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The Genomics and Genetics of Oxygen Homeostasis

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Abstract

Human survival is dependent upon the continuous delivery of O_2 to each cell in the body in sufficient amounts to meet metabolic requirements, primarily for ATP generation by oxidative phosphorylation. Hypoxia-inducible factors (HIFs) regulate the transcription of thousands of genes to balance O_2 supply and demand. The HIFs are negatively regulated by O_2-dependent hydroxylation and ubiquitination by prolyl hydroxylase domain (PHD) proteins and the von Hippel–Lindau (VHL) protein. Germline mutations in the genes encoding VHL, HIF-2α, and PHD2 cause hereditary erythrocytosis, which is characterized by polycythemia and pulmonary hypertension and is caused by increased HIF activity. Evolutionary adaptation to life at high altitude is associated with unique genetic variants in the genes encoding HIF-2α and PHD2 that blunt the erythropoietic and pulmonary vascular responses to hypoxia.

INTRODUCTION

Human survival is dependent upon the continuous delivery of O_2 to each of the approximately 100 trillion cells in the adult body in sufficient amounts to meet their metabolic requirements. The synthesis of DNA, RNA, and protein—as well as the biochemical activity of many proteins—requires ATP, which is produced primarily through the process of mitochondrial oxidative phosphorylation. When O_2 demand exceeds supply, cells execute changes in gene expression that serve to increase O_2 delivery and decrease O_2 consumption (88). As a result, a significant proportion (i.e., thousands) of genes expressed in any given cell will show changes in expression at the RNA and/or protein level in response to reduced O_2 availability (hypoxia). Whereas overall rates of RNA and protein synthesis decrease in response to hypoxia, the expression of a battery of genes is induced at the transcriptional level to mediate adaptive responses designed to maintain oxygen homeostasis. This review summarizes our current insights into the molecular mechanisms underlying this fundamental physiological system for maintaining oxygen homeostasis.

ERYTHROPOIESIS AND OXYGEN SENSING

The process of scientific discovery leading to the elucidation of mechanisms underlying coordinated changes in the expression of thousands of genes across the genome began as an effort to answer a simple question: How does the body control the production of red blood cells? The existence of a humoral factor that stimulates red cell production was deduced from studies of parabiotic rats, in which exposure of one member of the pair to hypoxia was sufficient to activate erythropoiesis in the bone marrow of the other (85), and from the demonstration that plasma from donor anemic rabbits stimulated erythropoiesis in recipient nonanemic rabbits (24). Nephrectomy experiments suggested that an erythropoietic hormone is produced in the kidneys of adult mammals (46). The purification and partial amino acid sequence analysis of the human erythropoietin (EPO) protein (71) led to the isolation of cDNA sequences (45, 62), which enabled the production of recombinant human EPO for administration to patients with chronic kidney disease, who are anemic due to lack of adequate EPO production (25). In addition, the ability to measure EPO mRNA and protein levels revealed that specialized EPO-producing cells in the kidney have the capacity to sense reduced O_2 availability (hypoxia) and respond by increasing *EPO* gene transcription (53, 93). But how is *EPO* transcription modulated?

Short DNA sequences required for hypoxia-induced transcription, designated the hypoxia response element (HRE), were identified in the 3′-flanking region of the human and mouse genes encoding EPO, based on the demonstration that insertion of the HRE into a heterologous reporter gene led to hypoxia-inducible expression of the gene product (5, 83, 99). Electrophoretic mobility-shift assays demonstrated the existence of a factor that was present specifically in nuclear extracts from hypoxic cells and bound to a double-stranded oligonucleotide containing an 18-base-pair DNA sequence from the HRE; furthermore, nucleotide substitutions that blocked binding of the factor, which was designated hypoxia-inducible factor 1 (HIF-1), also eliminated the ability of the HRE to induce reporter gene transcription (100). EPO is produced in response to hypoxia in a limited number of cell types, yet HIF-1 DNA-binding activity was induced by hypoxia in every mammalian cell type analyzed (118), which immediately suggested that HIF-1 plays a broader role than simply as a regulator of EPO production.

Protein purification by DNA affinity chromatography revealed that HIF-1 is a heterodimer composed of HIF-1α and HIF-1β subunits (119). The isolation of cDNA sequences enabled deduction of the amino acid sequences of the encoded proteins. These proteins share basic helix-loop-helix (bHLH) and Per-Arnt-Sim (PAS) domains in their amino-terminal halves (117), which

mediate heterodimerization and DNA binding (47). The bHLH domain is utilized by a large family of transcription factors (56). By contrast, the PAS domain is found in a small subset of bHLH proteins (39). Whereas HIF-1α represented a novel member of the bHLH-PAS protein family, HIF-1β was identical to the aryl hydrocarbon nuclear translocator (ARNT) protein, which was originally identified as the dimerization partner of the aryl hydrocarbon receptor, another bHLH-PAS protein (39). ARNT is a common subunit for multiple bHLH-PAS proteins (reviewed in 123).

The isolation of cDNA also enabled the generation of antibodies against HIF-1α and HIF-1β, which revealed that HIF-1α protein progressively accumulated over several hours after cells were transferred from standard tissue culture conditions (95% air and 5% CO_2 = 20% O_2) to hypoxic conditions (1% O_2, 5% CO_2, and 94% N_2) and that HIF-1α protein dramatically vanished within minutes after reoxygenation (117). Furthermore, HIF-1α protein accumulation occurred as a graded response: the greater the reduction in ambient O_2, the greater the levels of HIF-1α protein that were induced over a 4-h period (48).

The rapid degradation of HIF-1α in posthypoxic cells was mediated by the ubiquitin-proteasome system (87). O_2-dependent degradation required amino acid residues 400–600 of HIF-1α (40) and the von Hippel–Lindau (VHL) protein (70). The signaling mechanism was remarkably direct: Enzymes that utilize O_2 as a substrate insert one oxygen atom into a prolyl residue (Pro-402 or Pro-564 of human HIF-1α; **Figure 1**), and the other atom is used to split α-ketoglutarate into CO_2 and succinate, with prolyl hydroxylation required for VHL binding, ubiquitination, and degradation of HIF-1α (23, 43, 44, 126).

Whereas the amino-terminal half of HIF-1α contains the domains required for dimerization with HIF-1β and DNA binding (47), and the middle portion contains the O_2-dependent degradation domain (40), the carboxyl-terminal half contains the transactivation domain (TAD) (49, 82), which recruits the coactivators p300 and CREB-binding protein (CBP) (3) and is negatively regulated by factor inhibiting HIF-1 (FIH-1) (68). Just as hydroxylation of two proline residues negatively regulates HIF-1α protein stability by promoting VHL binding, hydroxylation of an asparagine residue (Asn-803; **Figure 1**) by FIH-1 negatively regulates HIF-1α transcriptional activity in an O_2-dependent manner by blocking the binding of p300 and CBP (57, 58). Taken together, these findings indicate that hydroxylation provides a molecular mechanism that directly transduces changes in O_2 availability to changes in gene expression through modulation of HIF-1 transcriptional activity.

The hypothesis that HIF-1 controls the expression of genes in addition to *EPO* was confirmed by the identification of a HIF-dependent HRE in the *VEGF* gene, which encodes vascular endothelial growth factor (27), thereby providing a mechanism by which hypoxic cells stimulate the formation of new blood vessels to increase O_2 delivery. HIF-dependent HREs were also identified in the *ALDOA*, *ENO1*, and *LDHA* genes encoding glycolytic enzymes (98), thereby providing a mechanism by which cells adapt their metabolism, and thereby decrease O_2 consumption, in response to hypoxia.

Thus, within the course of a dozen years from 1991 to 2002, a small number of laboratories elucidated the fundamental transcriptional mechanism that underlies oxygen homeostasis in virtually every metazoan species. All that remained was to fill in the details, such as the many roles of this system in embryonic development, postnatal physiology, disease pathophysiology, and therapeutics—in short, an ever-expanding universe of investigation, various aspects of which have been the topics of several recent Annual Reviews articles (17, 42, 84, 94, 95, 97). The remainder of the present review will honor this journal's mission by focusing specifically on the genomics and genetics of oxygen homeostasis.

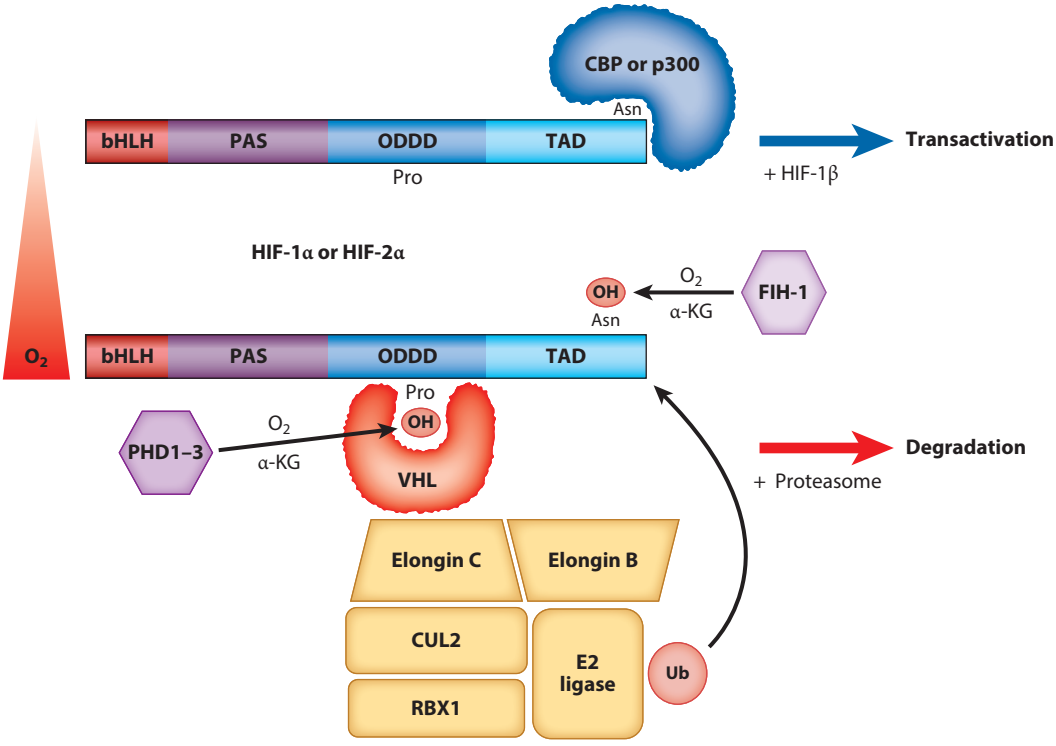

Figure 1

Regulation of hypoxia-inducible factor (HIF) degradation and transactivation by O_2-dependent hydroxylation. Two proline (Pro) residues in the oxygen-dependent degradation domain (ODDD) of HIF-1α or HIF-2α are hydroxylated under normoxic conditions by the prolyl hydroxylase domain (PHD) proteins PHD1–3, which is required for binding of the von Hippel–Lindau (VHL) protein. VHL recruits a protein-ubiquitin ligase complex [consisting of elongin B and C, Cullin 2 (Cul2), ring-box 1 (RBX1), and an E2 ligase] that ubiquitinates HIF-α, leading to proteasomal degradation. In addition, factor inhibiting HIF-1 (FIH-1) hydroxylates an asparagine residue (Asn) in the transactivation domain (TAD) of the HIF-α subunits, which blocks binding of the coactivator proteins p300 and CREB-binding protein (CBP) under normoxic conditions. The catalytic activity of PHDs and FIH-1 is inhibited under hypoxic conditions, leading to stabilization and activation of the HIF-α subunits, which heterodimerize with the HIF-1β subunit through interaction of basic helix-loop-helix (bHLH) and Per-Arnt-Sim (PAS) domains to form proteins that are competent to bind to DNA and activate transcription. Additional abbreviations: α-KG, α-ketoglutarate; Ub, ubiquitin.

GENES ENCODING COMPONENTS OF THE HIF PATHWAY

Genes Encoding HIFs

The naming of HIF-1 begged the question, Were there other HIFs? Fortunately for the author, the answer was yes (**Table 1**). Database searches using the HIF-1α sequence as the query revealed the existence of an ortholog, now known as HIF-2α or endothelial PAS domain protein 1 (EPAS1), which was regulated by prolyl and asparaginyl hydroxylation and heterodimerized with HIF-1β and appeared to be the major physiological regulator of *EPO* transcription in the kidney during postnatal life, whereas HIF-1α was required for embryonic erythropoiesis (36, 60, 111, 122). A third member of the family, HIF-3α, has been identified but is the least well studied of the trio (reviewed in 21). Whereas HIF-1α and HIF-1β are encoded in the genomes of even primitive metazoans (64) and are expressed in all mammalian tissues studied (120), HIF-2α and HIF-3α are present only in vertebrates and are expressed in a cell-type-restricted manner (21, 34, 64, 111, 121). As a result of alternative splicing, the *HIF1A*, *EPAS1*, and *HIF3A* genes each encode multiple

Table 1 Core components of the HIF pathway in humans

Gene name	Protein name
HIF1A	HIF-1α
EPAS1	HIF-2α
HIF3A	HIF-3α
ARNT	HIF-1β
EGLN1	PHD2
EGLN2	PHD1
EGLN3	PHD3
HIF1AN	FIH-1
VHL	VHL
TP300	p300
CREBBP	CBP

isoforms, some of which have opposing functions (reviewed in 12, 81)—an aspect of HIF biology that has been somewhat understudied.

Genes Encoding HIF Hydroxylases and O_2-Regulated HIF-Binding Proteins

Three prolyl hydroxylase domain (PHD) proteins (PHD1–3) mediating HIF prolyl hydroxylation have been identified in mammals (10, 23). These proteins have been characterized with regard to their propensity to hydroxylate different proteins (e.g., HIF-1α versus HIF-2α) or different sites in the same protein (e.g., Pro-402 versus Pro-564 of human HIF-1α) (16). A single protein (FIH-1) mediating asparaginyl hydroxylation of HIF-α subunits has been identified in mammals (**Table 1**). A single protein (VHL) binds specifically to prolyl-hydroxylated HIF-α subunits to trigger ubiquitination. Two coactivator proteins (CBP and p300) bind specifically to the TAD of HIF-α subunits, provided that the latter are not asparagine-hydroxylated (57, 58). CBP and p300 support transactivation of HIF target genes under hypoxic conditions through their activity as histone acetyltransferases and through protein–protein interactions with general transcription factors that form a multiprotein complex with RNA polymerase II (Pol II) (37, 76).

OTHER MODULATORS OF HIF ACTIVITY

The activation of HIFs in response to hypoxia is modulated by two principal mechanisms: protein–protein interaction and posttranslational modification. Hundreds of protein–protein interactions have been identified (reviewed in 96), which can be subdivided into two types. In the first type, "protein X" interacts with a HIF-α subunit and thereby alters its stability or transcriptional activity. Interacting proteins that directly affect transactivation by HIF-1α (and have no effect on HIF-1α stability) are summarized in **Table 2** (for references, see 96). In the second type, "protein Y" interacts with protein X, thereby modulating the interaction of the latter with a HIF-α subunit. For example, dozens of proteins are known to interact with VHL and positively or negatively regulate its ubiquitination of HIF-α subunits. The known posttranslational modifications of HIF-1α, the residues affected, and the functional consequences are summarized in **Table 3** (for references, see 96).

HIF-DEPENDENT DIRECT ACTIVATION OF TARGET GENES

Extensive work has been performed to identify thousands of mammalian genes that are directly activated by the binding of HIF-1 or HIF-2 to an HRE containing the HIF binding site

Table 2 Proteins that interact with HIF-1α and affect transactivation

Protein	Mechanism of action
CBP	Histone acetyltransferase (regulated by N803 hydroxylation)
EAF2	Blocks interaction with CBP/p300
FBP1	Inhibits TAD[a]
FHL2	Inhibits TAD (N803 hydroxylation independent)
FIH-1	Asparaginyl hydroxylase
JMJD2C	Histone demethylase
KAT5	Lysine acetyltransferase
MCM3	Inhibits TAD (N803 hydroxylation dependent)
MUC1	Stabilizes interaction of p300 with HIF-1α
P300	Histone acetyltransferase (regulated by N803 hydroxylation)
PARP1	Co-activator[a]
PCAF	Enhances p300 recruitment
PKM2	Enhances p300 recruitment and chromatin occupancy by HIF-1
PONTIN	Enhances p300 recruitment
REPTIN	Co-repressor; recruits HDAC1 to a subset of HIF target genes
SET7	Blocks binding of HIF-1 to DNA
SIRT1	Deacetylates HIF-1α on K674 to inhibit p300 recruitment
SIRT6	Co-repressor[a]
SRC1	Co-activator[a]
STAT3	Co-activator[a]
TAZ	Co-activator; promotes HIF-1 occupancy of HRE
TIF2	Co-activator[a]
TSGA10	Blocks nuclear localization
XBP1s	Co-activator[a]

[a]Precise mechanism of action is unknown.

sequence 5′-RCGTG-3′ (98). Currently, the two stringent criteria for direct HIF target genes are (*a*) hypoxia-induced mRNA expression in wild-type cells but not in cells in which HIF-1α and/or HIF-2α expression has been silenced, and (*b*) hypoxia-induced binding of HIF-1 (i.e., HIF-1α and HIF-1β) and/or HIF-2 (i.e., HIF-2α and HIF-1β) to the gene, as demonstrated by chromatin immunoprecipitation (ChIP) assays using antibodies that recognize each subunit and PCR primers that flank a 5′-RCGTG-3′ site. In any given cell, hundreds of mRNAs will be induced by hypoxia in a HIF-dependent manner.

Many genes will be induced by hypoxia in cell type A but not in cell type B and vice versa, despite the presence of HIF activity in all hypoxic cells, indicating that the battery of hypoxia-inducible genes in a given cell type reflects the activity of many transcription factors in addition to the HIFs. A general observation is that if gene X is induced by hypoxia in cell type A, then sites of hypoxia-induced HIF binding in gene X are often located in regions of chromatin that are sensitive to deoxyribonuclease (DNase) digestion, even under nonhypoxic conditions (92); this open (nucleosome-free) chromatin configuration allows HIFs, once they are induced by hypoxia, to immediately access their DNA binding sites. In fact, the identification of a DNase hypersensitive site in the 3′-flanking region of the human *EPO* gene led to the discovery of the first HRE and HIF binding site (99, 100). By contrast, if gene X is not induced by hypoxia in cell type B, then the HIF binding site sequence is more likely to be located in chromatin that is not sensitive to DNase digestion (a closed chromatin configuration), and HIFs cannot access those binding sites, which

Table 3 Posttranslational modifications of HIF-1α

Modifier	AA residue(s)[a]	Type of PTM[b]	Functional consequence
ATM	696	Phosphorylation	Protein stabilization
CBX4	391, 477	Sumoylation	Transcriptional activation
CDK1	668	Phosphorylation	Protein stabilization
CDK5	687	Phosphorylation	Protein stabilization
CK1δ	247	Phosphorylation	Inhibition of dimerization
CK2	796	Phosphorylation	Transcriptional activation
ERK1/2	641, 643	Phosphorylation	Nuclear localization
GSK-3β	551, 555, 589	Phosphorylation	Protein degradation
HDAC4	10, 11, 12, 19, 21	Deacetylation	Protein stabilization
LSD1	32	Demethylation	Protein stabilization
p300	709	Acetylation	Protein stabilization
PCAF	674	Acetylation	Transcriptional activation
PIASY	ND	Sumoylation	Protein degradation
PLK3	576, 657	Phosphorylation	Protein degradation
PRKACA	63, 692	Phosphorylation	Protein degradation
SENP1	ND	Desumoylation	Protein stabilization
SET7	32	Methylation	Protein degradation and transcriptional repression
SIRT1	674	Deacetylation	Transcriptional repression
SIRT2	709	Deacetylation	Protein degradation
STUB1	ND	Ubiquitination	Protein degradation
TRAF6	ND	Ubiquitination	Protein stabilization
VHL	538, 547, 632	Ubiquitination	Protein degradation

For literature citations, see Reference 96. Abbreviations: AA, amino acid; ND, not determined; PTM, posttranslational modification.

[a]AA residue of the 826-AA human HIF-1α that is modified.

[b]Type of PTM of HIF-1α.

are packaged into nucleosomes that consist of 146 base pairs of DNA wound around a histone core consisting of two copies each of H2A, H2B, H3, and H4 (67).

The readiness of gene X in cell type A to respond to hypoxia described above extends beyond the accessibility of HIF binding sites and requires a more detailed understanding of the mechanism of transcription. Pol II assembles a preinitiation supercomplex consisting of the multiprotein complexes designated transcription factor IIA (TFIIA) (2 subunits), TFIIB (1 subunit), TFIID (19 subunits), TFIIE (2 subunits), TFIIF (2 subunits), TFIIH (10 subunits), Core Mediator (26 subunits), and Pol II (13 subunits) (reviewed in 1). Cyclin-dependent kinase 7 (CDK7), which along with its allosteric regulator cyclin H is a component of TFIIH, phosphorylates serine residue 5 (Ser-5) in a heptad repeat (YSPTSPS) that is present in 52 copies in the carboxyl-terminal domain (CTD) of the large subunit of Pol II, leading to recruitment of the histone methyltransferase SETD1, which trimethylates histone H3 on lysine residue 4 to form the H3K4me3 mark that is associated with transcription initiation (reviewed in 1, 30, 50). Occupancy of the HIF target gene *ANKRD37* by Pol II phosphorylated on Ser-5 of the CTD was induced in hypoxic HCT116 colon carcinoma cells in a HIF-1α-dependent manner (29), indicating that HIF-1 stimulates transcriptional initiation.

Under nonhypoxic conditions, many HIF target genes that will be induced by hypoxia have already engaged Pol II, which binds to the promoter and initiates transcription, only to pause and

await binding of HIF-1 to trigger transcriptional elongation (29). What causes Pol II to pause after the transcription of only 30–60 nucleotides? Perhaps proteins that are critical for establishment of the initiation site, such as TFIID, which contains the TATA-binding protein, prevent further movement of Pol II. Or perhaps Pol II encounters a roadblock: its first nucleosome. A key event in jump-starting paused Pol II is the recruitment of positive transcription elongation factor b (P-TEFb), which consists of CDK9 and its allosteric activator cyclin T (reviewed in 50). CDK9 has three critical targets: (*a*) Phosphorylation of subunit E of the negative elongation factor (NELF) leads to expulsion of the latter from the Pol II complex; (*b*) phosphorylation of the SPT5 subunit of DRB sensitivity inducing factor (DSIF), another negative elongation factor, turns it into a positive elongation factor; and (*c*) phosphorylation of heptad repeat residue Ser-2 (YSPTSPS) in the CTD of Pol II allows it to engage positive elongation factors, such as SPT6, and chromatin-modifying enzymes, such as the histone methyltransferase SETD2, which trimethylates histone H3 on lysine residue 36 to form the H3K36me3 mark that is associated with transcription elongation (reviewed in 50). Occupancy of the *ANKRD37* gene by Pol II phosphorylated on Ser-2 of the CTD and by CDK9 was induced in hypoxic HCT116 cells in a HIF-1α-dependent manner (29), indicating that HIF-1 stimulates release of paused Pol II by CDK9 recruitment.

The HIF-1α-dependent recruitment of complexes that contain CDK8, cyclin C, Mediator protein 12 (MED12), and either MED13 or MED13L appears to be required for hypoxic induction of many HIF target genes in HCT116 cells, based on CDK8 knockdown and pulldown experiments (29). During transcription initiation, Pol II interacts with the Core Mediator, a complex that consists of two dozen subunits, including MED26, which interacts with TFIID to facilitate initiation (reviewed in 1). MED26 also interacts with P-TEFb-associated factors to facilitate elongation (107). ChIP assays revealed that hypoxia-induced binding of CDK9 and MED26 to *ANKRD37* was CDK8 dependent and that hypoxia-induced binding of CDK8 was HIF-1α dependent (29). In contrast to CDK9, the proteins that are phosphorylated by CDK8 to facilitate elongation in hypoxic HCT116 cells have not been identified. Additional factors are required in order to displace (and then replace) nucleosomes during elongation (reviewed in 50), and further studies are required to investigate whether HIF-1α interacts with the histone chaperones and chromatin-remodeling factors that facilitate transcription elongation.

HIF-DEPENDENT REPRESSION OF GENE EXPRESSION

The levels of hundreds of mRNAs decrease in response to hypoxia; as in the case of hypoxia-inducible mRNAs, for the majority of hypoxia-repressible mRNAs, the response to hypoxia is HIF dependent (35, 69, 72, 124). However, whereas most hypoxia-inducible mRNAs are the products of direct HIF target genes in which hypoxia-inducible binding of HIF-1 and/or HIF-2 can be demonstrated by ChIP, the vast majority of hypoxia-repressible genes do not show hypoxia-induced binding of HIF-1 or HIF-2 (72), suggesting that HIFs function primarily as transcriptional activators and that mechanisms of repression must be indirect. Several mechanisms are described below.

HIFs Activate Transcription of Genes Encoding Repressors

Several of the target genes directly activated by HIFs encode transcriptional repressors (**Table 4**). Many of these proteins bind to DNA in a sequence-specific manner but repress, rather than activate, transcription of their target genes. The HIF target gene RE1-silencing transcription factor (*REST*) encodes a zinc-finger transcription factor that binds to the 21-base-pair repressor element 1 and thereby regulates approximately 20% of the >1,000 hypoxia-repressed genes in HEK293 cells (13). Among the target genes bound by REST is the *HIF1A* gene encoding HIF-1α,

Table 4 HIF target genes encoding transcriptional repressors

Gene name	Protein name(s)	Mechanism
BHLHE40	BHLHB2, DEC1, SHARP2	DNA binding, protein interaction
BHLHE41	BHLHB3, DEC2, SHARP1	DNA binding, protein interaction
MXI1	MXI1	Inhibition of MYC-MAX dimerization
REST	REST	DNA binding
SNAI1	SNAIL	DNA binding
SNAI2	SLUG	DNA binding
TCF3	E2A, E47, TCF3	DNA binding
TWIST1	TWIST	DNA binding
ZEB1	ZFHX1A	DNA binding
ZEB2	ZFHX1B, SIP1	DNA binding

suggesting the existence of a feedback loop that may mediate either termination of the hypoxic response or transition from an acute hypoxic response, driven by HIF-1α, to a chronic response, driven by HIF-2α (12). In another remarkable example, the differentiation of mesenchymal progenitor cells into adipocytes is inhibited under hypoxic conditions due to HIF-1-dependent expression of the bHLH factor DEC1 (also known as SHARP2), which represses transcription of the *PPARG* gene encoding the transcription factor peroxisome proliferator activated receptor gamma (PPARγ) (127). Note that in this latter case, there are two degrees of separation between HIF-1 and the PPARγ target genes that are repressed in response to hypoxia.

Another general mechanism of repressor action is by protein–protein interaction. Hypoxia induces HIF-dependent expression of MXI1, which competes with MYC for heterodimerization with MAX, thereby repressing MYC transcriptional activity and protecting cells from MYC-induced apoptosis (18). As described above for DEC1, SHARP1 (also known as DEC2 or BHLHE41) is a bHLH-type DNA-binding protein, but it also interacts with HIF-1α and triggers proteasomal degradation in an O_2-independent manner, thereby repressing HIF-1 transcriptional activity (73), which represents another potential negative feedback loop, as described above for REST.

HIFs Activate Transcription of Genes That Affect mRNA Stability and Translation

HIFs indirectly regulate the rate of degradation or translation of many mRNAs by activating the transcription of genes encoding microRNAs (54). The induction of microRNAs represents another hierarchy of responses, as each microRNA regulates the expression of multiple proteins by binding to mRNAs and either inducing their degradation or blocking their translation. In addition to microRNAs, another mechanism by which HIFs indirectly regulate mRNA stability and translation is through transactivation of the *ALKBH5* and *ZNF217* genes (110, 128, 129). ALKBH5 is an eraser that demethylates adenosine residues in RNA that are methylated at the N6 position (m^6A), whereas ZNF217 blocks assembly of the writer methyltransferase that mediates m^6A modification of RNA, which is recognized by reader proteins that bind in an m^6A-dependent manner and alter the rate of mRNA degradation or translation (28).

HERITABLE DISORDERS OF OXYGEN SENSING

Von Hippel–Lindau Syndrome

In 1904, Eugen von Hippel, an ophthalmologist in Heidelberg, described a "very rare disease of the retina" characterized by the presence of angiomas in multiple family members (113).

Twenty-two years later, the Swedish ophthalmologist Arvid Lindau reported the association of retinal angiomas, cerebellar hemangioblastomas, and kidney tumors (63). In 1993, the *VHL* gene was identified, and affected individuals were found to be heterozygous for germline loss-of-function mutations (60). A subgroup of patients with VHL syndrome have pheochromocytomas, either alone (type 2C) or in association with hemangioblastomas and either a high (type 2B) or low (type 2A) risk of clear cell renal cell carcinoma (RCC), in contrast to type 1 patients, who are at increased risk for the development of clear cell RCC but not pheochromocytoma (51). Although large deletions in the *VHL* gene are often found in type 1 patients, specific missense mutations are associated with types 1 (C162F), 2A (Y98H), 2B (R167Q), and 2C (L188V) (20).

VHL is a classical tumor suppressor gene in which one mutant allele is present in the germline and the second allele is mutated or inactivated by methylation in tumor cells. All of the mutant alleles associated with hemangioblastomas and RCC (types 1, 2A, and 2B) encode VHL protein that fails to ubiquitinate and degrade HIF-1α and HIF-2α in an O_2-dependent manner. These mutations affect the binding of VHL to hydroxylated HIF-α subunits and/or binding of VHL to elongin C (**Figure 1**). By contrast, mutations associated only with pheochromocytoma (type 2C) do not impair the ability of VHL to regulate the stability of the HIF-α subunits. All of the type 2 *VHL* mutations are associated with decreased neuronal apoptosis in response to growth factor deprivation (51), which provides a molecular mechanism underlying the pathogenesis of pheochromocytoma.

RCC is a major cause of mortality in patients with VHL syndrome. In addition, patients with RCC not associated with VHL syndrome (the vast majority of RCC patients) also have VHL loss of function in their tumor tissue (but not in the germline). Patients with metastatic RCC have a five-year survival of less than 12% due to the lack of effective therapy. Recently, a phase 1 trial of PT2385, a small-molecule inhibitor of HIF-2 that binds to HIF-2α and prevents its dimerization with HIF-1β to inhibit HIF target expression and tumor growth (115), showed remarkable effects, with 1 complete response, 5 partial responses, and 10 individuals with stable disease among the 50 trial participants, all of whom had previously treated, advanced-stage RCC (19). Phase 2 trials are currently underway.

Congenital Polycythemia (Hereditary Erythrocytosis)

The key role of the HIF pathway in regulating erythropoiesis has been dramatically demonstrated by human genetics. Patients with hereditary erythrocytosis (also known as congenital polycythemia) are characterized by excess red blood cell (RBC) production. Affected individuals have been identified with one or more germline mutations at the *VHL*, *EGLN1*, or *EPAS1* locus.

Mutations in *VHL*. In 1997, congenital elevation of RBC mass (polycythemia or erythrocytosis) was reported as an endemic familial disorder in the Chuvash region of the Russian Federation (101). Affected individuals had significantly increased hemoglobin (Hgb), hematocrit (Hct), and RBC counts, with normal white blood cell counts (**Table 5**). The increased RBC mass was associated with significantly increased blood viscosity, which may cause reduced blood flow (sludging) in cerebral capillaries, leading to headaches, which were the most common symptom, reported by two-thirds of affected individuals (101). In a cohort of 20 individuals with Chuvash polycythemia (CP) and elevated Hct (median = 66%), phlebotomy performed to lower their Hct to the high normal range (median = 48%) led to an increase in the median serum EPO level from 51 to 121 U/mL (101).

The effect of phlebotomy indicated that CP individuals responded to normal RBC mass as if it were abnormally low, suggesting that an alteration in oxygen sensing might underlie the disorder.

Table 5 Hematologic parameters in Chuvash polycythemia

Parameter	CP (mean ± SD)	Normal range
Hemoglobin (g/dL)	21.8 ± 2.5	12–16 (F), 14–18 (M)
Hematocrit (%)	76.5 ± 7.6	37–47 (F), 40–53 (M)
RBC count ($\times 10^6/\mu L$)	7.3 ± 0.8	3.8–5.2 (F), 4.4–5.9 (M)
WBC count ($\times 10^3/\mu L$)	6.1 ± 1.9	4.0–10.0
Viscosity (a.u.)	15 ± 4.5	3.9–5.3

Data are from Reference 101. Abbreviations: a.u., arbitrary units; CP, Chuvash polycythemia; F, female; M, male; RBC, red blood cell; SD, standard deviation; WBC, white blood cell.

To test this hypothesis, a candidate gene approach was taken, and polymorphisms at the *VHL* locus segregated with the disease phenotype in an autosomal recessive manner (2). All affected individuals were homozygous for a missense mutation that changed arginine to tryptophan at residue 200 of the VHL protein. The R200W substitution impairs the interaction of VHL with hydroxylated HIF-1α or HIF-2α, leading to increased HIF target gene expression (2).

Individuals with CP were found to have striking cardiopulmonary responses to decreased inspired O_2 levels with increases in heart rate, respiratory rate, and pulmonary vascular tone that were much greater than those in control subjects (105). These findings underscored the global consequences of altered oxygen sensing in CP and complemented prior studies in mice, which had demonstrated that both HIF-1 and HIF-2 play critical roles in the pathophysiology of hypoxia-induced pulmonary hypertension (102). CP is associated with decreased life span, and a major contributing factor is an increased risk of arterial and venous thrombosis, which, remarkably, is inversely related to Hct (109). The HIF target genes that mediate this pathology have not been identified.

Following the demonstration of the R200W mutation in CP, individuals with polycythemia were found to have other missense mutations in the *VHL* gene, including H191D, L188V, S183L, P138L, D126N, and T124A (9, 59, 66, 90, 112), in either the homozygous or compound heterozygous state, such that all affected individuals have missense mutations in both *VHL* alleles. Homozygosity for the D126N allele was associated with polycythemia and severe pulmonary hypertension, leading to the death of an affected child at two years of age (90).

Individuals homozygous for R200W or the other *VHL* mutations described above do not have increased risk of RCC, pheochromocytoma, or any of the other tumors that are observed in patients with VHL syndrome; likewise, individuals heterozygous for a VHL syndrome allele do not have polycythemia (109). One potential explanation is that the $VHL^{R200W/R200W}$ genotype is associated with <50% of normal VHL activity, which is not sufficient to properly regulate erythropoiesis but is sufficient to prevent tumorigenesis, even if one of the mutant alleles is lost (i.e., $VHL^{R200W/-}$). By contrast, heterozygosity for a VHL syndrome allele (e.g., $VHL^{C162F/+}$) will result in ~50% VHL activity, which is sufficient to prevent polycythemia, but when the wild-type allele is lost (i.e., $VHL^{C162F/-}$), the residual VHL activity is insufficient to prevent tumorigenesis. Remarkably, a *VHL* germline mutation in intron 1 or a synonymous coding sequence mutation in exon 2 causes alternative splicing of the primary VHL RNA and is associated with either congenital polycythemia or VHL syndrome, depending on the pattern of alternative splicing induced by the mutation (61).

Mutations in *EGLN1*. In some families, congenital polycythemia was not associated with *VHL* mutations. Instead, affected individuals were heterozygous for missense mutations in the *EGLN1* gene encoding PHD2, specifically, P317R or R371H (78, 80). Remarkably, P317 and R371 are

conserved in all metazoan PHD2 protein sequences, indicating their critical role in PHD2 activity. These substitutions involve amino acid residues located in the catalytic center of the enzyme, and in the case of P317R, the mutation was reported to reduce the binding of HIF-1α to PHD2. The amino acid substitutions are located close to residues that bind Fe^{2+} in the catalytic center of the enzyme, suggesting an alternative mechanism for loss of PHD2 activity. A third mutant allele, H374R, involves one of the three conserved amino acids that are required for Fe^{2+} binding and is remarkable for the fact that affected individuals develop paragangliomas as well as congenital erythrocytosis (55). One affected individual was heterozygous for the H374R allele in the germline, but both alleles of *EGLN1* were mutated in the tumor, suggesting that PHD2 functions as a tumor suppressor with respect to the development of paraganglioma (55). Polycythemic individuals who are heterozygous for a nonsense or frameshift mutation in *EGLN1* have also been identified (32).

Most recently, a 14-year-old girl with polycythemia (Hgb = 18 g/dL, Hct = 54%, RBC count = $6.5 \times 10^6/\mu L$) was found to be homozygous for a C42R missense mutation in *EGLN1* (104). Like the mutated residues described above, C42 is conserved in all metazoan PHD2 proteins. However, unlike the amino acid residues described above, C42 is located not in the catalytic domain but in a zinc-finger domain that has been proposed to interact with heat shock protein 90 (106). Both of the proband's parents were heterozygous for the mutant allele and had normal Hgb and Hct, whereas her brother was homozygous and had elevated RBC indices (104), indicating autosomal recessive inheritance of polycythemia in this family. Similar to the VHL^{R200W} allele, it is likely that the $EGLN1^{C42R}$ allele is associated with only a modest loss of protein function, such that homozygosity for the hypomorphic allele is required to result in polycythemia.

Mutations in *EPAS1*. In a family with polycythemia in three generations, all affected individuals were heterozygous for a G537W missense mutation in the *EPAS1* gene encoding HIF-2α (79). Subsequently, additional *EPAS1* alleles were identified in other families, including G537R, M535S, M535I, and F540L (77). M535, G537, and F540 are conserved in all vertebrate HIF-2α sequences and are located in proximity to a residue (P531) that is hydroxylated. Several of the missense mutations reduce the interaction between HIF-2α and PHD2 (77). It should be noted that, unlike the mutations in *VHL* and *EGLN1*, which increase the expression of both HIF-1α and HIF-2α, the *EPAS1* mutations increase only HIF-2α expression. Several individuals in a four-generation pedigree segregating the G537R allele had pulmonary hypertension in addition to polycythemia (31). By contrast, the marked increase in ventilation that was observed when individuals with CP inspired a hypoxic gas mixture (105) was not observed in subjects with polycythemia due to the $EPAS1^{G537R}$ allele (26), whereas both groups showed increased heart rate and cardiac output. Thus, increased HIF-2α expression is sufficient to increase RBC mass, cardiac output, and pulmonary vascular tone but not ventilation.

It should be noted that, unlike the systemic arterial system, in which arterioles dilate in response to hypoxia in order to increase O_2 delivery, the pulmonary arterial system constricts in response to hypoxia. This is adaptive in the setting of pneumonia in which one lobe or segment of the lung is not capable of gas exchange due to the infection and resulting inflammatory response. Arterial constriction enables blood flow to be diverted to better-ventilated areas of the lung in order to maximize O_2 uptake. However, constriction of the entire pulmonary vascular bed, as occurs in pulmonary hypertension, dramatically reduces O_2 uptake and increases strain on the right ventricle, which must pump against increased pressure. If untreated, pulmonary hypertension will ultimately result in death due to heart failure and hypoxemia.

The role of HIF-2α in the maintenance of pulmonary artery (PA) tone is further illustrated by the bovine disorder known as brisket disease. On many Colorado ranches, the cattle spend spring grazing in the meadows, but as summer progresses, they work their way up the mountains

in search of more food and less heat. Some of the cattle develop a swelling in the brisket (chest) and die of pulmonary hypertension (with the brisket swelling due to pulmonary edema). Cattle on ranches at 4,850, 7,200, and 8,590 feet were catheterized and genotyped, which revealed that the majority of cattle with PA hypertension (PA pressure > 50 mm Hg) carried an *EPAS1* allele with A606T and G610S missense variants, whereas the majority of cattle without PA hypertension did not carry these variants (75).

The study of congenital polycythemia has provided compelling genetic evidence supporting the critical role of the HIF-PHD-VHL system in the regulation of erythropoiesis and, more broadly, in the maintenance of oxygen homeostasis in humans. Whereas the erythropoietic response to hypoxia occurs over days to weeks, cardiovascular and respiratory responses to hypoxia occur over seconds to minutes and involve activation of the sympathetic nervous system. Recent mouse studies have provided genetic evidence that development of the sympathetic nervous stem is impaired by conditional knockout of HIF-1α (8). Thus, although increased HIF-2α expression is sufficient to cause pathological erythropoietic, cardiovascular, and respiratory responses, it is likely that both HIF-1α and HIF-2α are necessary for physiological responses to hypoxia.

Finally, the identification of individuals with polycythemia due to missense mutations in the *EGLN1* gene demonstrated that modestly decreased PHD2 activity is sufficient to cause increased erythropoiesis. The discovery that the prolyl hydroxylases were (*a*) key negative regulators of HIF-dependent EPO production and (*b*) enzymes that use α-ketoglutarate as a substrate immediately suggested the possibility that α-ketoglutarate analogs could be developed as HIF prolyl hydroxylase inhibitors to stimulate erythropoiesis. HIF prolyl hydroxylase inhibitors may have benefits over recombinant human EPO, including convenience of administration (oral rather than parenteral), decreased risk of cardiovascular side effects and immune responses that occur in some individuals treated with recombinant human EPO, and increased iron availability in bone marrow (due to HIF-2-dependent expression of genes required for intestinal iron absorption and delivery to bone marrow). More than 25,000 subjects with anemia due to chronic kidney disease are currently enrolled in phase 3 trials involving three different HIF prolyl hydroxylase inhibitors (89).

GENETIC VARIATION AT THE *HIF1A* LOCUS AND CORONARY ARTERY DISEASE

In patients with coronary artery disease (CAD), the formation of an atherosclerotic plaque results in narrowing (stenosis) of a major coronary artery, which reduces blood flow to heart tissue, resulting in ischemic pain (angina). If the plaque ruptures, complete obstruction of the vessel occurs, resulting in a heart attack (myocardial infarction). Angiographic evidence of coronary arterial narrowing of 70% or more requires intervention, which consists of a coronary artery bypass graft or the insertion of a stent to enlarge the luminal diameter. Among patients with significant coronary stenosis, approximately two-thirds have collateral vessels that provide blood flow distal to the area of stenosis, and CAD patients with collaterals are more likely to survive a myocardial infarction than patients without collaterals. Genetic analysis of 100 consecutive patients with angiographic evidence of critical stenosis revealed that the frequency of a coding single-nucleotide polymorphism (cSNP, also called a nonsynonymous SNP) that changes proline to serine at residue 582 (P582S) in the HIF-1α protein sequence was fivefold higher in CAD patients without coronary collaterals compared with patients with collaterals (0.188 versus 0.037, $p < 0.001$) (86). These results suggest that CAD patients with the *HIF1A*^{P582S} allele either have impaired collateral formation or present with symptoms (angina) earlier in the disease process.

Whereas the study described above focused only on a single cSNP at the *HIF1A* locus, a genome-wide SNP study revealed that multiple SNPs at the *HIF1A* locus (including the P582S

variant) were the best predictors of whether patients with CAD initially presented with angina or myocardial infarction (38). The interpretation of these results is not clear because angiographic data regarding the severity of CAD and the presence or absence of collaterals were not presented. However, taken together, the findings of the two studies described in this section indicate that genetic variation at the *HIF1A* locus influences the clinical presentation of CAD. A subsequent study reported an association between the $HIF1A^{P582S}$ allele and reduced coronary artery branching (22), providing further genetic evidence that HIF-1 is an important regulator of the coronary vasculature. Conditional knockout of the mouse *Hif1a* gene in cardiomyocytes also resulted in abnormal coronary artery development (8).

KABUKI SYNDROME

Loss-of-function mutations in the gene encoding the histone H3K4 methyltransferase KMT2D cause Kabuki syndrome, which is characterized by craniofacial malformations, intellectual disability, visuospatial memory impairment, and decreased neural stem/progenitor cells in the dentate gyrus of the hippocampus (7). Analysis of KMT2D-deficient neural stem/progenitor cells revealed decreased expression of hypoxia response genes and decreased hypoxia-induced nuclear localization of HIF-1α (11), suggesting a critical functional interaction between HIF-1α and KMT2D in these cells.

EVOLUTIONARY ADAPTATION TO HIGH-ALTITUDE HYPOXIA

Humans have lived continuously on the Tibetan plateau at altitudes of 3,000–4,500 m for more than 20,000 years. More than 400,000 people currently live in Lhasa, the capital of Tibet, where they are exposed to an ambient O_2 concentration of 14%, or two-thirds of the O_2 available at sea level. When lowlanders ascend to such high altitudes, they respond to the ambient hypoxia with increased erythropoiesis. However, polycythemia is not a beneficial long-term adaptation, especially in female highlanders, as it is associated with poor pregnancy outcomes (premature birth, small-for-gestational-age birth, and stillbirth), and on this basis, it is likely that there has been a powerful negative evolutionary selection against polycythemia in highland populations (15). Although most Tibetans are adapted to life at high altitude, some develop chronic mountain sickness, which is characterized by polycythemia and pulmonary hypertension, and this life-threatening condition can be relieved only by transport to low altitude.

Polymorphisms at the *EPAS1* Locus Show Evidence of Evolutionary Selection

The power of high-throughput DNA sequencing made it possible to identify genetic variants contributing to adaptation to high-altitude hypoxia. Whereas genome-wide association studies (GWASs) often require tens of thousands of subjects to identify variants associated with various phenotypes, it required exome sequencing of only 50 unrelated Tibetan highlanders and 40 ethnic Han Chinese from Beijing (HapMap CHB, the reference lowland population) to identify a SNP in the *EPAS1* gene that had a frequency of 87% in Tibetans as compared with 9% in Han Chinese (population branch statistic, $P < 10^{-6}$) and was significantly associated with decreased Hgb levels in Tibetans (125). Luckily, the exomes included bordering sequences, as the *EPAS1* SNP was actually located in an intron.

Of course, the doubters (frustrated GWASers) argued that such a small sample size demanded replication. An analysis of 502,722 SNPs genome-wide in 35 Tibetans residing at 3,200–3,500 m and 84 lowland Han HapMap controls identified eight SNPs from the *EPAS1* locus, which

formed an extended haplotype with a frequency of 46% in Tibetans and 2% in Han Chinese, and these were the only SNPs with P values $< 5 \times 10^{-7}$, the threshold for genome-wide significance (4). The *EPAS1* SNPs were significantly associated with reduced Hgb concentration in the Tibetans, and the results were replicated in a second cohort of Tibetans residing at 4,200 m, in which 31 *EPAS* SNPs in high linkage disequilibrium were associated with significantly decreased Hgb concentration (4).

The story became even more exciting with resequencing of DNA encompassing the *EPAS1* locus in 40 Tibetan and 40 Han individuals at >200× genomic coverage, which led to the identification of 477 SNPs in a region of 129 kb (41). In particular, a 2.5-kb region encompassed a five-SNP haplotype that was seen in most Tibetans and none of the Han; furthermore, the haplotype was not observed in any of the world populations studied in the 1000 Genomes Project except for a single southern Han Chinese (CHS) individual and a single Beijing Han Chinese (CHB) individual. However, the five-SNP haplotype was found in the genome sequence that was obtained from DNA extracted from a small finger bone and large molar tooth found in a cave in Denisova, Siberia (41), which belonged not to a member of *Homo sapiens* but to an evolutionary dead-end distinct from Neanderthals. Whereas Neanderthal-specific variants account for 1.5–2% of variation in some modern-day European populations, Denisovan variants account for 4–6% of the genome in some modern-day Melanesian and Southeast Asian populations (114). The introgression of the *EPAS1* Denisovan variants into the Tibetan genome was estimated to have occurred approximately 40,000 years ago (41), which is similar to the ballpark estimate of when sustained habitation of the Tibetan plateau commenced.

Still, Tibet is a long uphill trek from Siberia (the Denisovan cave is at an altitude of 700 m), and so the story required a leap of faith. Enter a Buddhist monk, who, while meditating in Baishiya Karst Cave, located at 3,200 m on the Tibetan plateau, found a jawbone with exceptionally large molars, which the monk recognized as unusual enough to present to the sixth Gung-Thang living Buddha, who in turn gave the fossil to Lanzhou University, where it languished for almost four decades (33). Fortunately, the jawbone was shown to a visiting paleoanthropologist, whose suspicion that it might be Denisovan in origin was confirmed by amino acid sequence analysis of collagen extracted from the bone, which was estimated to be 160,000 years old (14), providing plenty of time for introgression of those five SNPs at the *EPAS1* locus that contribute to the ability of modern-day Tibetans to live at high altitude without excessive RBC production.

Finally, humans are not the only species to inhabit the Tibetan plateau and adapt to life under conditions of reduced O_2 availability. Whole-genome sequencing of indigenous canine populations from Tibet and Qinghai province (altitude > 2,500 m) and two indigenous Chinese lowland canine populations from Beijing and Guangzhou (altitude ∼50 m) ($N = 40$ total in each group) revealed that the strongest signal for selection was at the *EPAS1* locus, based on the root mean square of the F statistic [F_{ST}(rms)] for 2.4 million SNPs (116). Analysis of 225 SNPs in 145 kb encompassing the *EPAS1* locus revealed four cSNPs: G305S [F_{ST}(rms) = 0.95], D494E [F_{ST}(rms) = 0.93], V500M [F_{ST}(rms) = 0.95], and P750S [F_{ST}(rms) = 0.92]. The G305S substitution is particularly noteworthy because glycine is found in this position in all vertebrate HIF-2α proteins, and the residue is located in a cavity that is the site of binding for chemical ligands that block heterodimerization with HIF-1β (52, 91). The frequency of the G305S allele was 95% among 61 highland dogs and 6% among 149 lowland dogs (116). It should be noted that the highland canine populations, like the highland human populations, showed no significant increase in mean Hgb level compared with the lowland counterparts. However, there was no analysis of whether Tibetan dogs with the *EPAS1*^{G305S} allele have lower mean Hgb levels than Tibetan dogs without the variant allele. Functional studies are also required to determine whether G305S or any of the other cSNPs affect HIF-2 transcriptional activity. The remarkable finding of evolutionary

selection of *EPAS1* variants in two species underscores the importance of changes in HIF-2 activity in the adaptation to life on the Tibetan plateau.

cSNPs at the *EGLN1* Locus Show Evidence of Evolutionary Selection

With a 240-candidate-gene approach, a study compared 31 unrelated Tibetans with HapMap Chinese (CHB) and Japanese (JPT) lowland populations, using the cross-population extended haplotype homozygosity (XP-EHH) statistic to identify alleles that have increased in frequency to the point of near fixation in one of the populations along with the integrated haplotype score (iHS), a statistic based on the decay of linkage disequilibrium surrounding a variant subjected to natural selection (103). Ten genes from the candidate list were in the top 1% of the empirical distribution of XP-EHH or iHS. Six of these (including *EPAS1*) were identified by XP-EHH, four (including *PPARA*) were identified by iHS, and one (*EGLN1*) was identified by both tests. *PPARA* is one of the few target genes in which HIF-1 binding has been associated with transcriptional repression (74). Among Tibetan individuals, variant alleles at the *EPAS1* and *PPARA* loci showed significant negative correlations with Hgb levels: For each copy of a variant allele at either locus, Hgb decreased by ~1.7 g/dL (103).

To top it off, in an independent study, analysis of 905,747 SNPs in 49 ethnic Tibetans and 49 Andeans (another high-altitude population) revealed evidence of selection at the *EGLN1* locus in both groups; however, different *EGLN1* SNPs were selected in Tibetans and Andeans, consistent with the fact that the two groups evolved independently (6). This study also reported evidence of selection at the *EPAS1* locus in Tibetans but not in Andeans, who have resided at high altitude only for approximately 10,000 years (and had no contact with Denisovans). Andeans on average have higher Hgb levels and higher incidence of chronic mountain sickness (i.e., are less well adapted) than Tibetans.

All of the SNPs described thus far suffer from the fact that they are in noncoding DNA sequences, and their functional effect on gene expression has not been determined. It was therefore noteworthy when a cSNP was identified in the *EGLN1* gene that changed amino acid residue 127 in PHD2 from cysteine to serine (C127S); remarkably, this mutation appeared to have occurred ~8,000 years ago on an *EGLN1* gene that was already carrying a D4E cSNP, and the frequency of the $EGLN1^{D4E;C127S}$ allele in the population increased with altitude in different Tibetan villages (65, 108).

Biochemical characterization of recombinant $PHD2^{D4E;C127S}$ protein revealed that the K_m for O_2 (110 ± 30 μM) was significantly decreased compared with recombinant wild-type PHD2 (150 ± 60 μM), $PHD2^{D4E}$ (150 ± 30 μM), or $PHD2^{C127S}$ (160 ± 50 μM), indicating that the $PHD2^{D4E;C127S}$ protein retains its catalytic activity at lower O_2 levels than the wild-type protein (65), which would result in lower HIF activity, decreased EPO synthesis, and decreased RBC production under hypoxic conditions. It is likely that the PHD-HIF axis also has a cell autonomous effect on erythroid progenitor cells because, compared with bone marrow cells from $EGLN1^{WT}$ donors, erythroid progenitors from individuals carrying the $EGLN1^{D4E;C127S}$ allele have smaller erythroid colonies, and whereas erythroid colonies from $EGLN1^{WT}$ donors grow better at 5% than 20% O_2, erythroid colonies from $EGLN1^{D4E;C127S}$ donors grow less well in 5% than 20% O_2 (65). Taken together, the data suggest that the $EGLN1^{D4E;C127S}$ allele, in combination with the Denisovan *EPAS1* haplotype, has been selected in high-altitude Tibetan populations due to its negative effect on the erythropoietic and pulmonary vascular responses to chronic hypoxia.

CONCLUSION

The HIF-PHD-VHL pathway plays a critical role in oxygen homeostasis, regulating O_2 supply and demand by affecting transcriptional activation and repression as well as RNA stability and

translation. Germline mutations in the pathway that increase HIF activity result in hereditary erythrocytosis, whereas variants in pathway genes that are present at high frequency only in high-altitude populations appear to decrease HIF activity and blunt the erythropoietic response to hypoxia. It is likely that additional insights into oxygen homeostasis will be gleaned from further analysis of genetic variants in high-altitude populations, most notably, metabolic adaptations that enable efficient oxidative phosphorylation under hypoxic conditions.

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LITERATURE CITED

1. Allen BL, Taatjes DJ. 2015. The Mediator complex: a central integrator of transcription. *Nat. Rev. Mol. Cell Biol.* 16:155–66
2. Ang SO, Chen H, Hirota K, Gordeuk VR, Jelinek J, et al. 2002. Disruption of oxygen homeostasis underlies congenital Chuvash polycythemia. *Nat. Genet.* 32:614–21
3. Arany Z, Huang LE, Eckner R, Bhattacharya S, Jiang C, et al. 1996. An essential role for p300/CBP in the cellular response to hypoxia. *PNAS* 93:12969–73
4. Beall CM, Cavalleri GL, Deng L, Elston RC, Gao Y, et al. 2010. Natural selection on *EPAS1* (*HIF2α*) associated with low hemoglobin concentration in Tibetan highlanders. *PNAS* 107:11459–64
5. Beck I, Ramirez S, Weinmann R, Caro J. 1991. Enhancer element at the 3′-flanking region controls transcriptional response to hypoxia in the human erythropoietin gene. *J. Biol. Chem.* 266:15563–66
6. Bigham A, Bauchet M, Pinto D, Mao X, Akey JM, et al. 2010. Identifying signatures of natural selection in Tibetan and Andean populations using dense genome scan data. *PLOS Genet.* 6:e1001116
7. Bjornsson HT, Benjamin JS, Zhang L, Weissman J, Gerber EE, et al. 2014. Histone deacetylase inhibition rescues structural and functional brain deficits in a mouse model of Kabuki syndrome. *Sci. Transl. Med.* 6:256ra135
8. Bohuslavova R, Cerychova R, Papousek F, Olejnickova V, Bartos M, et al. 2019. HIF-1α is required for development of the sympathetic nervous system. *PNAS* 116:13414–23
9. Bond J, Gale DP, Connor T, Adams S, de Boer J, et al. 2011. Dysregulation of the HIF pathway due to *VHL* mutation causing severe erythrocytosis and pulmonary hypertension. *Blood* 117:3699–701
10. Bruick RK, McKnight SL. 2001. A conserved family of prolyl-4-hydroxylases that modify HIF. *Science* 294:1337–40
11. Carosso GA, Boukas L, Augustin JJ, Nguyen HN, Winer BL, et al. 2019. Precocious neuronal differentiation and disrupted oxygen responses in Kabuki syndrome. *JCI Insight* 4:e129375
12. Cavadas MA, Mesnieres M, Crifo B, Manresa MC, Selfridge AC, et al. 2015. REST mediates resolution of HIF-dependent gene expression in prolonged hypoxia. *Sci. Rep.* 5:17851
13. Cavadas MA, Mesnieres M, Crifo B, Manresa MC, Selfridge AC, et al. 2016. REST is a hypoxia-responsive transcriptional repressor. *Sci. Rep.* 6:31355
14. Chen F, Welker F, Shen C, Bailey SE, Bergmann I, et al. 2019. A late Middle Pleistocene Denisovan mandible from the Tibetan Plateau. *Nature* 569:409–12
15. Cho JI, Basnyat B, Jeong C, Di Rienzo A, Childs G, et al. 2017. Ethnically Tibetan women in Nepal with low hemoglobin concentration have better reproductive outcomes. *Evol. Med. Public Health* 2017:82–96
16. Chowdhury R, Leung IK, Tian YM, Abboud MI, Ge W, et al. 2016. Structural basis for oxygen degradation domain selectivity of the HIF prolyl hydroxylases. *Nat. Commun.* 7:12673

17. Colgan SP, Campbell EL, Kominsky DJ. 2016. Hypoxia and mucosal inflammation. *Annu. Rev. Pathol. Mech. Dis.* 11:77–100
18. Corn PG, Ricci MS, Scata KA, Arsham AM, Simon MC, et al. 2005. Mxi1 is induced by hypoxia in a HIF-1-dependent manner and protects cells from c-Myc-induced apoptosis. *Cancer Biol. Ther.* 4:1285–94
19. Courtney KD, Infante JR, Lam ET, Figlin RA, Rini BI, et al. 2018. Phase I dose-escalation trial of PT2385, a first-in-class hypoxia-inducible factor 2α antagonist in patients with previously treated advanced clear cell renal cell carcinoma. *J. Clin. Oncol.* 36:867–74
20. Couvé S, Ladroue C, Laine E, Mahtouk K, Guégan J, et al. 2014. Genetic evidence of a precisely tuned dysregulation in the hypoxia signaling pathway during oncogenesis. *Cancer Res.* 74:6554–64
21. Duan C. 2016. Hypoxia-inducible factor 3 biology: complexities and emerging themes. *Am. J. Physiol. Cell Physiol.* 310:C260–69
22. Duran J, Gotzens V, Carballo J, Martin E, Petit M, et al. 2012. The *HIF1A* C85T polymorphism influences the number of branches of the human coronary tree. *Am. Heart J.* 154:1035–42
23. Epstein AC, Gleadle JM, McNeill LA, Hewitson KS, O'Rourke J, et al. 2001. *C. elegans* EGL-9 and mammalian homologs define a family of dioxygenases that regulate HIF by prolyl hydroxylation. *Cell* 107:43–54
24. Erslev A. 1953. Humoral regulation of red cell production. *Blood* 8:347–57
25. Eschbach JW, Egrie JC, Downing MR, Browne JK, Adamson JW. 1987. Correction of the anemia of endstage renal disease with recombinant human erythropoietin. *N. Engl. J. Med.* 316:73–78
26. Formenti F, Beer PA, Croft QP, Dorrington KL, Gale DP, et al. 2011. Cardiopulmonary function in two human disorders of the hypoxia-inducible factor (HIF) pathway: von Hippel-Lindau disease and HIF-2α gain-of-function mutation. *FASEB J.* 25:2001–11
27. Forsythe JA, Jiang BH, Iyer NV, Agani F, Leung SW, et al. 1996. Activation of vascular endothelial growth factor gene transcription by hypoxia-inducible factor 1. *Mol. Cell. Biol.* 16:4604–13
28. Fu Y, Dominissini D, Rechavi G, He C. 2014. Gene expression regulation through reversible m^6A RNA methylation. *Nat. Rev. Genet.* 15:293–306
29. Galbraith MD, Allen MA, Bensard CL, Wang X, Schwinn MK, et al. 2013. HIF1A employs CDK8-Mediator to stimulate RNAPII elongation in response to hypoxia. *Cell* 153:1327–39
30. Galbraith MD, Donner AJ, Espinosa JM. 2010. CDK8: a positive regulator of transcription. *Transcription* 1:4–12
31. Gale DP, Harten SK, Reid CD, Tuddenham EG, Maxwell PH. 2008. Autosomal dominant erythrocytosis and pulmonary arterial hypertension associated with an activating *HIF-2α* mutation. *Blood* 112:919–21
32. Gardie B, Percy MJ, Hoogewijs D, Chowdhury R, Bento C, et al. 2014. The role of PHD2 mutations in the pathogenesis of erythrocytosis. *Hypoxia* 2:71–90
33. Gibbons A. 2019. Ancient jaw gives elusive Denisovans a face. *Science* 364:418–19
34. Graham AM, Presnell JS. 2017. Hypoxia-inducible factor (HIF) transcription factor family expansion, diversification, divergence and selection in eukaryotes. *PLOS ONE* 12:e0179545
35. Greijer AE, van der Groep P, Kemming D, Shvarts A, Semenza GL, et al. 2005. Up-regulation of gene expression by hypoxia is mediated predominantly by hypoxia-inducible factor 1 (HIF-1). *J. Pathol.* 206:291–304
36. Gruber M, Hu CJ, Johnson RS, Brown EJ, Keith B, et al. 2007. Acute postnatal ablation of Hif-2α results in anemia. *PNAS* 104:2301–6
37. Henry RA, Kuo YM, Andrews AJ. 2013. Differences in specificity and selectivity between CBP and p300 acetylation of histone H3 and H3/H4. *Biochemistry* 52:5746–59
38. Hlatky MA, Quertermous T, Boothroyd DB, Priest JR, Glassford AJ, et al. 2007. Polymorphisms in hypoxia inducible factor 1 and the initial clinical presentation of coronary disease. *Am. Heart J.* 154:1035–42
39. Hoffman EC, Reyes H, Chu FF, Sander F, Conley LH, et al. 1991. Cloning of a factor required for activity of the Ah (dioxin) receptor. *Science* 252:954–58
40. Huang LE, Gu J, Schau M, Bunn HF. 1998. Regulation of hypoxia-inducible factor 1α is mediated by an O_2-dependent degradation domain via the ubiquitin-proteasome pathway. *PNAS* 95:7987–92

41. Huerta-Sánchez E, Jin X, Asan Bianba Z, Peter BM, et al. 2014. Altitude adaptation in Tibetans is caused by introgression of Denisovan-like DNA. *Nature* 512:194–97
42. Islam MS, Leissing TM, Chowdhury R, Hopkinson RJ, Schofield CJ. 2018. *Annu. Rev. Biochem.* 87:585–620
43. Ivan M, Kondo K, Yang H, Kim W, Valiando J, et al. 2001. HIFα targeted for VHL-mediated destruction by proline hydroxylation: implications for O_2 sensing. *Science* 292:464–68
44. Jaakkola P, Mole DR, Tian YM, Wilson MI, Gielbert J, et al. 2001. Targeting of HIF-α to the von Hippel-Lindau ubiquitylation complex by O_2-regulated prolyl hydroxylation. *Science* 292:468–72
45. Jacobs K, Shoemaker C, Rudersdorf R, Neill SD, Kaufman RJ, et al. 1985. Isolation and characterization of genomic and cDNA clones of human erythropoietin. *Nature* 313:806–10
46. Jacobson LO, Marks E, Gaston E, Goldwasser E. 1957. Role of the kidney in erythropoiesis. *Nature* 179:633–34
47. Jiang B-H, Rue E, Wang GL, Roe R, Semenza GL. 1996. Dimerization, DNA binding, and transactivation properties of hypoxia-inducible factor 1. *J. Biol. Chem.* 271:17771–78
48. Jiang B-H, Semenza GL, Bauer C, Marti HH. 1996. Hypoxia-inducible factor 1 levels vary exponentially over a physiologically relevant range of O_2 tension. *Am. J. Physiol. Cell Physiol.* 271:C1172–80
49. Jiang B-H, Zhang JZ, Leung SW, Roe R, Semenza GL. 1997. Transactivation and inhibitory domains of hypoxia-inducible factor 1α: modulation of transcriptional activity by oxygen tension. *J. Biol. Chem.* 272:19253–60
50. Jonkers I, Lis JT. 2015. Getting up to speed with transcription elongation by RNA polymerase II. *Nat. Rev. Mol. Cell Biol.* 16:167–77
51. Kaelin WG Jr. 2008. The von Hippel-Lindau tumor suppressor protein: O_2 sensing and cancer. *Nat. Rev. Cancer* 8:865–73
52. Key J, Scheuermann TH, Anderson PC, Daggett V, Gardner KH. 2009. Principles of ligand binding within a completely buried cavity in HIF-2α PAS-B. *J. Am. Chem. Soc.* 131:17647–54
53. Koury ST, Bondurant MC, Koury MJ. 1988. Localization of erythropoietin synthesizing cells in murine kidneys by in situ hybridization. *Blood* 71:524–27
54. Kulshreshtha R, Ferracin M, Wojcik SE, Garzon R, Alder H, et al. 2007. A microRNA signature of hypoxia. *Mol. Cell. Biol.* 27:1859–67
55. Ladroue C, Carcenac R, Leporrier M, Gad S, Le Hello C, et al. 2008. *PHD2* mutation and congenital erythrocytosis with paraganglioma. *N. Engl. J. Med.* 359:2685–92
56. Lambert SA, Jolma A, Campitelli LF, Das PK, Yin Y, et al. 2018. The human transcription factors. *Cell* 172:650–65
57. Lando D, Peet DJ, Gorman JJ, Whelan DA, Whitelaw ML, et al. 2002. FIH-1 is an asparaginyl hydroxylase enzyme that regulates the transcriptional activity of hypoxia-inducible factor. *Genes Dev.* 16:1466–71
58. Lando D, Peet DJ, Whelan DA, Gorman JJ, Whitelaw ML. 2002. Asparagine hydroxylation of HIF transactivation domain: a hypoxic switch. *Science* 295:858–61
59. Lanikova L, Lorenzo F, Yang C, Vankayalapati H, Drachtman R, et al. 2013. Novel homozygous *VHL* mutation in exon 2 is associated with congenital polycythemia but not with cancer. *Blood* 121:3918–24
60. Latif F, Tory K, Gnarra J, Yao M, Duh FM, et al. 1993. Identification of the von Hippel-Lindau disease tumor-suppressor gene. *Science* 260:1317–20
61. Lenglet M, Robriquet F, Schwarz K, Camps C, Couiturier A, et al. 2018. Identification of a new *VHL* exon and complex splicing alterations in familial erythrocytosis or von Hippel-Lindau disease. *Blood* 132:469–83
62. Lin F-K, Suggs S, Browne JK, Smalling R, Egrie JC, et al. 1985. Cloning and expression of the human erythropoietin gene. *PNAS* 82:7580–84
63. Lindau A. 1926. Studien ber kleinbirncysten bau: pathogenese und beziehungen zur angiomatosis rentinae. *Acta Radiol. Microbiol. Scand.* 1(Suppl.):1–128
64. Loenarz C, Coleman ML, Boleininger A, Schierwater B, Holland PW, et al. 2011. The hypoxia-inducible transcription factor pathway regulates oxygen sensing in the simplest animal, *Trichoplax adherens*. *EMBO Rep.* 12:63–70

65. Lorenzo FR, Huff C, Myllymaki M, Olenchock B, Swierczek S, et al. 2014. A genetic mechanism for Tibetan high-altitude populations. *Nat. Genet.* 46:951–56
66. Lorenzo FR, Yang C, Lanikova L, Butros L, Zhuang Z, et al. 2013. Novel compound VHL heterozygosity (*VHL* T124A/L188V) associated with congenital polycythemia. *Br. J. Hematol.* 162:851–53
67. Luger K, Mader AW, Richmond RK, Sargent DF, Richmond TJ. 1997. Crystal structure of the nucleosome core particle at 2.8 Å resolution. *Nature* 389:251–60
68. Mahon PC, Hirota K, Semenza GL. 2001. FIH-1: a novel protein that interacts with HIF-1α and VHL to mediate repression of HIF-1 transcriptional activity. *Genes Dev.* 15:2675–86
69. Manalo DJ, Rowan A, Lavoie T, Natarajan L, Kelly BD, et al. 2005. Transcriptional regulation of vascular endothelial cell responses to hypoxia by HIF-1. *Blood* 105:659–69
70. Maxwell PH, Wiesener MS, Chang G-W, Clifford SC, Vaux EC, et al. 1999. The tumor suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. *Nature* 399:271–75
71. Miyake T, Hung CKH, Goldwasser E. 1977. Purification of human erythropoietin. *J. Biol. Chem.* 252:5558–64
72. Mole DR, Blancher C, Copley RR, Pollard PJ, Gleadle JM, et al. 2009. Genome-wide association of hypoxia-inducible factor HIF-1α and HIF-2α DNA binding with expression profiling of hypoxia-inducible transcripts. *J. Biol. Chem.* 284:16767–75
73. Montagner M, Enzo E, Forcato M, Zanconato F, Parenti A, et al. 2012. SHARP1 suppresses breast cancer metastasis by promoting degradation of hypoxia-inducible factors. *Nature* 487:380–84
74. Narravula S, Colgan SP. 2001. Hypoxia-inducible factor 1-mediated inhibition of peroxisome proliferator-activated receptor α expression during hypoxia. *J. Immunol.* 166:7543–48
75. Newman JH, Holt TN, Cogan JD, Womack B, Phillips JA III, et al. 2015. Increased prevalence of *EPAS1* variant in cattle with high-altitude pulmonary hypertension. *Nat. Commun.* 6:6863
76. Ogryzko VV, Schiltz RL, Russanova V, Howard BH, Nakatani Y. 1996. The transcriptional coactivators p300 and CBP are histone acetyltransferases. *Cell* 87:953–59
77. Percy MJ, Chung YJ, Harrison C, Mercieca J, Hoffbrand AV, et al. 2012. Two new mutations in the *HIF2A* gene associated with erythrocytosis. *Am. J. Hematol.* 87:439–42
78. Percy MJ, Furlow PW, Beer PA, Lappin TR, McMullin MF, et al. 2007. A novel erythrocytosis-associated PHD2 mutation suggests the location of a HIF binding groove. *Blood* 110:2193–96
79. Percy MJ, Furlow PW, Lucas GS, Li X, Lappin TR, et al. 2008. A gain-of-function mutation in the *HIF2A* gene in familial erythrocytosis. *N. Engl. J. Med.* 358:162–68
80. Percy MJ, Zhao Q, Flores A, Harrison C, Lappin TR, et al. 2006. A family with erythrocytosis establishes a role for prolyl hydroxylase domain protein 2 in oxygen homeostasis. *PNAS* 103:654–59
81. Prabhakar NR, Semenza GL. 2012. Adaptive and maladaptive cardiorespiratory responses to continuous and intermittent hypoxia mediated by hypoxia-inducible factors 1 and 2. *Physiol. Rev.* 92:967–1003
82. Pugh CW, O'Rourke JF, Nagao M, Gleadle JM, Ratcliffe PJ. 1997. Activation of hypoxia-inducible factor 1: definition of regulatory domains within the α subunit. *J. Biol. Chem.* 272:11205–14
83. Pugh CW, Tan CC, Jones RW, Ratcliffe PJ. 1991. Functional analysis of an oxygen-regulated transcriptional enhancer lying 3′ to the mouse erythropoietin gene. *PNAS* 88:10553–57
84. Ramakrishnan SK, Shah YM. 2016. Role of intestinal HIF-2α in health and disease. *Annu. Rev. Physiol.* 78:301–25
85. Reissmann KR. 1950. Studies on the mechanism of erythropoietic stimulation in parabiotic rats during hypoxia. *Blood* 5:372–80
86. Resar JR, Roguin A, Voner J, Nasir K, Hennebry TA, et al. 2005. Hypoxia-inducible factor 1α polymorphism and coronary collaterals in patients with ischemic heart disease. *Chest* 128:787–91
87. Salceda S, Caro J. 1997. Hypoxia-inducible factor 1α (HIF-1α) protein is rapidly degraded by the ubiquitin-proteasome system under normoxic conditions. *J. Biol. Chem.* 272:22642–47
88. Samanta D, Semenza GL. 2018. Metabolic adaptation of cancer and immune cells mediated by hypoxia-inducible factors. *Biochim. Biophys. Acta Rev. Cancer* 1870:15–22
89. Sanghani NS, Haase VH. 2019. Hypoxia-inducible factors in renal anemia: current clinical experience. *Adv. Chronic Kidney Dis.* 26:253–66

90. Sarangi S, Lanikova L, Kapralova K, Acharya S, Swierczek S, et al. 2014. The homozygous *VHL*^{D126N} missense mutation is associated with dramatically elevated erythropoietin levels with consequent polycythemia and early onset severe pulmonary hypertension. *Pediatr. Blood Cancer* 61:2104–6
91. Scheuermann TH, Tomchick DR, Machius M, Guo Y, Bruick RK, et al. 2009. Artificial ligand binding within the HIF-2α PAS-B domain of the HIF-2 transcription factor. *PNAS* 106:450–55
92. Schödel J, Oikonomopoulos S, Ragoussis J, Pugh CW, Ratcliffe PJ, et al. 2011. High-resolution genome-wide mapping of HIF-binding sites by ChIP-seq. *Blood* 117:e207–17
93. Schuster SJ, Badiavas EV, Costa-Giomi P, Weinmann R, Erslev AJ, et al. 1989. Stimulation of erythropoietin gene transcription during hypoxia and cobalt exposure. *Blood* 73:13–16
94. Semenza GL. 2014. Hypoxia-inducible factor 1 and cardiovascular disease. *Annu. Rev. Physiol.* 76:39–56
95. Semenza GL. 2014. Oxygen sensing, hypoxia-inducible factors, and disease pathophysiology. *Annu. Rev. Pathol. Mech. Dis.* 9:47–71
96. Semenza GL. 2017. A compendium of proteins that interact with HIF-1α. *Exp. Cell Res.* 356:128–35
97. Semenza GL. 2019. Pharmacologic targeting of hypoxia-inducible factors. *Annu. Rev. Pharmacol. Toxicol.* 59:379–403
98. Semenza GL, Jiang B-H, Leung SW, Passantino R, Concordet JP, et al. 1996. Hypoxia response elements in the aldolase A, enolase 1, and lactate dehydrogenase gene promoters contain essential binding sites for hypoxia-inducible factor 1. *J. Biol. Chem.* 271:32529–37
99. Semenza GL, Nejfelt MK, Chi SM, Antonarakis SE. 1991. Hypoxia-inducible nuclear factors bind to an enhancer element located 3′ to the human erythropoietin gene. *PNAS* 88:5680–84
100. Semenza GL, Wang GL. 1992. A nuclear factor induced by hypoxia via de novo protein synthesis binds to the human erythropoietin gene enhancer at a site required for transcriptional activation. *Mol. Cell. Biol.* 12:5447–54
101. Sergeyeva A, Gordeuk VR, Tokarev YN, Sokol L, Prchal JF, et al. 1997. Congenital polycythemia in Chuvashia. *Blood* 89:2148–54
102. Shimoda LA, Yun X, Sikka G. 2019. Revising the role of hypoxia-inducible factors in pulmonary hypertension. *Curr. Opin. Physiol.* 7:33–40
103. Simonson TS, Yang Y, Huff CD, Yun H, Qin G, et al. 2010. Genetic evidence for high-altitude adaptation in Tibet. *Science* 329:72–74
104. Sinnema M, Song D, Guan W, Janssen JW, van Wijk R, et al. 2018. Loss-of-function zinc finger mutation in the *EGLN1* gene associated with erythrocytosis. *Blood* 132:1455–58
105. Smith TG, Brooks JT, Balanos GM, Lappin TR, Layton DM, et al. 2006. Mutation of von Hippel-Lindau tumor suppressor and human cardiopulmonary physiology. *PLOS Med.* 3:e290
106. Song D, Li LS, Heaton-Johnson KJ, Arsenault PR, Master SR, et al. 2013. Prolyl hydroxylase domain protein 2 (PHD2) binds a Pro-Xaa-Leu-Glu motif, linking it to the heat shock protein 90 pathway. *J. Biol. Chem.* 288:9662–74
107. Takahashi H, Parmely TJ, Sato S, Tomomori-Sato C, Banks CA, et al. 2011. Human Mediator subunit MED26 functions as a docking site for transcription elongation factors. *Cell* 146:92–104
108. Tashi T, Reading ST, Wuren T, Zhang X, Moore LG, et al. 2017. Gain-of-function *EGLN1* prolyl hydroxylase (PHD2 D4E:C127S) in combination with *EPAS1* (HIF-2α) polymorphism lowers hemoglobin concentration in Tibetan highlanders. *J. Mol. Med.* 95:665–70
109. Tashi T, Song J, Prchal JT. 2019. Congenital and evolutionary modulations of hypoxia sensing and their erythroid phenotype. *Curr. Opin. Physiol.* 7:27–32
110. Thalhammer A, Bencokova Z, Poole R, Loenarz C, Adam J, et al. 2011. Human AlkB homologue 5 is a nuclear 2-oxoglutarate dependent oxygenase and a direct target of hypoxia-inducible factor 1α (HIF-1α). 2011. *PLOS ONE* 6:e16210
111. Tian H, McKnight SL, Russell DW. 1997. Endothelial PAS domain protein 1 (EPAS1), a transcription factor selectively expressed in endothelial cells. *Genes Dev.* 11:72–82
112. Tomasic NL, Piterkova L, Huff C, Bilic E, Yoon D, et al. 2013. The phenotype of polycythemia due to Croatian homozygous VHL (571C>G;H191D) mutation is different from that of Chuvash polycythemia (VHL 598CS>T;R200W). *Haematologica* 98:560–67

113. von Hippel E. 1904. Uber eine sehr self seltene erkrankung der netzhaut. *Klin. Beob. Arch. Ophthalmol.* 59:83–106
114. Wall JD, Yoshihara Caldeira Brandt D. 2016. Archaic admixture in human history. *Curr. Opin. Genet. Dev.* 41:93–97
115. Wallace EM, Rizzi JP, Han G, Wehn PM, Cao Z, et al. 2016. A small-molecule antagonist of HIF-2α is efficacious in preclinical models of renal cell carcinoma. *Cancer Res.* 76:5491–500
116. Wang GD, Fan RX, Zhai W, Liu F, Wang L, et al. 2014. Genetic convergence in the adaptation of dogs and humans to the high-altitude environment of the Tibetan plateau. *Genome Biol. Evol.* 6:2122–28
117. Wang GL, Jiang B-H, Rue EA, Semenza GL. 1995. Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O_2 tension. *PNAS* 92:5510–14
118. Wang GL, Semenza GL. 1993. General involvement of hypoxia-inducible factor 1 in transcriptional responses to hypoxia. *PNAS* 90:4304–8
119. Wang GL, Semenza GL. 1995. Purification and characterization of hypoxia-inducible factor 1. *J. Biol. Chem.* 270:1230–37
120. Wiener CM, Booth G, Semenza GL. 1996. In vivo expression of mRNAs encoding hypoxia-inducible factor 1. *Biochem. Biophys. Res. Commun.* 225:485–88
121. Wiesener MS, Jurgensen JS, Rosenberger C, Scholze CK, Horstrup JH, et al. 2003. Widespread hypoxia-inducible expression of HIF-2α in distinct cell populations of different organs. *FASEB J.* 17:271–73
122. Wiesener MS, Turley H, Allen WE, Willam C, Eckardt KU, et al. 1998. Induction of endothelial PAS domain protein-1 by hypoxia: characterization and comparison with hypoxia-inducible factor 1α. *Blood* 92:2260–68
123. Wu D, Rastinejad F. 2017. Structural characterization of mammalian bHLH-PAS transcription factors. *Curr. Opin. Struct. Biol.* 43:1–9
124. Xia X, Lemieux ME, Li W, Carroll JS, Brown M, et al. 2009. Integrative analysis of HIF binding and transactivation reveals its role in maintaining histone methylation homeostasis. *PNAS* 106:4260–65
125. Yi X, Liang Y, Huerta-Sanchez E, Jin X, Cuo ZX, et al. 2010. Sequencing of 50 human exomes reveals adaptation to high altitude. *Science* 329:75–78
126. Yu F, White SB, Zhao Q, Lee FS. 2001. HIF-1α binding to VHL is regulated by stimulus-sensitive proline hydroxylation. *PNAS* 98:9630–35
127. Yun Z, Maecker HL, Johnson RS, Giaccia AJ. 2002. Inhibition of *PPARγ2* expression by the HIF-1-regulated gene *DEC1/Stra13*: a mechanism for regulation of adipogenesis by hypoxia. *Dev. Cell* 2:331–341
128. Zhang C, Samanta D, Lu H, Bullen JW, Zhang H, et al. 2016. Hypoxia induces the breast cancer stem cell phenotype by HIF-dependent and ALKBH5-mediated m^6A-demethylation of NANOG mRNA. *PNAS* 113:E2047–56
129. Zhang C, Zhi WI, Lu H, Samanta D, Chen I, et al. 2016. Hypoxia-inducible factors regulate pluripotency factor expression by ZNF217- and ALKBH5-mediated modulation of RNA methylation in breast cancer cells. *Oncotarget* 7:64527–42