nonspecific, non-HRE effects of iron depletion.

The role of iron-deficiency versus concomitant anemia in regulating cardiopulmonary function and structure is complex. Dietary iron-deficiency anemia in rats results in cardiac hypertrophy with increased diameter of cardiac myocytes, depletion of cardiac norepinephrine, increased plasma norepinephrine levels, increased urinary norepinephrine excretion, increased cardiac inotropic response to intravenous norepinephrine, impaired thermogenesis, and low thyroid hormone levels secondary to impaired thyroid-stimulating hormone secretion.[70,71] The correction of anemia by exchange transfusion corrected the thermogenesis and thyroid/thyroid-stimulating hormone defects but had no effect on the disordered catecholamine metabolism.[70] By using a similar iron-deficient anemic rat model, chronic administration of reserpine resulted in the complete prevention of cardiac hypertrophy, although it had no effect in another model of cardiac hypertrophy, aortic banding.[72,73] Ultrastructural studies have shown that the myocyte hypertrophy in iron deficiency differs dramatically from that in aortic banding in that iron deficiency uniquely results in a large increase in the cellular mitochondrial volume fraction.[74,75] Similar changes were noted in erythroblasts. In a cohort of 72 women with prolonged iron-deficiency anemia, 42% showed evidence of significant chronic intrinsic pulmonary disease (abnormal forced vital capacity, forced expiratory volume in 1 second, forced expiratory volume in 1 second/forced vital capacity, MEF_{50}, MEF_{75}), suggesting that severe airway dysfunction and pulmonary vascular function may be associated with iron deficiency.[76] Thus, although specific effects of iron deficiency on PAP or pulmonary artery morphology have not been reported, the reported iron-deficiency–induced disorder of the autonomic nervous system may affect autonomic control of PA pressure and airway dysfunction is suggestive of a broad spectrum of pulmonary disease.

Accordingly, the possible confounding role of iatrogenic iron deficiency in the reported CP subjects of Smith et al[65] suggests that future studies of both PAP and ventilation status in the CP population should be performed with careful attention to normalizing iron status.

Mechanism of the Normal Hypoxic PAP Response: Hypoxic Pulmonary Hypertension

In general, cardiac output has a relatively small effect on systolic PAP, rendering systolic PAP as essentially a direct measure of pulmonary vascular smooth muscle tone and collagenous arterial wall properties.[69] There are 2 components to hypoxic pulmonary hypertension (HPH): acute hypoxic pulmonary vasoconstriction (HPV) and subsequent chronic vascular remodeling characterized by medial thickening of small pulmonary arteries and right ventricular hypertrophy.[77] Although numerous humoral mediators have been nominated as responsible, no conclusive evidence exists for any humoral mediator in human beings.

Endothelin has received substantial study as a possible mediator of acute HPV. Plasma endothelin-1 (ET-1) levels are increased in human beings exposed acutely to high altitude and ET-1 was related inversely to the partial pressure of arterial oxygen and correlated with PAP. An increase in F_{IO_2} to 0.35 normalized the partial pressure of arterial oxygen and tended to decrease ET-1 levels and decreased PAP.[78] Although animal studies have shown attenuation of HPH by ET_A receptor antagonism, the human acute HPV response was unaltered by systemic ET_A antagonism of sufficient magnitude to result in significant decreases in both basal normoxic pulmonary and systemic vascular resistance values.[79] The potential for a chronic effect of ET-1 on HPV or HPH in human beings remains unexplored.

Additional factors have been found to contribute to acute or chronic HPH in animals, but have not yet been confirmed in human beings. These factors include oxidative stress as inhibited by allopurinol,[80] altered arachidonic acid metabolism favoring the production of PA constrictors (eg, thromboxane B2),[81] platelet-activating factor,[82] calcineurin,[83] serotonin,[84] histamine,[85] and atrial natriuretic peptide.[86] A consistent role for angiotensin in animal studies has not been found.[87] In the case of platelet-activating factor antagonism, attenuation of HPH and right ventricular hypertrophy (RVH) occurred after 7 days of hypoxia in rats, but platelet-activating factor antagonism had no demonstrable effect on increased PAP or RVH produced by $CoCl_2$, an agent known to stabilize HIF-α.[82]

Experimental Systemic Hypoxia Typically is Accompanied by Respiratory Alkalosis: Understanding the Possible Roles of pHi and HIF-α in the Cause of HPH Requires Complex Study Designs

Because experimentally induced hypoxia, as caused by reductions in F_{IO_2}, invariably is accompanied by systemic respiratory alkalosis, experiments designed to examine the role of PA smooth muscle cell (PA SMC) pHi in modulating HPH require control for the induction of systemic (and pulmonary) extracellular alkalosis. Regarding the mechanism of chronic PA SMC mitogenic responses, it has been reported that cell proliferation in response to growth factors in vitro is associated with increased pHi and that pharamacologic blockade of Na/H exchange with the Na/H inhibitor, dimethyl amiloride, prevented cell proliferation.[88] Accordingly, in rats, induction of chronic normobaric hypoxemia resulted in a significant increase in PAP, which was prevented by infusion of either dimethyl amiloride or ethylisopropyl amiloride, both of which inhibit Na/H exchange and can prevent cell alkalinization.[89] In similar studies in rats with acute (30 minute) normobaric hypoxia, however, acute pulmonary hypertension was not prevented by dimethyl amiloride.[89] Increased PA SMC pHi has been found in mice with chronic normobaric hypoxia and Na/H activity was shown to be increased.[90] These studies in mice also reported that Na/H exchanger isoform Na/H Exchanger 1 (NHE1) mRNA and protein abundance both were increased in PA SMCs by chronic hypoxia.

Recent studies have implicated a role for HIF-1α in the PA SMC alkalinization response to chronic hypoxia. By using chronically hypoxic mice with partial deletion of HIF-1α, PA SMCs did not show an increase in the intracellular pHi or in NHE1 expression observed in wild-type mice.[91] These investigators found that 48 hours of hypoxia in vitro in normal PA SMCs was sufficient to increase NHE1 expression, as was vector-delivered HIF-1α overexpression without hypoxia. The critical question of whether an increase in HIF-1α abundance is required for either cell alkalinization or hypoxic PAP increases was not addressed. Moreover, because pHi measurements taken under ex vivo conditions cannot be expected to reflect those of the in vivo circumstance when hypobaric hypoxia generates pHi changes as a result of both systemic respiratory alkalosis and of putative hypoxia-specific changes in pHi, mechanistic conclusions regarding any role for PA SMC pHi for generating HPH are premature. Accordingly, in future experiments it will be desirable to control for the extracellular acid-base state that accompanies both hypocapnic and isocapnic normobaric hypoxia and the pHi responses to hypoxia as they relate to changes in PAP. It also will be desirable to perform studies examining pHi changes in the setting of physiologically relevant changes in HIF-α protein levels in PA SMCs rather than the large changes presumably induced by gene deletion.

Similarly, it is difficult to interpret the reported finding that partial HIF-1α–deleted mice with chronic normobaric hypoxia showed a significant amelioration of HPH and right ventricular hypertrophy in association with attenuated increases in pulmonary arteriolar wall thickness.[92] Whether physiologically relevant modulation of HIF-α protein levels can affect either the acute or chronic PAP response to hypoxia remains to be determined, as does the related question of whether a primary increase of PA SMC HIF-α can increase basal normoxic PAP.

In summary, the roles of systemic, cellular, and cellular compartmental acidosis on HIF-α stabilization and its consequences are emerging rapidly. Further understanding of in vivo effects will require experimental designs that control for both systemic hypoxia and for its secondary respiratory alkalosis. Likewise, in vitro studies must be designed with careful attempts to mimic in vivo circumstances and to control for expected in vivo simultaneous changes in oxygenation and acid-base equilibrium. In addition to acid-base effects on HIF biology and its multitude of downstream gene-transcription effects, altered HIF stabilization per se has the potential to affect acid-base equilibrium, although the unduly large magnitude of gene-deletion experimental effects to date make assessing the magnitude of physiologic effects difficult.

References

1. Maxwell PH, Wiesener MS, Chang GW, et al: The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. Nature 399:271-275, 1999
2. Iyer NV, Leung SW, Semenza GL: The human hypoxia-inducible factor 1alpha gene: HIF1A structure and evolutionary conservation. Genomics 52:159-165, 1998
3. Wang GL, Jiang BH, Rue EA, et al: Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O2 tension. Proc Natl Acad Sci U S A 92:5510-5514, 1995
4. D'Angelo G, Duplan E, Boyer N, et al: Hypoxia up-regulates prolyl hydroxylase activity: A feedback mechanism that limits HIF-1 responses during reoxygenation. J Biol Chem 278:38183-38187, 2003
5. Huang LE, Pete EA, Schau M, et al: Leu-574 of HIF-1alpha is essential for the von Hippel-Lindau (VHL)-mediated degradation pathway. J Biol Chem 277:41750-41755, 2002
6. Gray MJ, Zhang J, Ellis LM, et al: HIF-1alpha, STAT3, CBP/p300 and Ref-1/APE are components of a transcriptional complex that regulates Src-dependent hypoxia-induced expression of VEGF in pancreatic and prostate carcinomas. Oncogene 24:3110-3120, 2005
7. Kallio PJ, Pongratz I, Gradin K, et al: Activation of hypoxia-inducible factor 1alpha: Posttranscriptional regulation and conformational change by recruitment of the Arnt transcription factor. Proc Natl Acad Sci U S A 94:5667-5672, 1997
8. Bing OH, Brooks WW, Messer JV: Heart muscle viability following hypoxia: Protective effect of acidosis. Science 180:1297-1298, 1973
9. Penttila A, Trump BF: Extracellular acidosis protects Ehrlich ascites tumor cells and rat renal cortex against anoxic injury. Science 185:277-278, 1974
10. Kaku DA, Giffard RG, Choi DW: Neuroprotective effects of glutamate antagonists and extracellular acidity. Science 260:1516-1518, 1993
11. Currin RT, Gores GJ, Thurman RG, et al: Protection by acidotic pH against anoxic cell killing in perfused rat liver: Evidence for a pH paradox. FASEB J 5:207-210, 1991
12. Willam C, Warnecke C, Schefold JC, et al: Inconsistent effects of acidosis on HIF-alpha protein and its target genes. Pflugers Arch 451:534-543, 2006
13. Christou H, Bailey N, Kluger MS, et al: Extracellular acidosis induces heme oxygenase-1 expression in vascular smooth muscle cells. Am J Physiol 288:H2647-H2652, 2005
14. Mekhail K, Gunaratnam L, Bonicalzi ME, et al: HIF activation by pH-

dependent nucleolar sequestration of VHL. Nat Cell Biol 6:642-647, 2004
15. Mekhail K, Khacho M, Carrigan A, et al: Regulation of ubiquitin ligase dynamics by the nucleolus. J Cell Biol 170(5):733-44, 2005 Aug 29.
16. Amirand C, Mentre P, van de Geijn S, Waksmundzka M, Debey P. Intracellular pH in one-cell mouse embryo differs between subcellular compartments and between interphase and mitosis. Biol Cell 92(6): 409-19, 2000 Sep.
17. Mekhail K, Khacho M, Gunaratnam L, Lee S. Oxygen sensing by H+: implications for HIF and hypoxic cell memory. Cell Cycle 3:1027-1029, 2004
18. Seagroves TN, Ryan HE, Lu H, et al: Transcription factor HIF-1 is a necessary mediator of the Pasteur effect in mammalian cells. Mol Cell Biol 21:3436-3444, 2001
19. Luo F, Liu X, Yan N, et al: Hypoxia-inducible transcription factor-1alpha promotes hypoxia-induced A549 apoptosis via a mechanism that involves the glycolysis pathway. BMC Cancer 6:26, 2006
20. Savai R, Schermuly RT, Voswinckel R, et al: HIF-1alpha attenuates tumor growth in spite of augmented vascularization in an A549 adenocarcinoma mouse model. Int J Oncol 27:393-400, 2005
21. Acker T, Diez-Juan A, Aragones J, et al: Genetic evidence for a tumor suppressor role of HIF-2alpha. Cancer Cell 8:131-141, 2005
22. Kim JW, Tchernyshyov I, Semenza GL, et al: HIF-1-mediated expression of pyruvate dehydrogenase kinase: A metabolic switch required for cellular adaptation to hypoxia. Cell Metab 3:177-185, 2006
23. Lim JH, Park JW, Kim MS, et al: Bafilomycin induces the p21-mediated growth inhibition of cancer cells under hypoxic conditions by expressing HIF-1α. Mol Pharmacol 70:1856-1865, 2006
24. Semenza GL: Baffled by Bafilomycin: An anti-cancer agent that induces HIF-1α expression. Mol Pharmacol 70:1841-1843, 2006
25. Sennoune SR, Bakunts K, Martinez GM, et al: Vacuolar H+-ATPase in human breast cancer cells with distinct metastatic potential: Distribution and functional activity. Am J Physiol 286:C1443-C1452, 2004
26. Martinez-Zaguilan R, Lynch RM, Martinez GM, et al: Vacuolar-type H(+)-ATPases are functionally expressed in plasma membranes of human tumor cells. Am J Physiol 265:C1015-C1029, 1993
27. Martinez-Zaguilan R, Martinez GM, Gomez A, et al: Distinct regulation of pHi and [Ca2+]i in human melanoma cells with different metastatic potential. J Cell Physiol 176:196-205, 1998
28. Ihnatko R, Kubes M, Takacova M, et al: Extracellular acidosis elevates carbonic anhydrase IX in human glioblastoma cells via transcriptional modulation that does not depend on hypoxia. Int J Oncol 29:1025-1033, 2006
29. Lu H, Forbes RA, Verma A: Hypoxia-inducible factor 1 activation by aerobic glycolysis implicates the Warburg effect in carcinogenesis. J Biol Chem 277:23111-23115, 2002
30. Shuin T, Yamasaki I, Tamura K, et al: Von Hippel-Lindau disease: Molecular pathological basis, clinical criteria, genetic testing, clinical features of tumors and treatment. Jpn J Clin Oncol 36:337-343, 2006
31. Pastore Y, Jedlickova K, Guan Y, et al: Mutations of von Hippel-Lindau tumor-suppressor gene and congenital polycythemia [erratum in: Am J Hum Genet 74:598, 2004]. Am J Hum Genet 73:412-419, 2003
32. Bento MC, Chang KT, Guan Y, et al: Congenital polycythemia with homozygous and heterozygous mutations of von Hippel-Lindau gene: Five new Caucasian patients. Haematologica 90:128-129, 2005
33. Ang SO, Chen H, Hirota K, et al: Disruption of oxygen homeostasis underlies congenital Chuvash polycythemia. Nat Genet 32:614-621, 2002
34. Rathmell WK, Hickey MM, Bezman NA, et al: In vitro and in vivo models analyzing von Hippel-Lindau disease-specific mutations. Cancer Res 64:8595-8603, 2004
35. Hoffman MA, Ohh M, Yang H, et al: von Hippel-Lindau protein mutants linked to type 2C VHL disease preserve the ability to downregulate HIF. Hum Mol Genet 10:1019-1027, 2001
36. Maranchie JK, Vasselli JR, Riss J, et al: The contribution of VHL substrate binding and HIF1-alpha to the phenotype of VHL loss in renal cell carcinoma. Cancer Cell 1:247-255, 2002
37. Roe JS, Youn HD: The positive regulation of p53 by the tumor suppressor VHL. Cell Cycle 15:5, 2006 [E-pub ahead of print]
38. Okuda H, Saitoh K, Hirai S, et al: The von Hippel-Lindau tumor suppressor protein mediates ubiquitination of activated atypical protein kinase C. J Biol Chem 276:43611-43617, 2001
39. Datta K, Nambudripad R, Pal S, et al: Inhibition of insulin-like growth factor-I-mediated cell signaling by the von Hippel-Lindau gene product in renal cancer. J Biol Chem 275:20700-20706, 2000
40. Roe JS, Kim H, Lee SM, et al: p53 stabilization and transactivation by a von Hippel-Lindau protein. Mol Cell 22:395-405, 2006
41. Nakamura E, Abreu-e-Lima P, Awakura Y, et al: Clusterin is a secreted marker for a hypoxia-inducible factor-independent function of the von Hippel-Lindau tumor suppressor protein. Am J Pathol 168:574-584, 2006
42. Pollard PJ, El-Bahrawy M, Poulsom R, et al: Expression of HIF-1α, HIF-2α (EPAS1), and their target genes in paraganglioma and phaeochromocytoma with VHL and SDH mutations. J Clin Endocrinol Metab 2006 [E-pub ahead of print]
43. Ma W, Tessarollo L, Hong SB, et al: Hepatic vascular tumors, angiectasis in multiple organs, and impaired spermatogenesis in mice with conditional inactivation of the VHL gene. Cancer Res 63:5320-5328, 2003
44. Elson DA, Thurston G, Huang LE, et al: Induction of hypervascularity without leakage or inflammation in transgenic mice overexpressing hypoxia-inducible factor-1alpha. Genes Dev 15:2520-2532, 2001
45. Kim WY, Safran M, Buckley MR, et al: Failure to prolyl hydroxylate hypoxia-inducible factor alpha phenocopies VHL inactivation in vivo. EMBO J 2006 [E-pub ahead of print]
46. Percy MJ, Zhao Q, Flores A, et al: A family with erythrocytosis establishes a role for prolyl hydroxylase domain protein 2 in oxygen homeostasis. Proc Natl Acad Sci U S A 103:654-659, 2006
47. Seeley TW, Langsetmo I, Stephenson R, et al: FG-2216: Tumor progression studies and correction of anemia of chronic disease in xenograft models (meeting abstract). J Am Soc Nephrol 16:481A, 2005
48. Gordeuk VR, Sergueeva AI, Miasnikova GY, et al: Congenital disorder of oxygen sensing: Association of the homozygous Chuvash polycythemia VHL mutation with thrombosis and vascular abnormalities but not tumors. Blood 103:3924-3932, 2004
49. Krapf R, Beeler I, Hertner D, et al: Chronic respiratory alkalosis. The effect of sustained hyperventilation on renal regulation of acid-base equilibrium. N Engl J Med 324:1394-1401, 1991
50. Lugliani R, Whipp BJ, Seard C, et al: Effect of bilateral carotid-body resection on ventilatory control at rest and during exercise in man. N Engl J Med 285:1105-1111, 1971
51. Wade JG, Larson CP Jr, Hickey RF, et al: Effect of carotid endarterectomy on carotid chemoreceptor and baroreceptor function in man. N Engl J Med 282:823-829, 1970
52. Lahiri S, Roy A, Baby SM, et al: Oxygen sensing in the body. Prog Biophys Mol Biol 91:249-286, 2006
53. Joseph V, Soliz J, Soria R, et al: Dopaminergic metabolism in carotid bodies and high-altitude acclimatization in female rats. Am J Physiol 282:R765-R773, 2002
54. Hui AS, Striet JB, Gudelsky G, et al: Regulation of catecholamines by sustained and intermittent hypoxia in neuroendocrine cells and sympathetic neurons. Hypertension 42:1130-1136, 2003
55. Baby SM, Roy A, Mokashi AM, et al: Effects of hypoxia and intracellular iron chelation on hypoxia-inducible factor-1alpha and -1beta in the rat carotid body and glomus cells. Histochem Cell Biol 120:343-352, 2003
56. Peng YJ, Yuan G, Ramakrishnan D, et al: Heterozygous hif-1 deficiency impairs carotid body-mediated cardio-respiratory responses and ROS generation in mice exposed to chronic intermittent hypoxia. J Physiol 2006 [E-pub ahead of print]
57. Roy A, Li J, Baby SM, et al: Effects of iron-chelators on ion-channels and HIF-1alpha in the carotid body. Respir Physiol Neurobiol 141:115-123, 2004
58. Schmitt P, Garcia C, Soulier V, et al: Influence of long-term hypoxia on tyrosine hydroxylase in the rat carotid body and adrenal gland. J Auton Nerv Syst 40:13-19, 1992
59. Quik M, Sourkes TL: The effect of chronic iron deficiency on adrenal tyrosine hydroxylase activity. Can J Biochem 55:60-65, 1977
60. Moudgil R, Michelakis ED, Archer SL: Hypoxic pulmonary vasoconstriction. J Appl Physiol 98:390-403, 2005

61. Gruppo Italiano Studio Policitemia: Polycythemia vera: The natural history of 1213 patients followed for 20 years. Ann Intern Med 123: 656-664, 1995
62. Dingli D, Utz JP, Krowka MJ, et al: Unexplained pulmonary hypertension in chronic myeloproliferative disorders. Chest 120:801-808, 2001
63. Garcia-Manero G, Schuster SJ, Patrick H, et al: Pulmonary hypertension in patients with myelofibrosis secondary to myeloproliferative diseases. Am J Hematol 60:130-135, 1999
64. Kadikoylu G, Onbasili A, Tekten T, et al: Functional and morphological cardiac changes in myeloproliferative disorders (clinical study). Int J Cardiol 97:213-220, 2004
65. Smith TG, Brooks JT, Balanos GM, et al: Mutation of von Hippel-Lindau tumour suppressor and human cardiopulmonary physiology. PLoS Med 3:e290, 2006 [E-pub ahead of print]
66. Bushuev VI, Miasnikova GY, Sergueeva AI, et al: Endothelin-1, vascular endothelial growth factor and systolic pulmonary artery pressure in patients with Chuvash polycythemia. Haematologica 91:744-749, 2006
67. Lee KH, Choi E, Chun YS, et al: Differential responses of two degradation domains of HIF-1alpha to hypoxia and iron deficiency. Biochimie 88:163-169, 2006
68. Ren X, Dorrington KL, Maxwell PH, et al: Effects of desferrioxamine on serum erythropoietin and ventilatory sensitivity to hypoxia in humans. J Appl Physiol 89:680-686, 2000
69. Balanos GM, Dorrington KL, Robbins PA: Desferrioxamine elevates pulmonary vascular resistance in humans: Potential for involvement of HIF-1. J Appl Physiol 92:2501-2507, 2002
70. Beard J, Green W, Miller L, et al: Effect of iron-deficiency anemia on hormone levels and thermoregulation during cold exposure. Am J Physiol 247:R114-R119, 1984
71. Turner LR, Premo DA, Gibbs BJ, et al: Adaptations to iron deficiency: Cardiac functional responsiveness to norepinephrine, arterial remodeling, and the effect of beta-blockade on cardiac hypertrophy. BMC Physiol 2:1, 2002
72. Rossi MA, Carillo SV: Pathogenesis of cardiac hypertrophy in iron deficiency anaemia: The role of noradrenaline. Br J Exp Pathol 63:269-277, 1982
73. Rossi MA, Carillo SV: Does norepinephrine play a central causative role in the process of cardiac hypertrophy? Am Heart J 109:622-624, 1985
74. Dallman PR, Goodman JR: Enlargement of mitochondrial compartment in iron and copper deficiency. Blood 35:496-505, 1970
75. Goodman JR, Warshaw JB, Dallman PR: Cardiac hypertrophy in rats with iron and copper deficiency: Quantitative contribution of mitochondrial enlargement. Pediatr Res 4:244-256, 1970
76. Golovin AA, Konvai VD, Red'kin IUV, et al: The external respiratory function of patients with iron-deficiency anemia and the possible mechanisms of its disorder. Ter Arkh 62:53-55, 1990
77. Barnes PJ, Liu SF: Regulation of pulmonary vascular tone. Pharmacol Rev 47:87-131, 1995
78. Goerre S, Wenk M, Bartsch P, et al: Endothelin-1 in pulmonary hypertension associated with high-altitude exposure. Circulation 91:359-364, 1995
79. Johnson W, Nohria A, Garrett L, et al: Contribution of endothelin to pulmonary vascular tone under normoxic and hypoxic conditions. Am J Physiol 283:H568-H575, 2002
80. Hoshikawa Y, Ono S, Suzuki S, et al: Generation of oxidative stress contributes to the development of pulmonary hypertension induced by hypoxia. J Appl Physiol 90:1299-1306, 2001
81. Fike CD, Kaplowitz MR, Pfister SL: Arachidonic acid metabolites and an early stage of pulmonary hypertension in chronically hypoxic newborn pigs. Am J Physiol 284:L316-L323, 2003
82. Ono S, Westcott JY, Voelkel NF: PAF antagonists inhibit pulmonary vascular remodeling induced by hypobaric hypoxia in rats. J Appl Physiol 73:1084-1092, 1992
83. Koulmann N, Novel-Chate V, Peinnequin A, et al: Cyclosporin A inhibits hypoxia-induced pulmonary hypertension and right ventricle hypertrophy. Am J Respir Crit Care Med 174:699-705, 2006
84. Marcos E, Adnot S, Pham MH, et al: Serotonin transporter inhibitors protect against hypoxic pulmonary hypertension. Am J Respir Crit Care Med 168:487-493, 2003 [E-pub 2003 May 28]
85. Russell PC, Wright CE, Barer GR, et al: Histamine induced pulmonary vasodilatation in the rat: Site of action and changes in chronic hypoxia. Eur Respir J 7:1138-1144, 1994
86. Chen YF, Feng JA, Li P, et al: Atrial natriuretic peptide-dependent modulation of hypoxia-induced pulmonary vascular remodeling. Life Sci 79:1357-1365, 2006
87. Hubloue I, Rondelet B, Kerbaul F, et al: Endogenous angiotensin II in the regulation of hypoxic pulmonary vasoconstriction in anaesthetized dogs. Crit Care 8:R163-R171, 2004
88. Quinn DA, Dahlberg CG, Bonventre JP, et al: The role of Na+/H+ exchange and growth factors in pulmonary artery smooth muscle cell proliferation. Am J Respir Cell Mol Biol 14:139-145, 1996
89. Quinn DA, Du HK, Thompson BT, et al: Amiloride analogs inhibit chronic hypoxic pulmonary hypertension. Am J Respir Crit Care Med 157:1263-1268, 1998
90. Rios EJ, Fallon M, Wang J, et al: Chronic hypoxia elevates intracellular pH and activates Na+/H+ exchange in pulmonary arterial smooth muscle cells. Am J Physiol 289:L867-L874, 2005
91. Shimoda LA, Fallon M, Pisarcik S, et al: HIF-1 regulates hypoxic induction of NHE1 expression and alkalinization of intracellular pH in pulmonary arterial myocytes. Am J Physiol 291:L941-L949, 2006
92. Yu AY, Shimoda LA, Iyer NV, et al: Impaired physiological responses to chronic hypoxia in mice partially deficient for hypoxia-inducible factor 1alpha. J Clin Invest 103:691-696, 1999