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Hypoxia-inducible factor signaling in pulmonary hypertension

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Pulmonary hypertension (PH) is characterized by pulmonary artery remodeling that can subsequently culminate in right heart failure and premature death. Emerging evidence suggests that hypoxia-inducible factor (HIF) signaling plays a fundamental and pivotal role in the pathogenesis of PH. This Review summarizes the regulation of HIF isoforms and their impact in various PH subtypes, as well as the elaborate conditional and cell-specific knockout mouse studies that brought the role of this pathway to light. We also discuss the current preclinical status of pan- and isoform-selective HIF inhibitors, and propose new research areas that may facilitate HIF isoform-specific inhibition as a novel therapeutic strategy for PH and right heart failure.

HIF signaling and biology

The transcription factor hypoxia-inducible factor (HIF) is a master regulator of oxygen homeostasis that acts as a heterodimeric complex composed of the oxygen-sensitive α subunit (HIF-α; including HIF-1α, HIF-2α [EPAS1], and HIF-3α) and the oxygen-insensitive β subunit (HIF-β; including HIF-1β [aryl hydrocarbon receptor nuclear translocator, ARNT1], ARNT2, and ARNT3) (1). In oxygenized cells, the HIF-α subunit is inactivated via hydroxylation by prolyl hydroxylase domain proteins (PHDs) and factor inhibiting HIF (FIH), which allows the binding of von Hippel–Lindau (VHL) tumor suppressor protein, a component of an E3 ubiquitin ligase complex that subsequently targets hydroxylated HIF-α for proteasomal degradation. Under hypoxic conditions, oxygen becomes limited, leading to the attenuation of HIF-α hydroxylation and resulting in stabilization of HIF-α subunits. This initiates nuclear translocation and binding of the HIF-α subunit with the HIF-β subunit, and this activated HIF initiates an adaptive response to hypoxia by inducing or repressing a broad range of genes involved in regulation of vascular tone, angiogenesis, erythropoiesis, cellular metabolism, proliferation, survival, and autophagic response (1). However, nonhypoxic conditions, i.e., growth factors, hormones, or cytokines, also modulate HIF-α subunits at various levels (gene transcription, mRNA processing, protein-protein interactions, and posttranslational modifications; for a detailed description see refs. 1, 2) and regulate a plethora of signaling pathways. In the lung, HIFs orchestrate a physiological response to hypoxia and contribute to the pathogenesis of numerous disorders, including lung cancer, chronic obstructive pulmonary disease (COPD), pulmonary fibrosis (PF), and pulmonary hypertension.

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Regulation of HIF signaling pathways in PH patients

Pulmonary hypertension (PH) is a severe pulmonary vascular disorder characterized by excessive proliferation of vascular cells, increased extracellular matrix deposition, and accumulation of inflammatory cells within the pulmonary vascular wall, collectively resulting in increased pulmonary vascular resistance (3). Despite extensive research in this field, the mechanisms underlying disease development and progression are incompletely understood (4). Among many dysregulated signaling pathways, HIF signaling has been identified as one underlying mechanism determining disease progression not only in pulmonary arterial hypertension (PAH; group I PH), but also in PH due to lung diseases and/or hypoxia, including PH associated with chronic high altitude exposure (5, 6), COPD (7), and PF (8) (group III PH). Notably, augmented expression of HIF-1α has been observed in lung tissues of patients with PAH (9–13), chronic thromboembolic PH (14), and idiopathic PF-associated PH (15, 16), while HIF-2α has been associated with congenital diaphragmatic hernia–associated PH (17). In addition, neonatal patients with acute respiratory disease–associated PH also display increased circulating levels of HIF-1α (18). Moreover, HIF-1α and its target genes vascular endothelial growth factor (*VEGF*) and erythropoietin (*EPO*) are upregulated in peripheral blood cells of newborns with cyanosis and persistent PH, therefore representing early markers of generalized hypoxia (19). Similarly, increases in circulating bone marrow–derived progenitor cells observed in PAH patients are regulated by HIF-1α–driven C-X-C motif chemokine ligand 12 (*CXCL12*) expression in pulmonary artery endothelial cells (PAECs) (20). The cellular sources of increased HIF-1α expression in lung tissue of PH patients are PAECs (16, 21) and pulmonary artery smooth muscle cells (PASMCs) (22, 23). While some reports show HIF-1α upregulation in the pulmonary arteries of PAH patients (24), others provide evidence of decreased HIF-1α in PASMCs isolated from idiopathic PAH (IPAH) patients (25, 26). PAH patients also display increased

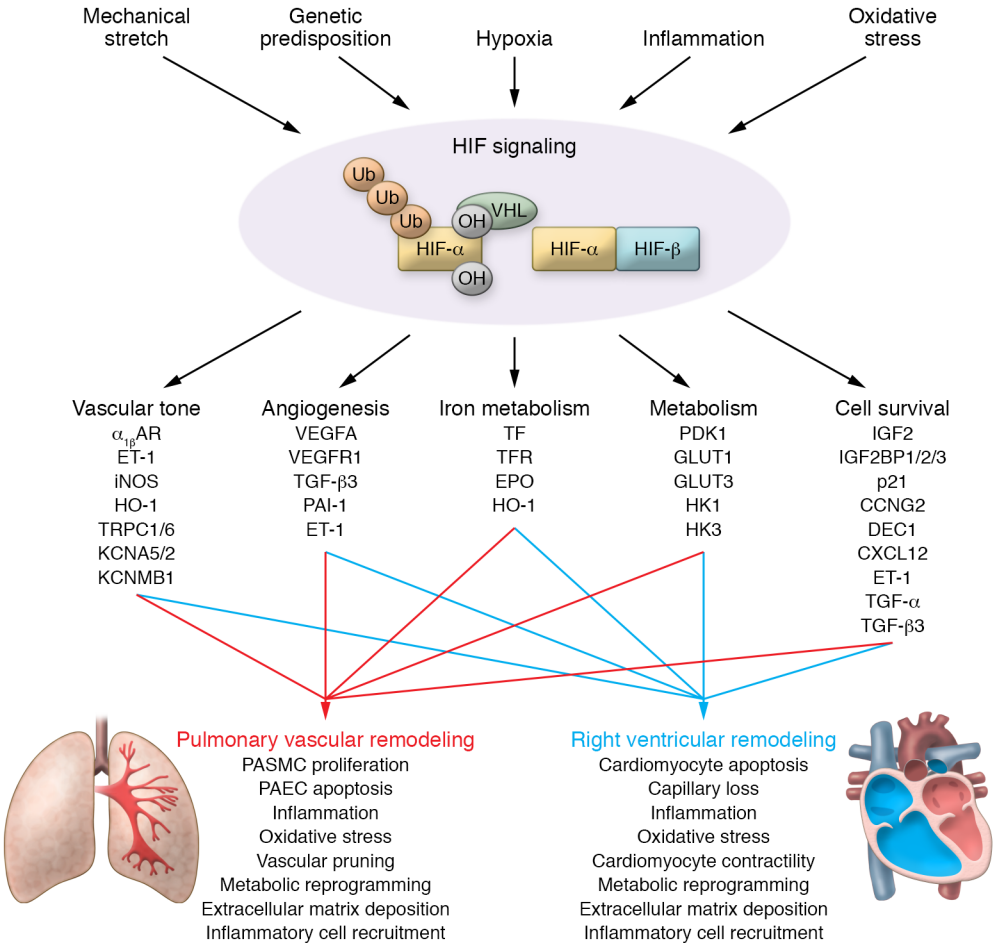

Figure 1. Emerging concepts of HIF signaling in pulmonary hypertension. Emerging evidence shows that many pro-PH factors apart from hypoxia, such as inflammation, mechanical stretch, oxidative stress, and genetic predisposition, converge on HIF signaling pathways, causing alterations in vascular tone, angiogenesis, metabolism, and cell survival that subsequently lead to pulmonary vascular and right ventricular remodeling. VHL, von Hippel–Lindau tumor suppressor; $\alpha_{1\beta}$AR, $\alpha_1\beta$-adrenergic receptor; iNOS, inducible nitric oxide synthase; HO-1, heme oxygenase-1; TRPC1, transient receptor potential canonical 1; KCNA5, potassium voltage-gated channel, shaker-related subfamily, member 5; KCNMB1, calcium-activated potassium channel subunit beta-1; PAI-1, plasminogen activator inhibitor-1; TF, transferrin; TFR, transferrin receptor; EPO, erythropoietin; PDK1, pyruvate dehydrogenase kinase 1; IGF2BP1, insulin-like growth factor 2 mRNA-binding protein 1; p21, cyclin-dependent kinase inhibitor 1; CCNG2, cyclin-G2; DEC1, deleted in esophageal cancer 1.

pulmonary expression of HIF-1β (11), and similarly, the expression of HIF-2α has been found to be increased in pulmonary arteries of patients with PAH (24, 27) and IPF-associated PH (16). The cellular sources of increased HIF-2α expression in the lung tissue of PH patients are mainly PAECs (16, 27, 28), suggesting cell type– and context-specific regulation of HIF isoforms in PH.

Upstream regulators of the HIF system in PH

Activation of HIFs in various subtypes of PH suggests that along with chronic hypoxia, other factors responsible for the initiation of PH (gene variants, vasoconstriction, endothelial dysfunction, mitochondrial abnormalities, dysregulated cell growth, and inflammation) can activate HIF signaling pathways to trigger alterations in pulmonary vascular cells, inflammatory cells, and cardiac cells that remodel lung vasculature and right ventricle (RV) (Figure 1).

Gene variants of the HIF pathway. Gene variants of HIF pathway molecules identified in high-altitude populations and in patients with Chuvash polycythemia illustrate the HIF pathway's importance in pulmonary vascular adaptation and remodeling. Chuvash polycythemia is characterized by the presence of the R200W (598C>T) missense mutation in VHL, which reduces its binding to hydroxylated HIF-α subunits and thus increases HIF-1α and HIF-2α levels (29). This leads to expression of HIF target genes including *EPO* and *VEGF* and results in development of polycythemia. In Chuvash polycythemia, apart from erythrocytosis, several VHL loss-of-function mutations, including D126N (376G>A) (30), D126N (376G>A)/S183L (548C>T) (31), and M54I (162G>C), were found to be associated with higher resting pulmonary artery pressure (PAP), severe PH, and RV dysfunction (32–35). Moreover, mutation in HIF-2α G537R, which impairs HIF-2α hydroxylation, causing familial erythrocytosis, is also associated with PH (36).

Several genome-wide selection studies have been performed on high-altitude populations, including Tibetans, Ethiopians, and Andeans (5, 6), and identified signals of positive selection for gene variants in and around the HIF pathway enabling these populations to adapt to life at high-altitude hypoxia. However, long-term high-altitude residency may lead to a sustained increase in PAP

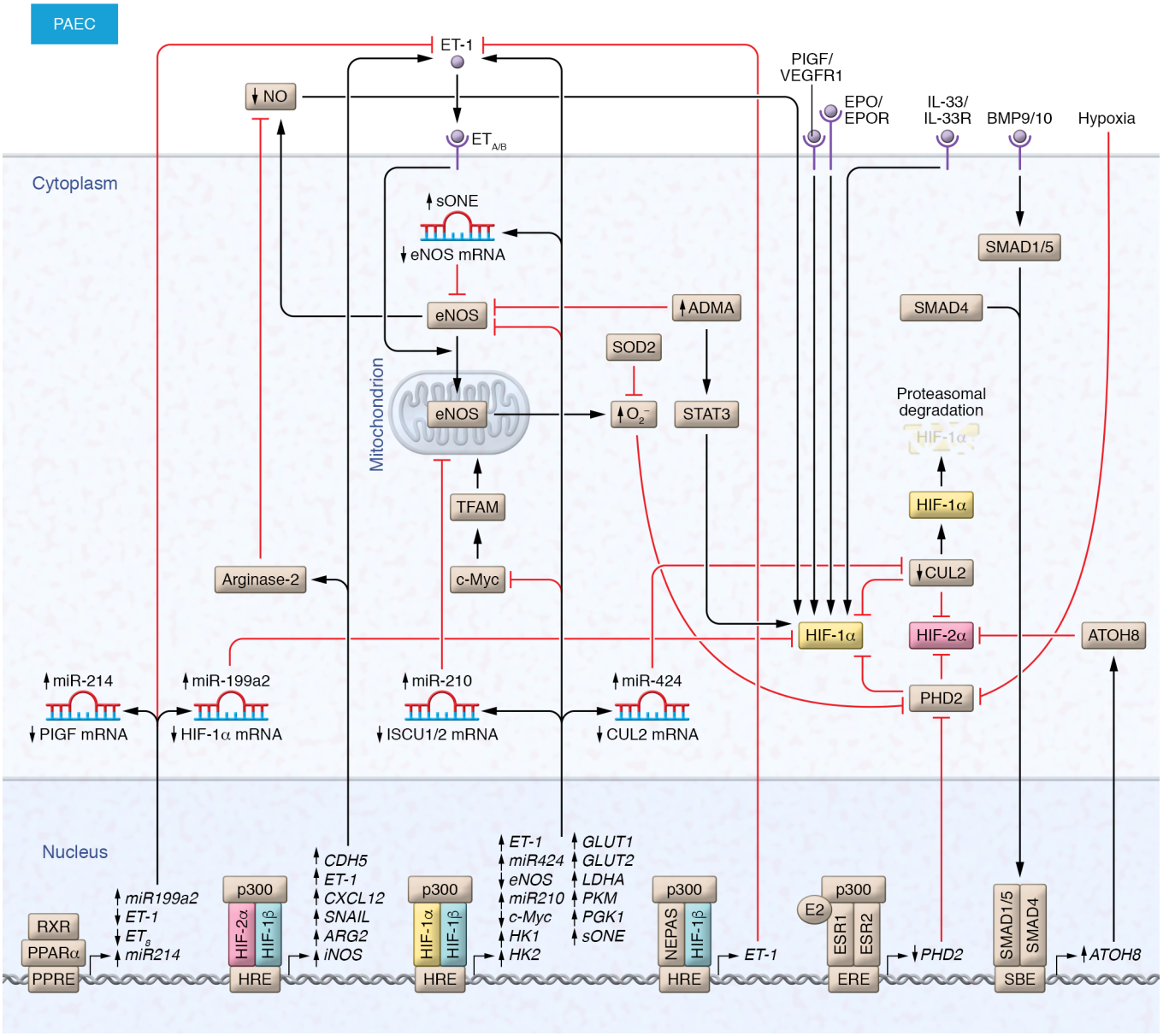

Figure 2. HIF signaling in PH: upstream and downstream modulators in pulmonary artery endothelial cells. Vasomodulatory, mitochondrial, and inflammatory growth factors and epigenetic abnormalities associated with PH regulate HIF isoform stability and transcriptional activity in pulmonary artery endothelial cells (PAECs). Subsequently, HIF isoforms transcriptionally activate a series of genes that participate in vascular tone, angiogenesis, metabolism, and cell proliferation. Long black lines with arrows indicate an activating effect; blocked red lines, an inhibiting effect; ↑, activation or upregulation; ↓, inactivation or downregulation. TFAM, mitochondrial transcription factor A; PIGF, placental growth factor; EPOR, erythropoietin receptor; $ET_{A/B}$, endothelin receptor type A and B; sONE, antisense mRNA; ADMA, asymmetric dimethylarginine; Cul2, Cullin 2; ATOH8, atonal BHLH transcription factor 8; ISCU1/2, iron-sulfur (Fe-S) cluster assembly proteins 1 and 2; PGK1, phosphoglycerate kinase 1; PKM, pyruvate kinase M.

and development of PH. Among all high-altitude populations, Tibetans have the lowest PAP (37). A candidate gene study based on the results of genome-wide analyses that identified gene variants associated with high-altitude adaptation found that *EPAS1* (HIF-2α) variants are associated with lower PAP in Tibetans (38). Furthermore, Tibetans who live at low altitudes but harbor gene variants in *EPAS1* (encoding HIF-2α) and *EGLN1* (encoding PHD2) display blunted hypoxic pulmonary vasoconstriction (39). Thus, future studies are required to delineate the role of HIF pathway gene variants in driving high-altitude PH susceptibility or resistance among indigenous high-altitude populations.

Vasomodulatory factors. Various vasomodulatory factors have been shown to regulate HIF isoform stability and transcriptional activity in PAECs (Figure 2 and Table 1). Nitric oxide (NO) maintains pulmonary vascular tone, and its downregulation is implicated in PH pathogenesis. Recent studies indicate that the NO plays a central role in hypoxia/HIF axis regulation. For example, in PAECs, hypoxia leads to post-transcriptional negative regulation of endothelial NO synthase (eNOS) expression by the *cis*–natural antisense RNA sONE (40), which results in lower levels of NO and increased HIF-1α stability. Accordingly, low levels of NO due to eNOS deletion also cause HIF-1α stabilization and migration

Table 1. Summary of studies investigating upstream regulators of HIF signaling pathway in PH

Upstream	Target	Cell type	Mechanisms	Effects implicated in cellular processes	Reference
miR-17/92	PHD2	PASMCs	miR-17/92 targets 3′-UTR of PHD2 mRNA		134
miR-322	HIF-1α	PASMCs	miR-322 promotes HIF-1α stability under hypoxia	miR-322 increases cell proliferation	79
miR-103/107	HIF-1β	PASMCs	miR-103/107 targets 3′-UTR of HIF-1β mRNA	Hypoxia induces miR-103/107 downregulation leading to HIF-1β upregulation resulting in increased cell proliferation	135
CD146	HIF1α	PASMCs	CD146 activates HIF-1α expression through activation of NF-κB	CD146 mediates PASMC proliferation and migration and enhances PASM synthetic markers (COL1A1, FN1, and VIM)	55
RASSF1A	HIF-1α	PASMCs	ROS-mediated RASSF1A stabilization protects HIF-1α from degradation and enhances its transcriptional activity	RASSF1A increases PASMC proliferation and induces HIF-1α–dependent glycolytic gene induction (PDK1, LDHA, and HK2) in PASMCs	69
Src	HIF-1α	PASMCs	Phosphorylated Src activates Akt/mTOR signal pathway leading to HIF-1α protein stabilization	Src/Akt/mTOR signaling increases PASMC proliferation and migration and decreases apoptosis	136
15-HETE	HIF-1α	PASMCs	15-HETE increases both HIF-1α expression and transcriptional activity	15-HETE inhibits PASMC apoptosis	137
KLF5	HIF-1α	PASMCs	KLF5 interacts with HIF-1α protein leading to its stability under hypoxia	KLF5 stabilizes HIF-1α protein leading to increases cell proliferation and migration and promotes cell apoptosis	138
PAK1	HIF-1α	PASMCs	PAK1 activates HIF-1α expression through NF-κB signaling	Thrombin-activated PAK1 leads to HIF-1α expression resulting in increased ROS production and cell proliferation	139
SENP-1	HIF1-α	PASMCs	SENP1 induces deSUMOylation of HIF-1α leading to its stability under hypoxia	SENP1-mediated deSUMOylation of HIF-1α leads to increased PASMC proliferation in response to hypoxia	62
Pyk2	HIF-1α	PASMCs	Hypoxia-induced activation of Pyk2 leads to ROS-mediated HIF-1α upregulation	Pyk2 promotes cell proliferation and migration under hypoxia	140
SUMO-1	HIF-1α	PASMCs	SUMO-1 directly binds to HIF-1α to induce its SUMOylation resulting in its increased stability under hypoxia		61
ADMA	HIF-1α	PAECs, PASMCs	ADMA stabilizes HIF-1α through activating STAT3 signaling pathways	ADMA decreases eNOS expression and increases cytokine expression, cellular proliferation, and calcium influx	141
S1P	HIF-1α	PAECs, PASMCs	S1P leads to HIF-1α stabilization independent of hypoxia	S1P induces HIF-1α–dependent gene expression	142
miR-199a2	HIF-1α	PAECs	miR-199a2 targets 3′-UTR of HIF-1α mRNA	miR-199a2 downregulation leads to HIF-1α–mediated ET-1 upregulation	48
ATOH8	HIF-2α	PAECs	ATOH8 directly interacts with HIF-2α to mediate its degradation	ATOH8 deficiency promotes HIF-2α transcriptional activity with its target gene induction (DLL4 and ANGPT2) in PAECs	53
CPS1-IT	HIF-1α	PAECs	CPS1-IT directly binds to HIF-1α to reduce its transcriptional activity	Reduced CPS1-IT leads to NF-κB–mediated IL-1β expression through HIF-1α activation	143
IRP1	HIF-1α	PAECs	IRP1 binds to HIF-1α mRNA IRE and prevents translation	IRP1 deletion leads to HIF-1α protein expression resulting in HIF-1α–dependent upregulation of ET-1	144
SOD2	HIF-1α	PAECs	SOD2 converts superoxide to hydrogen peroxide and diatomic oxygen in the mitochondria	Attenuated SOD2 expression in PAECs leads to HIF-1α stabilization	21
IL-33/ST2	HIF-1α	PAECs	IL-33/ST2/HIF-1α/VEGF signaling pathway promotes cell proliferation, adhesion, and angiogenesis	IL-33/ST2/HIF-1α/VEGF signaling pathway promotes cell proliferation, adhesion, and angiogenesis	47
Estradiol	HIF-1α	PAECs	Estradiol receptor recruits CREB-binding protein (CBP)/p300, thereby limiting CBP/p300 availability to HIF-1α	Estradiol attenuates hypoxia-induced ET-1 expression in PAECs	51
HIMF (FIZZ1/ RELMα)	HIF-1α	Pulmonary fibroblasts	HIMF upregulates HIF-1α through NF-κB signaling	HIMF induces IL-6 in HIF-1α–dependent manner	145

of normoxic endothelial cells (ECs) (41). Moreover, endogenous regulators of NO production such as arginase-2 and asymmetric dimethyl arginine affect cell proliferation and inflammatory gene expression by stabilizing HIF-1α in PAECs (42). Endothelin 1 (ET-1), a potent vasoconstrictor, also stabilizes HIF-1α, which in turn promotes the HIF-1α–induced glycolytic switch via eNOS-mediated reactive oxygen species (ROS) production in PAECs (43). Likewise, stimulation of normoxic PASMCs with ET-1 increases stability of HIF-1α as a result of increased Ca^{2+} and ROS and increases transcriptional activity of HIF-1α due to ERK1/2 pathway activation, which phosphorylates p300 to increase its binding to HIF-1α (44). Moreover, ET-1 promotes HIF-1α protein stabilization in normoxic PASMCs via calcineurin-dependent RACK1 dephosphorylation, which in turn inhibits PHD2 activity (45). Importantly, HIF has also been shown to regulate ET-1 synthesis. Mice with global deficiency for NEPAS, a transcript variant of HIF-3α, exhibit *ET-1* overexpression, which leads to pulmonary vascular remodeling and dilated cardiomyopathy due to excessive tissue vascularization that is evident from birth and progresses in later stages of life (46). Thus, HIF and ET-1 form a bidirectional regulatory loop that plays an important role in driving pulmonary vascular remodeling.

Inflammation, growth factors, and microRNAs. Hypoxia induces upregulation of IL-33 and its receptor ST2 in PAECs, which activates downstream HIF-1α/VEGF signaling resulting in enhanced proliferation, adhesion, and angiogenesis in an ST2-dependent fashion (47). Similarly, hormones and growth factors such as bone morphogenetic protein (BMP), placental growth factor (PlGF), platelet-derived growth factor (PDGF), EPO, estradiol, and sig-

naling molecules regulate HIF isoform transcriptional activity in PAECs. For example, in patients with sickle cell disease–associated (SCD-associated) PH, elevated levels of PlGF result in downregulation of microRNA-199a2 (miR-199a2), a negative regulator of HIF-1α. Furthermore, PPARα agonist–mediated transcription of miR-199a2 attenuates *ET-1* expression and HIF-1α level, ameliorating PH in a mouse model of SCD (48–50). Estradiol negatively regulates *ET-1* expression in PAECs by interfering with HIF activity, possibly through competition for limiting quantities of CBP/p300 (51). Estradiol also negatively regulates HIF-2α by promoting its degradation by estrogen receptor β–mediated (ERβ-mediated) PHD2 upregulation in hypoxic PAECs (52). By contrast, the BMP signaling molecules SMAD1 and SMAD5 transcriptionally activate atonal bHLH transcription factor 8 (*ATOH8*) expression, which interacts with HIF-2α to reduce its abundance and expression of its target genes delta-like protein 4 (*DLL4*) and angiopoietin-2 (*ANGPT2*) in hypoxia-exposed PAECs (53). ATOH8-KO mice spontaneously develop PH, suggesting an important role for BMP signaling in regulating the HIF pathway. Moreover, HIF-1α–driven expression of miR-322/424 in human ECs attenuates HIF-1α degradation by causing post-transcriptional repression of cullin-2, an E3 ubiquitin ligase scaffolding protein (54).

However, with regard to PASMCs, cross-regulation between the adhesion molecule CD146 and HIF-1α via the NF-κB pathway in PASMCs has been shown to trigger pulmonary vascular remodeling. Disruption of the CD146/HIF-1α axis in PASMCs blunts vascular remodeling and produces a marked attenuation of PH (55). Furthermore, exaggerated proliferation in PASMCs occurs after exposure to growth factors, such as epidermal growth factor (EGF), FGF2, PDGF, or is mediated by HIF-1α but not HIF-2α activation (56), suggesting that HIF-1α acts downstream of these growth factors, which are well established as disease-driving factors in PH. PPARγ agonists exert antiproliferative effects on PASMCs via PPARγ-mediated inhibition of HIF-1α and its downstream genes such as *PDK-1*, *TRPC1*, and *TRPC6* (57, 58). However, hypoxia induces PPARγ downregulation via HIF-1α in PASMCs (57, 58), suggesting a negative feedback loop mechanism between PPARγ and HIF-1α. Among other regulators, hypoxia-induced downregulation of miR-206 (59) and miR-150 (60) promotes a pro-proliferative and promigratory phenotype of PASMCs by targeting HIF-1α. Furthermore, in rat PASMCs, upregulation of molecules related to both SUMOylation (SUMO-1) and deSUMOylation (SENP1) leads to increased HIF-1α stability and transcriptional activity, thus increasing proliferation (61, 62).

Mitochondrial abnormalities. The interplay between mitochondrial abnormalities, NADPH oxidases (NOXs), and ROS has been established as an important activator of HIF-1α in pulmonary vascular cells in PH (63–65). For example, mitochondrial abnormalities that shift metabolism away from oxidative phosphorylation toward glycolysis (notably pyruvate dehydrogenase kinase [PDK] activation) lead to a normoxic impairment of electron flux and reduced mitochondrial ROS production (66). This pseudohypoxic signal is associated with nuclear translocation of HIF-1α. On the contrary, reports suggest that low levels of superoxide dismutase 2 (SOD2), as observed in PAECs from patients with IPAH, promote HIF-1α stabilization due to increased ROS levels (21). Mechanical stretch imposed on PASMCs due to pulmonary hemodynamic stress causes mitochondrial complex III–mediated ROS formation, which both induces the NF-κB pathway and inhibits Phd2, leading to HIF-1α activation (67), indicating that hemodynamic stress itself serves as an independent regulator of HIF-1α. Indeed, several molecules that are upstream regulators of ROS are implicated in HIF-1α activation. For instance, loss of sirtuin 3, a crucial regulator of mitochondrial function in PASMCs, causes mitochondrial dysfunction leading to ROS production and HIF-1α stabilization (68). As a consequence, sirtuin 3–KO mice develop spontaneous PH and RV hypertrophy (68). Recently, we identified a molecular mechanism in which a scaffold protein, Ras association domain family 1A (RASSF1A), acts as a crucial regulator of HIF-1α signaling in PASMCs. Upon hypoxia, HIF-1α upregulates *RASSF1A* expression, and RASSF1A is stabilized by ROS-driven and protein kinase C–mediated (PKC-mediated) phosphorylation. RASSF1A in turn stabilizes HIF-1α, leading to increased HIF-1 transcriptional activity (69). This crucial RASSF1A–HIF-1α feed-forward loop determines pro-proliferative and glycolytic switch of PASMCs and pulmonary artery adventitial fibroblasts (PAAFs) (69). Disruption of RASSF1A/HIF-1α crosstalk by genetic ablation of RASSF1A mitigates pulmonary vascular remodeling in mice exposed to chronic hypoxia.

HIFs' role in PH-associated cellular and molecular abnormalities

Under extended exposure to reduced oxygen levels in pulmonary vascular cells, HIF isoforms transcriptionally activate a series of genes (Figures 2 and 3) that regulate vascular tone, angiogenesis, metabolism, and proliferation. Moreover, recent studies highlighted the potential role of HIFs and the underlying molecular mechanisms in the dysregulation of the innate and adaptive immune system in PH.

PAECs. PAECs exhibit different phenotypes (proliferative, migratory, angiogenic, and/or endothelial-mesenchymal transition [EndoMT]) during PH pathogenesis, and HIF isoforms play a decisive role in defining these phenotypes. For example, HIF-1 induces cyclin-dependent kinase inhibitor 1B ($p27^{Kip1}$) upregulation and cyclin D1 downregulation, leading to decreased proliferation and migration of hypoxic PAECs (70). By contrast, HIF-2–driven octamer-binding transcription factor 4 (OCT4) expression via miR-130/131–mediated downregulation of PPARγ/apelin signaling results in increased proliferation of PAECs (71). In addition, both HIF-1 and HIF-2 in PAECs contribute to altered metabolic phenotype by modulating the expression of distinct mitochondrial enzymes such as pyruvate dehydrogenase kinase 1 (*PDK1*), hexokinase 1,2 (*HK1,2*), lactate dehydrogenase A (*LDHA*), and glucose transporter 1,3 (*GLUT1,3*) to regulate anaerobic glycolysis and the Warburg effect (aerobic glycolysis). The influence of HIF-1 on glycolytic metabolism is well established (72); the Warburg effect in IPAH could possibly be driven by HIF-1α stabilization, independently of the hypoxic environment. On the other hand, HIF-2, but not HIF-1, by upregulating SNAI1 transcription factors, triggers EndoMT, a mechanism potentially involved in the development of occlusive intimal/neointimal lesions and severe pulmonary vascular wall thickening in IPAH (28). Furthermore, EC HIF-2 influences the development of hypoxic PH via an arginase-1–dependent mechanism. The HIF-2/arginase-1 axis dysregu-

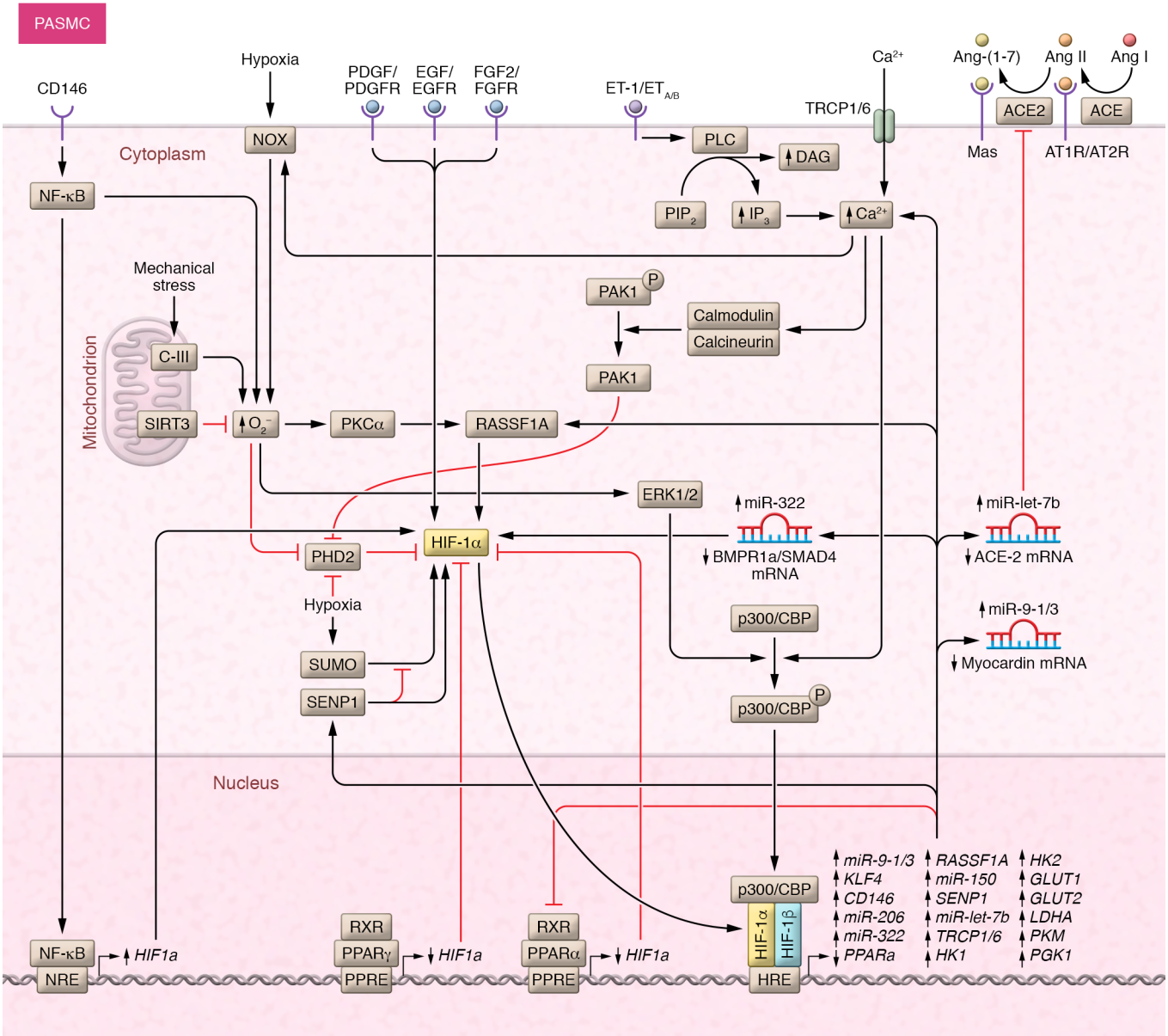

Figure 3. HIF signaling in PH: Upstream and downstream modulators in pulmonary artery smooth muscle cells. Signaling pathways associated with PH such as hypoxia, vasomodulation, growth factors, mechanical stress, and oxidative stress pathways regulate HIF isoform stability and transcriptional activity in PASMCs. This regulates genes related to cell proliferation and synthetic phenotypes, as well as genes related to Ca2+ modulation/ion channels, oxidative stress, mitochondrial fragmentation, and the renin-angiotensin-aldosterone system (RAAS) system. Long black lines with arrows indicate an activating effect; blocked red lines, an inhibiting effect; ↑, activation or upregulation; ↓, inactivation or downregulation. C-III, mitochondrial complex III; SIRT3, Sirtuin 3; TRPC6, transient receptor potential cation channel subfamily C member 1 or 6; FGFR, fibroblast growth factor receptor; Ang-I, angiotensin I; Ang-II, angiotensin II; Ang-(1-7), angiotensin (1-7); Mas, Ang-(1-7) receptor; ATR1/2, angiotensin receptor type 1 and 2; ACE, angiotensin converting enzyme; PIP2, phosphatidylinositol 4,5-bisphosphate; IP3, inositol trisphosphate; DAG, diacylglycerol; O2-, superoxide anion; PKCα, protein kinase C alpha; PAK1, P21 activated kinase 1; SENP-1, sentrin-specific protease 1.

lates vascular NO homeostasis (73, 74), resulting in PH in hypoxia-exposed mice (73). Consequently, EC arginase-1 loss attenuates PH in hypoxia-exposed mice (73), and arginase inhibition prevents PH in monocrotaline-injected (MCT-injected) rats (75). However, the expression of HIF-2–mediated angiopoietin-1 and -2 in ECs is shown to be essential to maintaining proper pulmonary vascular homeostasis (76, 77). These data propose that HIF-1 and HIF-2 exert pathogenic roles in PH by regulating distinct cellular processes in PAECs.

PASMCs. In PASMCs, HIF isoforms regulate not only genes related to cell proliferation and synthetic phenotypes but also genes related to vasoconstriction (Ca^{2+} modulation/ion channels), oxidative stress, mitochondrial fragmentation, and the renin-angiotensin-aldosterone system (Figure 3). Increased PASMC proliferation and the prosynthetic phenotypic switch observed in hypoxia are mediated by HIF-1–driven expression of miR-9-1 and miR-9-3, which negatively regulate myocardin (*MYOCD*) expression (78). Augmented proliferation of rat PASMCs is associated with inhibition of the BMP

pathway as a result of HIF-1–induced, but not HIF-2–induced, miR-322, which causes posttranslational repression of *Bmpr1a* and *Smad5* genes (79). HIF-1–dependent upregulation of miR-210 causes apoptosis resistance in PASMCs by targeting transcription factor E2F3 (80). By contrast, HIF-2 promotes hypoxia-responsive PASMC migration and contractility by upregulating thrombospondin-1 (81). Thus, multiple mechanisms contribute to the pro-proliferative, promigratory, and apoptosis resistance phenotypes of PASMCs. Furthermore, hypoxia-induced muscularization of nonmuscularized pulmonary arterioles involves preexisting smooth muscle cell (SMC) progenitor cells that undergo dedifferentiation, migration to the distal vessel, proliferation, and redifferentiation (82). Elegant studies by Sheikh et al. demonstrated that activation of these progenitor cells starts with HIF-1α–mediated PDGF-β expression (83), and progresses with expansion of these progenitor-derived SMCs via HIF-1α–mediated Krüppel-like factor 4 (*KLF4*) expression (84). These studies demonstrate a central role of HIF-1 in the initiation as well as progression of pulmonary artery muscularization in hypoxia-induced PH.

Changes in intracellular K^+ and intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) play a pivotal role in the regulation of contraction, migration, and proliferation of PASMCs (2). Notably, HIF-1 plays an essential role in modulating $[Ca^{2+}]_i$ levels in PASMCs by regulating the expression of various ion channels. HIF-1 promotes overexpression of the transient receptor potential (TRPC) channel members *TRPC1* and *TRPC6* and subsequently increases $[Ca^{2+}]_i$ in hypoxic PASMCs (85). In addition, HIF-1 via *ET-1* represses voltage-gated K^+ channels members that subsequently also increase $[Ca^{2+}]_i$ (86). On the other hand, HIF-1 activates expression of the β_1 subunit (*KCNMB1*) of the calcium-sensitive K^+ channel BKCa, which prevents an excessive rise in $[Ca^{2+}]_i$ in PASMCs (87).

PAAFs. Although both HIF-1α and HIF-2α are activated in PAAFs in response to hypoxia, HIF-2α induction appears to play the dominant role in the proliferation response, whereas both HIF-1 and HIF-2 increase PAAF migration to a similar extent (88). Furthermore, studies suggest that HIF-1 via the regulation of *ACE* and *ACE2* (a homolog of ACE that counterbalances the function of ACE) directly participates in the regulation of the renin-angiotensin-aldosterone system and consequently PAAF proliferation (89–91).

Inflammatory cells. Immune cells play an essential role in pulmonary vascular remodeling by regulating the functions of pulmonary vascular cells (92). For example, hypoxic PAAFs drive profibrotic macrophage phenotypes under the control of HIF-1, resulting in the release of various paracrine factors. Importantly, macrophage-produced VEGF and IL-6 are shown to promote pulmonary vascular remodeling (12).

The role of the HIF system in pulmonary vascular remodeling

Considering the multitude of cellular and mechanistic roles of the HIF pathway, it is not surprising that knockout mouse models of the HIF pathway (*Hif1/2α*, *VHL*, and *Phd2*) provided valuable insights on the HIF pathway in the hypoxic adaptation of the pulmonary vasculature and the development of PH (Table 2). Earlier studies revealed that the genes of the HIF pathway are crucial for embryonic development and that biallelic deletion of the majority of those genes is lethal. For example, homozygous deletion of *Phd2* in mice leads to embryonic lethality, although *Phd1/3* double knockout leads to viable and fertile mice (93). Complete deletion of *Hif1a* (94), *Hif2a* (95), or *Hif1b* (96) in mice results in embryonic lethality due to various developmental defects. In contrast, mice with global heterozygous deletion of either *Hif1a* or *Hif2a* reach adulthood and do not display phenotypes in homeostatic conditions, making them useful to study the role of HIFs in disease. For example, mice with heterozygous deletion of *Hif1a* exhibit attenuated PH and RV hypertrophy upon hypoxia exposure (97). By contrast, mice with heterozygous deletion of *Hif2a* are completely protected from hypoxia-induced PH (98). Further, to evaluate how global deletion of HIF isoforms during adult life affects hypoxia-induced PH, Hu et al. showed that global *Hif1a* deletion in mice did not prevent hypoxia-induced PH, whereas mice with global *Hif2a* deletion did not survive long-term hypoxia (99). Conversely, global partial *Hif2a* deletion diminishes development of hypoxia-induced PH at 5 weeks in adult mice (99).

Furthermore, to tease out the cell- and postnatal-specific role of HIF pathway components in PH pathogenesis, various studies have used constitutive or inducible cell-specific knockout mouse models. For example, Ball et al. reported that SMC-specific postnatal (inducible) deletion of *Hif1a* attenuated PH but did not affect RV hypertrophy (100). Meanwhile, in another study, mice with constitutive SMC-specific *Hif1a* deletion showed exacerbated hypoxia-induced PH (101). More recent studies demonstrated that mice with constitutive EC-specific *Hif1a* deletion are not protected from hypoxia-induced PH and RV hypertrophy (28, 73). However, in another study, mice with inducible EC *Hif1a* deletion were protected from PH and RV hypertrophy under hypoxia (84). Interestingly, inducible *Hif1a* deletion in either ECs and SMCs did not prevent hypoxia-induced PH and RV hypertrophy (102). In contrast, postnatal deletion of (PDGFR-β^+)/SMC marker$^+$ progenitors completely prevents PH and RV hypertrophy in hypoxia-exposed mice (84). With regard to HIF-2α, Skuli et al. reported that mice with EC-specific *Hif2a* deletion develop PH and RV dilatation (but not RV hypertrophy) due to vascular leakage into the lung parenchyma (103). Similarly, Tang et al. demonstrated that EC-specific deletion of *Hif2a*, but not *Hif1a*, prevents mice from developing PH under hypoxic conditions (28). Interestingly, simultaneous deletion of *Hif1a* and *Hif2a* in ECs also provides protection against bleomycin-induced PH and RV hypertrophy despite lung fibrosis development (16). These data suggest that the prominent role of *Hif2a* in ECs is critical in the initiation and progression of PH. However, results of EC-specific gene-deletion mouse models should be interpreted cautiously, since, depending on whether a Cre/ERT2 or Cre23 system was used, gene deletion may be exclusively EC-specific or target other cell types, respectively (104).

Notably, multiple groups have shown that mice with *Phd2* deletion in ECs spontaneously develop severe PH associated with massive pulmonary vascular lesions and adverse RV remodeling that is evident from the age of 1.5 months (10, 105, 106). Concomitant deletion of both *Phd2* and *Hif1a* or *Phd2* and *Hif2a* in ECs identified HIF-2α activation as a critical downstream modulator of PHD2 deficiency in PH development (10, 105). Interestingly, these mice show increased mortality within 6–9 months of age, presumably due to progressive RV failure (10, 105). Supporting this role of HIF-2α activity in the effect of PHD2 deficiency, mice with both heterozygous and homozygous *Hif2a* G536W gain-of-

Table 2. Summary of studies evaluating the phenotypes of genetic manipulation of HIF and HIF regulators in animal models of PH

Gene	Disease model (time)	Genotype	Tissue or cell/Gene deletion	RVP	PA remodeling	RV remodeling	Reference
Hif1a	HOX (5 wk)	Ubc^{CreERT}-HIF1-$\alpha^{fl/fl}$	Global/inducible (Tam)	↔	NA	↔	99
Hif1a	HOX (3 wk)	HIF-$1\alpha^{-/+}$	Global/constitutive	↓	↓	↓	97
Hif1a	HOX (3 wk)	EC $Alk1^{Cre}$-HIF-$1\alpha^{fl/fl}$	EC/constitutive	↔	↔	↔	73
Hif1a	HOX (3 wk)	EC $Tie2^{CreERT2}$-HIF-$1\alpha^{fl/fl}$	EC/inducible (Tam)	↔	NA	↔	28
Hif1a	HOX (3 wk)	SMC $SM22\alpha^{Cre}$-HIF-$1\alpha^{fl/fl}$	SMC/constitutive	↑	↔	NA	101
Hif1a	HOX (4 wk)	SMC $Smm^{CreERT2}$-HIF-$1\alpha^{fl/fl}$	SMC/inducible (Tam)	↓	↓	↔	100
Hif1a	HOX (3 wk)	EC $Cdh5^{CreERT2}$-HIF-$1\alpha^{fl/fl}$	EC/inducible (Tam)	↓	NA	↓	84
Hif1a	HOX (3 wk)	$Pdgfrb^{CreERT2}$-HIF-$1\alpha^{fl/fl}$	MC/inducible (Tam)	↓	NA	↓	84
Hif1a	HOX (3 wk)	$Cx3cr1^{Cre}$-Hif-$1\alpha^{fl/fl}$	Mono/constitutive	↓	↓	↓	110
Hif1a	HOX (3 wk)	$LyzM^{Cre}$-Hif-$1\alpha^{fl/fl}$	Myeloid cells	↓	↓	↓	109
Hif1a/Hif2a	Bleo (4 wk)	EC $Cdh5^{Cre}$-HIF-1α/HIF-$2\alpha^{fl/fl}$	EC/constitutive	↓	↓	↓	16
Hif1a/Hif2a	HOX (4 wk)	EC $Cdh5^{Cre}$-HIF-1α/HIF-$2\alpha^{fl/fl}$	EC/constitutive	↓	↓	↓	16
Hif2a	HOX (3 wk)	EC $Alk1^{Cre}$-HIF-$2\alpha^{fl/fl}$	EC/constitutive	↓	↓	↓	73
Hif2a	HOX (4 wk)	HIF-$2\alpha^{-/+}$	Global/constitutive	↓	↓	↓	98
Hif2a	HOX (4 wk)	HIF-$2\alpha^{-/+}$	Global/constitutive	↓	NA	↓	38
Hif2a	HOX (4 wk)	HIF-$2\alpha^{-/+}$	Global/constitutive	↓	NA	NA	146
Hif2a	HOX (4 wk)	EC $Cdh5^{Cre}$-HIF-$2\alpha^{fl/fl}$	EC/constitutive	↓	↓	↓	105
Hif2a	HOX (3 wk)	EC $Tie2^{CreERT2}$-HIF-$2\alpha^{fl/fl}$	EC/inducible (Tam)	↓	↓	↓	28
Hif2a	HOX (3 wk)	SMC SM^{Cre}-HIF-$2\alpha^{fl/fl}$	SMC/inducible (Tam)	↔	NA	↔	28
Hif2a	HOX (5 wk)	Global Ubc^{CreERT}-HIF-$2\alpha^{WT/fl}$	Global/inducible (Tam)	↔	NA	↓	99
Hif2a	HOX (4 wk)	EC $Cdh5^{Cre}$-HIF-$2\alpha^{fl/fl}$	EC/constitutive	↓	↓	↓	99
Hif2a	4–6 months old	$EPAS1^{G536W/G536}$	Global/constitutive	↑	↑	↑	107
Hif2a	3, 6, and 9 months old	EC $Cdh5^{Cre}$-HIF-$2\alpha^{fl/fl}$	EC/constitutive	↑	NA	↑	103
Egln1	15 months old	EC $Cdh5^{Cre}$-$Egln1^{loxP/loxP}$	EC/constitutive	↑	↑	↑	106
Egln1	10–12 weeks old	EC $Cdh5^{Cre}$-$Egln1^{fl/fl}$	EC/constitutive	↑	NA	↑	105
Egln1	3.5 months old	EC $Tie2^{Cre}$-$Egln1^{fl/fl}$	EC/constitutive	↑	NA	↑	10
Egln1	Spontaneous	EC $Tie2^{Cre}$-$Egln1^{fl/fl}$	EC/constitutive	↑	↑	↑	28
Egln1	HOX (4 wk)	SMC $Smmhc^{CreERT2}$-$Egnl1^{fl/fl}$	SMC/inducible (Tam)	↔	↑	↓	134
Egln1	HOX (4 wk)	SMC $Smmhc^{CreERT2}$-$Egnl1^{fl/fl}$	SMC/inducible (Tam)	↑	↔	↔	134
Egln1	HOX (3 wk)	Tibetan PHD2	Global	↔	NA	NA	146
Nepas (Hif3a)	Spontaneous	Global NEPAS/HIF-$3\alpha^{-/-}$	Global/constitutive	↓	↑	↑	46
Hif1a	rHIMF	HIF-$1\alpha^{-/+}$	Global/constitutive	↓	↓	↑	145
Hif2a	HOX (3 wk)	$Th^{CreERT2}$-HIF-$2\alpha^{fl/fl}$	CAC/constitutive	↑	↑	↔	147

↑, Significantly higher compared with WT counterparts; ↓, significantly lower compared with WT counterparts; ↔, no difference compared with WT counterparts. Bleo, bleomycin-induced; CAC, catecholaminergic cell; EC, endothelial cell; HOX, hypoxia-exposed; MC, mesenchymal cell; Mono, monocyte; PA, pulmonary artery; RVP, right ventricular systolic pressure; RV, right ventricular; SMC, smooth muscle cell; Tam, tamoxifen.

function mutations develop spontaneous PH and RV hypertrophy without RV dilatation (107). Furthermore, mice bearing homozygous knockin of a human R200W VHL mutation (as found in patients with Chuvash polycythemia) develop PH (108). Notably, development of PH in this model is attenuated in the setting of heterozygous deletion of *Hif2a*, but not of *Hif1a* (108), suggesting a prominent role of HIF-2α in PH induced by VHL loss of function.

Furthermore, in a study exploring the inflammatory- and immune-specific roles of HIF isoforms in the pathogenesis of PH, mice with EC-specific deletion of *Phd2* developed spontaneous PH due to *Hif2a* stabilization, which was partially attenuated by transplantation with WT bone marrow–derived cells, suggesting that HIF-2 activation in bone marrow–derived cells contributes to pulmonary vascular remodeling (10). In addition, mice with *Hif1a* deletion in myeloid cells are partially protected from hypoxia-induced PH and RV hypertrophy, mainly as a result of attenuated macrophage activity (109). Similarly, mice with monocyte-specific *Hif1a* deletion display attenuated PH, PA remodeling, and RV hypertrophy in response to hypoxia or hypoxia plus Sugen 5416 (SuHx) exposure (110). In addition to myeloid cells, the HIF pathway is also involved in the regulation of lymphoid cells. For example, *Hif2a* activation induced by *Phd2* deletion causes immunoregulatory dysfunction (111), which may partially explain PH development associated with reduced regulatory T cell function (112). Collectively, these studies suggest that both bone marrow–derived macrophages and thymus-derived T cells are involved in pulmonary vascular remodeling at least in part because of HIF pathway activation.

Taken together, global, inducible, and cell-specific deletion of HIF isoforms and HIF pathway molecules established cell type– and context-specific roles of HIF isoforms in early as well as late stages of PH development in adult mice.

Table 3. Summary of studies evaluating the effects of pharmacological agents targeting HIF signaling in animal models of PH

Agent	Mechanism of action	Treatment option	Disease model	Agent application details	RVP	PA remodeling	RV remodeling	Reference
R59949	PHD2 activator	Therapeutic	HOX mice	Injection (i.p.) daily 5 days a week, 0.125 mg/25 g BW	↔	↓	↔	134
2-ME2	HIF-1α inhibitor	Therapeutic	HOX rats	Implantation (s.c.), 60 μg/kg per day	↓	↓	↓	148
Topotecan	HIF-1α inhibitor	Preventive	HOX rats	Oral gavage daily, 1 mg/kg BW	↓	↓	↓	149
Topotecan	HIF-1α inhibitor	Preventive	HOX rats	Oral gavage daily, 10 mg/kg BW	↓	↓	↓	149
Digoxin	HIF-1α inhibitor	Preventive	HOX mice	Injection daily, 1.0 mg/kg BW	↓	NA	↓	150
Anti-CD146 mAb AA98	HIF-1α inhibitor	Therapeutic	HOX mice	Injection (i.p.) twice a week, 5 mg/kg BW	↓	↓	↓	55
Anti-CD146 mAb AA98	HIF-1α inhibitor	Therapeutic	MCT rats	Injection (i.p.) twice a week, 5 mg/BW	↓	↓	↓	55
CAPE	HIF-1α inhibitor	Therapeutic	MCT rats	Injection (i.p.) daily, 5 or 10 mg/kg BW	↓	↓	↓	151
Celastramycin	HIF-1α inhibitor	Preventive	HOX mice	Osmotic pump, 10 mg/kg BW	↓	↓	↓	22
Celastramycin	HIF-1α inhibitor	Preventive	MCT rats	Injection (s.c.) daily, 3 mg/kg BW	↓	↓	↓	22
Celastramycin	HIF1α inhibitor	Therapeutic	SuHx rats	Injection (s.c.) daily, 3 mg/kg BW	↓	↓	↓	22
YC-1	HIF-1α inhibitor	Preventive	HOX mice	Injection (i.p.) daily, 5 mg/kg BW	↓	↓	↓	152
YC-1	HIF-1α inhibitor	Therapeutic	HOX mice	Injection (i.p.) daily, 5 mg/kg BW	↓	↓	↓	152
HIF2α-ASO	HIF-2α inhibitor	Preventive	HOX mice	Injection (i.p.) twice a week, 50 mg/kg BW	↓	↓	↓	99
PT2567	HIF-2α inhibitor	Preventive	HOX rats	Oral gavage daily, 300 mg/kg BW	↓	↓	↓	99
C76	HIF-2α inhibitor	Preventive	EC Phd2-KO mice	Injection (i.p.) daily, 12.5 mg/kg BW	↓	↓	↓	27
C76	HIF-2α inhibitor	Therapeutic	SuHx rats	Injection (i.p.) daily, 12.5 mg/kg BW	↓	↓	↓	27
C76	HIF2α inhibitor	Therapeutic	MCT rats	Injection (i.p.) daily, 12.5 mg/kg BW	↓	↓	↓	27
Apigenin	HIF-1α inhibitor	Preventive	HOX rats	Oral gavage, daily, 50 mg/kg BW or 160 mg/kg BW	↓	↓	↓	153
Apigenin	HIF-1α inhibitor	Therapeutic	HOX rats	Oral gavage, daily, 50 mg/kg BW or 160 mg/kg BW	↓	↓	↓	153

↑, Significantly higher compared with placebo-treated counterparts; ↓, significantly lower compared with placebo-treated counterparts; ↔, no difference compared with placebo-treated counterparts. RVP, right ventricular pressure; PA, pulmonary artery; RV, right ventricle; CAPE, caffeic acid phenethyl ester; EC, endothelial cell; ET, ejection time; HOX, hypoxia-exposed; 2-ME2, 2-methoxyestradiol.

The role of the HIF system in RV remodeling

RV failure is one of the most common causes of morbidity and mortality in PAH (113). Upon PH onset, the RV undergoes remodeling to maintain its contractility, characterized by increased RV wall thickness and mass and moderate dilatation, mediated by cardiomyocyte hypertrophy and extracellular matrix deposition. However, at some point during the course of persistent pressure overload, the compensatory mechanisms of the RV expire, and the RV fails (113).

In physiological conditions, HIF-1α expression is significantly higher in the right than in the left ventricle (114). RV HIF-1α expression is increased in a number of animal models of PH, including MCT rats (115), hypoxia-exposed (HOX) rats (116), pulmonary artery–banded (PAB) rats (117), SuHx rats (117), and pulmonary embolism (PE) rats (118). Rats with PE display increased RV HIF-1α expression, and its level is positively correlated with RV hypertrophy and PAP (118). Interestingly, mice with *Hif2a* gain-of-function mutations develop RV hypertrophy but do not show signs of RV dilatation despite a substantial increase in PAP, suggesting preserved RV function (107). In patients with repaired tetralogy of Fallot, the presence of gain-of-function mutations in HIF-1α is associated with preserved RV function and better outcome due to increased TGF-β1 (*TGFB1*) expression and myocardial fibrosis (119). Collectively, these reports suggest that presumably mild to moderate activation of both HIFs is associated with preserved RV function. By contrast, strong activation of HIFs adversely affects RV function. For example, mice with global inducible deletion of *Phd2* display increased mortality due to severe polycythemia and dilated cardiomyopathy (120). Similarly, HIF-2 activation in mice with EC-specific *Phd2* deletion leads to spontaneous PH with high mortality due to severe RV failure (10). However, the roles of HIFs and PHDs have not been studied in fixed-afterload models of RV hypertrophy and failure using cell-specific gene knockout or overexpression.

Therapeutic potential of HIF targeting in PH

As we have discussed above, experiments in rodent models revealed that HIF-1/2α exerts a profound impact on pulmonary vascular remodeling. Moreover, antisense oligonucleotides to *Hif2* (but not to *Hif1*) reduced vessel muscularization, rises in PAPs, and RV hypertrophy in mice exposed to hypoxia, suggesting that inhibition of HIF-2α can provide a therapeutic approach to prevent or reverse the development of PH (99). Thus, great interest has arisen in developing therapeutics targeting this pathway. Studies have specifically targeted components of the HIF pathway such as PHD2, HIF-1α, or HIF-2α with pharmacological agents in various rodent models of PH. Most tested agents that directly or indirectly inhibit HIFs have been able to prevent or reverse experimental PH (Table 3). Agents/compounds that inhibit HIF-1α at the level of mRNA (topotecan and camptothecin), protein synthesis (2-methoxyestradiol, digoxin, celastramycin, caffeic acid phenethyl ester), protein accumulation and transcriptional activity (YC-1), and targeting of the molecules regulating the HIF

axis (anti-CD146, mAb AA98, apigenin), or that inhibit HIF-2α at the level of mRNA (C76) or at the level of heterodimerization and DNA binding (PT2567), have been evaluated. These inhibitors, given via different routes (intraperitoneal, intravenous, subcutaneous, oral), were shown to prevent as well as reverse PH in various animal models of PH (hypoxia, MCT, and SuHx). Notably, the HIF-2α inhibitor C76 showed strong anti-remodeling effects in three experimental models of PH (27), indicating that inhibition of HIF-2 may be a promising therapeutic approach for PH.

Conclusions and future directions

Data obtained from cell systems, animal models, and patient-derived materials have consistently confirmed that HIF isoforms are important components of PH pathogenesis. The hypoxic and pseudohypoxic states that occur in different groups of PH may vary in intensity and duration, thus allowing an intricate interplay between HIF-1 and HIF-2 in driving the pathological processes that underlie pulmonary vascular and RV remodeling. Animal models have helped elucidate the nonredundant and complementary roles of HIF-1 and HIF-2. For example, HIF-1 plays a major role in driving vasoconstriction, PASMC proliferation, angiogenesis, and RV contractility, whereas HIF-2 plays a major role in inflammatory cell recruitment and in EC phenotypic switch to a proinflammatory state and to EndoMT. These data suggest dynamic regulation of HIF isoforms as well as cell- and context-specific roles of HIF-1 and HIF-2 in the initiation, progression, and establishment phases of pulmonary vascular and RV remodeling. Although head-to-head comparisons of mice with cell-specific deletions of HIF-1α and HIF-2α at different time points of hypoxic exposure are still needed to determine their influence on pulmonary vasculature and RV, it is conceivable that HIF-2α may play a major role in the initiation of the disease, whereas HIF-1α may play a major role in the progression and perpetuation of the disease. However, in cancers, HIF-1α plays the dominant role in the response to acute hypoxia, whereas HIF-2α drives the response to chronic hypoxia, although both are involved in cancer progression (121). Intriguingly, this HIF switch is also observed in pulmonary vascular endothelial and smooth muscle cells upon exposure to hypoxia (122), which may allow HIF-1 and HIF-2 to play divergent and complementary roles during hypoxic and pseudohypoxic responses of pulmonary vascular and cardiac cells in PH.

These contradictory data in knockout mouse models versus in vitro cell models may be explained by the use of acute exposure to hypoxia (maximum exposure of 1–4 days) in the in vitro experiments versus the use of small-animal models (mice) for PH in the knockout studies. Thus, further studies with inducible deletion of HIF isoforms in severe animal models of PH (MCT, SuHx in mice and rats) and in vascular cells isolated from PH patients and large animals (cows), which exhibit and maintain their unique phenotypes in vitro (44), are needed to provide deeper insights into cell type– and context-specific roles of HIF isoforms in PH. Furthermore, understanding the molecular mechanisms that determine HIF-1/2 switches or activation of cell type– and context-specific HIF isoforms in PH will facilitate a better understanding of the pathophysiological roles exerted by HIF isoforms and the potential clinical implications of targeting them. For example, PHD2 has relatively more influence on HIF-1α, whereas PHD3 has relatively more influence on HIF-2α (123). In addition, molecules like sirtuin 1, hypoxia-associated factor (HAF), and heat shock proteins (HSP70, HSP90) differentially regulated the degradation and activities of different HIF isoforms in various cell types (121, 124). Furthermore, translation of HIF-2α (but not HIF-1α) is linked to iron metabolism due to an iron-responsive element in the 5′-untranslated region of HIF-2α (125, 126). However, no studies to date have explored the mechanisms regulating the HIF-1/2 switch in pulmonary vascular and cardiac cells upon exposure to hypoxia and other nonhypoxic PH stimuli. Considering the pathophysiological roles of iron metabolism, sirtuins, and heat shock proteins in PH (68, 126–128), the possibility that these mechanisms are operative in the putative HIF-1/2 switch associated with PH warrants further investigation.

Despite striking similarity in protein sequence, dimerization partners, and binding sites among HIF-1α and HIF-2α proteins, it is well documented that HIF-1 and HIF-2 activate different subsets of hypoxia-inducible genes in various pathological conditions, including PH. Recent studies suggest that despite sharing an identical consensus recognition sequence, each HIF isoform has an inherent property that determines its binding distribution across the genome. For example, HIF-1 binds closer to promoters, while HIF-2 binds distal enhancers, and their inherent distributions are unaffected by the degree or duration of hypoxia or the cell type (129). In addition, differential recruitment of other transcription factors underlies HIF-mediated cell-specific hypoxia responses. Indeed, accumulating evidence suggests that HIF-1α and HIF-2α form separate multifactorial complexes with other transcription factors, cofactors, and RNA polymerase II to mediate the distinct functions of HIF-1 or HIF-2 (130). Hence, it is important to identify these complexes and their common and unique target genes, not only to understand the distinct pathological processes mediated by HIF isoforms, but also to selectively inhibit HIF isoform functions as a therapeutic approach.

In summary, rapidly advancing research has brought to light the isoform-specific, context-specific, and cell-specific roles of the HIF pathway in regulating pulmonary vascular remodeling. This has introduced a novel therapeutic approach for the treatment of PH. Inline, HIF-2–selective inhibitors reversed PH in various animal models of PH without any significant side effects. Notably, a HIF-2–specific small-molecule inhibitor developed to treat renal cancer has demonstrated a favorable safety profile in a recent phase I trial (131), entered into phase II clinical trials, and will be considered for clinical trials among PH patients in the future. Head-to-head comparisons and multicenter preclinical studies of pan-HIF inhibitors and HIF-1– and HIF-2–selective inhibitors in various animal models of PH and RV dysfunction are warranted before moving into clinical development (132). Notably, further studies are needed to develop personalized therapeutics, i.e., to determine under what conditions and in which PH patients HIF inhibition can provide an optimal therapeutic strategy. In addition, given the myriad roles of HIFs and their possible influence on extrapulmonary manifestations in patients with PH (15, 133), it will be important to carefully assess the risk/benefit ratio of systemic versus pulmonary selective HIF inhibitors. Thus, more work needs to be done to identify novel, potent, and more specific inhibitors targeting clearly defined points in the HIF pathway, followed by

lung-selective delivery of these inhibitors, which will be the key to developing potential therapeutic strategies for PH.

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