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REVIEW

Complex role of the HIF system in cardiovascular biology

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Abstract The hypoxia-inducible factor (HIF) system was discovered as an oxygen-sensitive regulatory pathway that confers adaptive responses to hypoxia. Novel aspects of this growing network indicate that there is a significant, nonhypoxic contribution to regulation. Multiple lines of evidence suggest that components of the HIF pathway are intimately involved in the pathogenesis of cardiovascular disorders. This review highlights the functional context of the HIF system in cardiovascular pathobiology with possible therapeutic implications.

Keywords HIF · Developmental · Angiogenesis · Hypertension · Energy metabolism · Cardiomyopathy

Introduction

To date, various roles of hypoxia-inducible factors (HIF) have been recognized in human pathologies. This review focuses on cardiovascular aspects of HIFs delineating their complex contributions to cardiovascular physiology.

HIF-1 is a basic helix–loop–helix (bHLH) PAS heterodimeric transcription factor consisting of the constitutively expressed ARNT and a regulatory HIF-α subunit [1, 2]. ARNT may dimerize with HIF-1α, which regulates an overlapping but distinct set of genes with HIF-2α [3, 4] and HIF-3α [5], a competitive inhibitor of the other α-subunits.

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The HIF complex regulates a variety of genes with biological functions ranging from vessel growth, vasodilation, oxygen transport, and metal and energy metabolism to cell fate decisions [6, 7]. These target genes in turn mediate adaptive responses to hypoxia/ischemia at organism (erythropoiesis), organ (angiogenesis), and cellular levels (energy metabolism) [8].

Since most studies have examined only HIF-1α, but not HIF-2α (or HIF-3α), comments on HIF-α subunits will be directed towards HIF-1α unless otherwise indicated.

Oxygen-dependent regulation

The regulation of HIF-α subunits is primarily posttranslational (Fig. 1). In normoxia, HIF-α protein is hydroxylated on two specific proline residues in the oxygen-dependent degradation domain (ODD) by prolyl hydroxylase domain proteins (PHD1-3) [9, 10]. PHDs are O_2- and 2-oxoglutarate-dependent dioxygenases that oxidatively decarboxylate 2-oxoglutarate to form succinate and CO_2 [2]. PHDs contain Fe^{2+} in the catalytic center essential for enzymatic activity [11]. Prolyl hydroxylation provides the mechanistic explanation for O_2-dependent regulation of HIF-α stability.

OS-9 binds both PHD and HIF-1α to facilitate hydroxylation [12]. Hydroxylated HIF-α is bound by von Hippel–Lindau protein (pVHL), which recruits the Elongin C ubiquitin ligase complex, to ubiquitinate HIF-α [13]. SSAT2 stabilizes the interaction of pVHL with Elongin C to enhance ubiquitination of HIF-α [14] followed by proteosomic degradation [15]. In hypoxia, HIF-α protein, via the bHLH and PAS domains, heterodimerizes with ARNT in the nucleus. The complex binds to conserved hypoxia-responsive elements containing the core sequence RCGTG in the regulatory region of target genes [16].

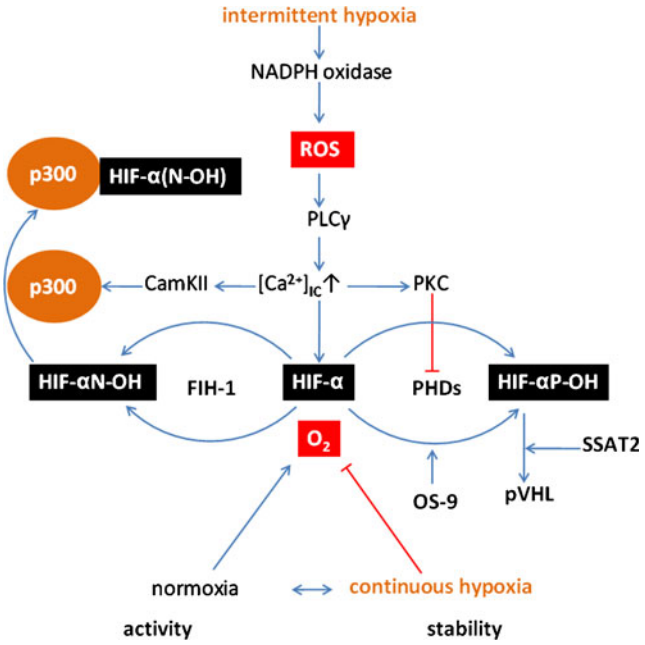

Fig. 1 Oxygen-dependent regulation of hypoxia-inducible factor (HIF)-α subunits. Continuous hypoxia deactivates prolyl hydroxylase domain proteins (PHDs) to hydroxylate HIF-α (HIF-αP-OH). OS-9 enhances the bond between PHD and HIF-1α. HIF-αP-OH is ubiquitinated by the von Hippel–Lindau protein (pVHL). SSAT2 pVHL-dependently enhances HIF-α ubiquitination. In normoxia, HIF-α transactivation domains are hydroxylated (HIF-αN-OH) by FIH-1, which prevents HIF-α from binding to co-activators p300/CBP. Intermittent hypoxia operates via NADPH oxidase-generated reactive oxygen species (ROS), phospholipase PLCγ, and increase of intracellular calcium. Elevated $[Ca^{2+}]_{IC}$ activates protein kinase C (PKC), which inhibits PHD activity. Increased $[Ca^{2+}]_{IC}$ induces Ca^{2+}/calmodulin-dependent protein kinase II (CamKII) that facilitates p300-HIF-α interaction

HIF-α proteins have C- and N-terminal transactivation domains (C-TAD and N-TAD; Fig. 1) [17, 18]. Hydroxylation of a specific asparagine residue by the O_2- and 2-oxoglutarate-dependent dioxygenase factor inhibiting HIF-1 (FIH-1) prevents HIF-α C-TAD from binding to co-activators p300/CBP [19, 20]. Having a lower K_M for O_2 than PHDs, FIH-1 remains active at a lower pO_2, where PHDs are inactive. FIH-1 preferentially hydroxylates HIF-1α [21].

The specificity of HIF-α isoforms is not solely via selective DNA-binding at the target gene locus [22] but is also dependent on N-TAD [23].

Continuous hypoxia regulating HIF-α occurs physiologically at high altitude or pathophysiologically in tissue ischemia (Fig. 1) [8].

Intermittent hypoxia takes place physiologically while swimming or pathophysiologically in obstructive sleep apnea [8]. HIF-α regulation by intermittent hypoxia involves NADPH oxidase-generated reactive oxygen species (ROS), phospholipase Cγ, and elevation of $[Ca^{2+}]_{IC}$ [8], followed by oxygen-independent events (Fig. 1). Regulation of HIF-α proteins during intermittent hypoxia includes isoform-specific events [24].

Oxygen-independent regulation

Nonhypoxic extracellular growth signal cues prepare cells about to grow for an increased need for O_2. This "a priori" alertness serves to prepare them for a period of higher self-maintenance. Thus, activation of G-protein-coupled receptor and receptor tyrosine kinase with ensuing activation of the PI3K and MAPK pathways increases HIF-α protein synthesis [8] (Fig. 2).

Iron chelators such as desferroxamine stabilize HIF-α in a PHD-dependent manner. The redox aspect of HIF-α regulation [25] (Fig. 2) is reflected by the observation that oxidation of Fe^{2+} to Fe^{3+} by ROS [26] underlies PHD inactivation and HIF-1α accumulation in disease states

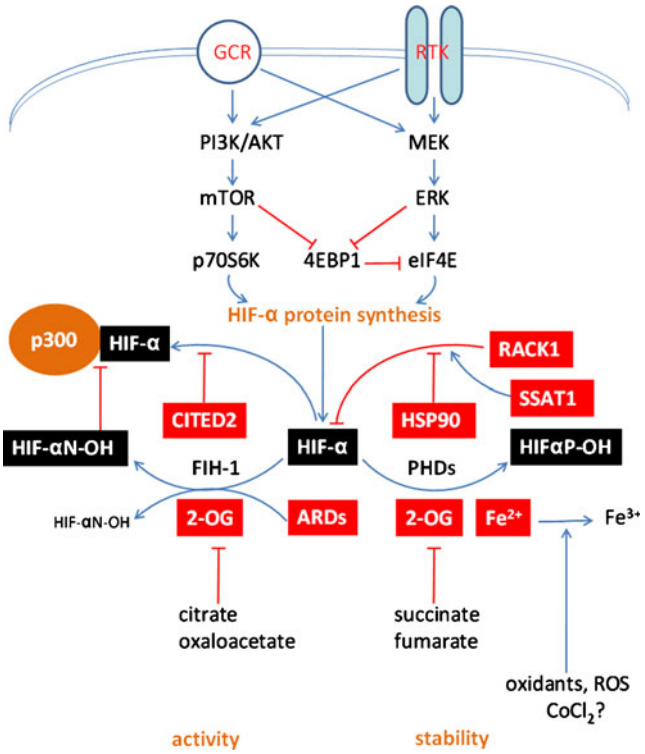

Fig. 2 Oxygen-independent regulation of hypoxia-inducible factor (HIF)-α subunits. G-coupled receptor (GCR) or receptor tyrosine kinase (RTK) activation starts a cascade of phosphorylation events leading to HIF-α protein synthesis. PHD activity is blocked when Fe^{2+} in the catalytic center is oxidized. 2-oxoglutarate (2-OG) may be competed out with other Krebs cycle metabolites inhibiting PHDs and FIH-1. RACK1 binds to HIF-α and facilitates ubiquitination. Heat shock protein 90 (HSP90) competes with RACK1 for binding to HIF-α; calcineurin dephosphorylates RACK1, preventing its functional dimerization. SSAT1 stabilizes the interaction of HIF-α with RACK1. CITED2 inhibits the interaction between HIF-α and co-activators p300/CBP. Ankyrin repeat domain-containing proteins (ARDs) compete with HIF-α for asparaginyl hydroxylation by FIH-1

[27–29]. Redox mechanisms such as ascorbate (whose cellular uptake is blocked by $CoCl_2$), glutathione, and cysteine play an important role in regulating basal HIF-1α turnover rate [27–29].

The PHDs have been found to be inhibited by various Krebs cycle metabolites (Fig. 2). Among them, succinate and fumarate inhibit all PHD isoforms [30, 31]. This inhibition is competitive with regard to 2-oxoglutarate and reversible with excess 2-oxoglutarate [32]. Mutations in succinate dehydrogenase or fumarate hydratase with ensuing succinate [33] or fumarate [34] accumulation, respectively, lead to normoxic HIF-1α stabilization. The observation that not all studies found fumarate and succinate to inhibit PHD activity [35] suggests a tissue-specific phenomenon [36]. The responsiveness of PHDs to relative changes in intermediary metabolites is an exciting new territory for HIF research.

HIF-α stability is also regulated in a PHD- and pVHL-independent manner (Fig. 2). RACK1 binds to HIF-1α, upon which RACK1 interacts with Elongin C recruiting an E3 ubiquitin–protein ligase complex that facilitates HIF-1α ubiquitination [37]. Heat shock protein 90 (HSP90) stabilizes HIF-1α, and HSP90 inhibitors induce proteosomal degradation of HIF-1α even in cells lacking pVHL [38]. RACK1 competes with HSP90 for binding to the PAS-A subdomain of HIF-1α [37]. Cyclosporine A inhibits hypoxia-induced HIF-1α stabilization [39] and abrogates calcineurin-induced dephosphorylation of RACK1, preventing its functional dimerization necessary for HIF-1α degradation [40]. SSAT1 stabilizes the interaction of HIF-1α with RACK1 [41].

Transcriptional activity of HIF-α is also regulated in an O_2-independent fashion. Ca^{2+}/calmodulin kinase II activation enhances HIF-1α transcriptional activity via increased binding to coactivator p300, independently of asparaginyl hydroxylation [42] (Fig. 1).

Metabolic regulation of FIH-1 works analogously to that of the PHDs although with different Krebs cycle substrate preference [30, 31].

Recruitment of p300/CBP by the HIF-1 complex requires an interaction between the p300/CBP cysteine-histidine-rich (CH1) region and HIF-1α C-TAD. CITED2 (cAMP-responsive element-binding protein (CBP)/p300-interacting transactivators with glutamic acid (E) and aspartic acid (D)-rich tail) inhibits this interaction [43] (Fig. 2).

A class of ankyrin repeat domain (ARD)-containing proteins from the IκB and Notch receptor families is hydroxylated by FIH-1 [44, 45]. Some ARDs show higher affinity to FIH-1 than HIF-α does, thereby efficiently competing for FIH-1-mediated hydroxylation [45] (Fig. 2).

Whether these regulatory mechanisms can be targeted pharmaceutically for therapeutic benefit remains a challenge for future studies.

PHD isoforms

The extent, time course, cellular localization, and regulation of expression of PHD isoforms vary in a cell-type specific manner [11], as does the affinity of PHDs to HIF-α isoforms [46].

PHD1 is expressed in the nucleus and is induced by estrogen [11]. PHD1 equally hydroxylates both ODDs [46]. It is highly expressed in testis, with low levels of expression in the heart [11].

PHD2 expression is induced by hypoxia, desferroxamine, $CoCl_2$, and all known PHD inhibitors [47, 48]. Basal expression levels are high in the heart [49]. PHD2 is predominantly localized to the cytoplasm [48]. It hydroxylates both ODDs, preferring HIF-1α over HIF-2α. Blockade of PHD2, but not of PHD1 or PHD3, is sufficient to stabilize HIF-1α in normoxia [50].

PHD3 is upregulated by hypoxia and its mimics [46, 51]. It distributes evenly in the cytoplasm and nucleus [48]. PHD3 retains significant activity in hypoxia [51], with a preference for HIF-2α and C-terminal ODD [46].

Whether the PHDs have targets other than HIF-α is a current area of intense research activity. A recently discovered PHD3-β2 adrenergic receptor (β2-AR) interaction allows for O_2-regulated adrenoceptor abundance with potential cardiac therapeutic implications [52].

Development

Intrauterine development occurs in a hypoxic microenvironment that acts as an essential developmental and differentiation stimulus [53]. The intact HIF system adapts cells to hypoxia. As a corollary, organs participating in nutrient/O_2 delivery or with high-energy dependence, such as cardiovascular, pulmonary, and nervous systems, are often affected when the pathway is dysregulated. A variety of genetic mouse models has been created to explore the roles of the HIF system in development.

Germ line deletion of HIF-1α is embryonic lethal (Table 1) with downregulation of genes implicated in angiogenesis, glucose metabolism, and cellular proliferation [54] (Table 1). Deficient HIF-1α abrogates vascularization and causes numerous extracardiac and cardiac malformations [54, 55], the latter featuring downregulation of core cardiac transcription factors [56]. Overexpression of HIF-2α is unable to rescue HIF-1α-deficient cells from hypoxia-induced cell death [3], which suggests non-redundancy of the two α-subunits in development. Mice with MLC2v-driven cardiac-restricted HIF-1α deletion are viable with slight reduction in cardiac vascularization, contractility, and high-energy phosphate content [57]. Thus, while HIF-1α is vital in early cardiac development, a lack of cardiac HIF-1α expression is compatible with postnatal survival.

Table 1 Developmental involvement of hypoxia-inducible factor pathway components

Genotype	Phenotype	Reference
HIF-1α −/−	Downregulation of hypoxia-inducible genes, lacking cephalic vascularization, abnormal neural development, numerical reduction of somites, cardiac malformations, embryonic lethal	[54–56]
MLC2v HIF-1α −/−	Reduction in cardiac vascularization, contractility, and high-energy phosphate content	[57]
HIF-2α −/−	Defective fetal catecholamine production	[58]
	Respiratory distress syndrome	[59]
	Vascular developmental disorder with hemorrhage, embryonic lethal	[60]
	Multiple organ abnormality including retinopathy, hepatic steatosis, cardiac hypertrophy, skeletal myopathy, hypocellular bone marrow, azoospermia, and metabolic dysfunction	[61]
NEPAS −/−	Impaired lung remodelling and right ventricular enlargement	[62]
PHD2 −/−	Placentation and cardiac malformations, embryonic lethal	[64]
PHD3 −/−	Abnormal sympathoadrenal development, systemic hypotension	[65]
CITED2 −/−	Cardiac and neural tube malformations, embryonic lethal	[66]
MLC2v pVHL −/−	Cardiac lipid accumulation, cardiac tumors, and heart failure	[68]

Germ line disruption of HIF-2α may cause distinct phenotypes depending on the mouse strain used: defective fetal catecholamine production [58], respiratory distress syndrome [59], a lethal vascular developmental disorder [60], or multiple organ abnormalities including myocardial hypertrophy [61]. The presence of multiple phenotypes makes dissection of the precise developmental role of HIF-2α elusive.

NEPAS (neonatal and embryonic PAS, an alternatively spliced HIF-3α form) suppresses reporter expression driven by HIF-1α and HIF-2α in vitro. Disruption of NEPAS in mice leads to impaired lung remodelling, right ventricular enlargement [62], and pulmonary endothelial cell endothelin-1 overexpression, potentially contributing to pulmonary hypertension [63].

Germ line deletion of PHD2 results in embryonic lethality with placental and cardiac malformations [64]. Lack of PHD3 causes abnormal sympathoadrenal development and systemic hypotension, where a role for HIF-2α is suggested [65]. An intriguing possibility raised by this model is that in the absence of PHD3, increased abundance of the β2-AR occurs, altering the β1-AR/β2-AR ratio, which may in turn contribute to hypotension in an HIF-independent manner [52].

Disruption of CITED2 leads to embryonic lethality with cardiac and neural tube malformations [43]. CITED2 knockout mice are partially rescued by HIF-1α heterozygosity [66], suggesting that unrepressed HIF-1α activity is an important contributor to the phenotype. Mutations in the CITED2 gene have been identified in patients with congenital heart defects [67].

Cardiac deletion of pVHL causes cardiac tumors, lipid accumulation, and heart failure in adulthood [68]. Concomitant deletion of HIF-1α prevents this phenotype [68], suggesting that chronic activation of HIF-1α is deleterious to the heart. A contribution of HIF-1α-independent mechanisms is possible.

A variety of different gene knockout models confirms that a delicate balance of HIF-α isoforms and their regulators is important for normal cardiovascular development (Table 1). Generally, the more severe phenotypes observed with deletions of components in the PHD2-HIF-1α rather than in PHD3-HIF-2α axis underscores the former's significance in hypoxic life. Finally, beside tissue-specific expression and non-redundancy of individual components, potential non-HIF targets may contribute to the observed phenotypes.

Ischemia-related disorders

Cardiac ischemia (Fig. 3a) arises from partial/complete interruption of coronary blood flow resulting in angina or myocardial infarction. HIF-1α loss-of-function studies suggest an increased susceptibility for ischemic injury [69, 70]. HIF-1α is expressed in human hearts with acute ischemia or infarction [71]. Promising animal [72] and human [73] gene therapy studies have been conducted in ischemic lower limb. Externally delivered HIF-1α improves myocardial perfusion and left ventricular function in infarct models [74]. Cardiac HIF-1α overexpression limits myocardial infarct size and promotes postischemic function and capillarization [75]. Small-molecule PHD inhibitors enhance protection against ischemia [76], while shRNA-targeting PHDs promote therapeutic revascularization [77] (Fig. 3a). These findings would suggest that activation of HIF-1α is beneficial in ischemic syndromes.

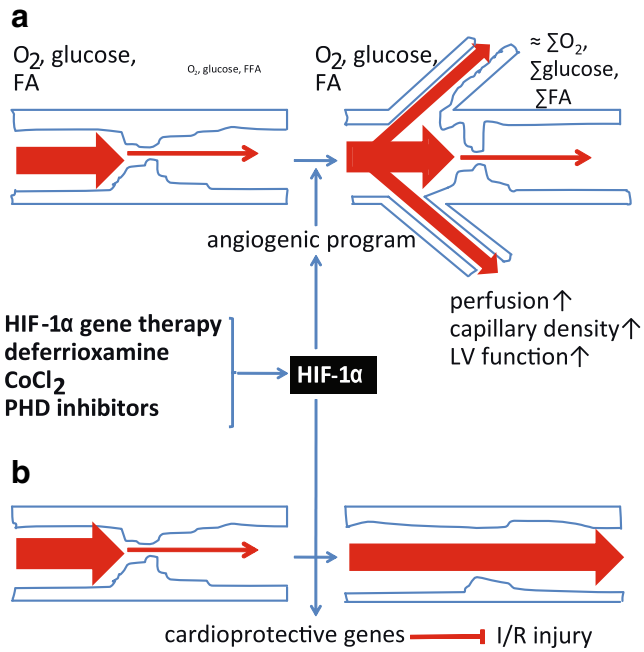

Fig. 3 Involvement of hypoxia-inducible factor (HIF)-1α in tissue ischemia, therapeutic revascularization (**a**), and cardioprotection against ischemia-reperfusion injury (**b**). **a** Arterial obliteration leads to reduced levels of O_2, glucose, and fatty acids (FA) vital for tissue maintenance. HIF-1α combats ischemia by transcriptionally activating angio- and arteriogenesis. **b** Reperfused ischemic tissue suffers from ischemia-reperfusion (I/R) injury diminished by HIF-1α-induced cardioprotective genes

Ischemic myocardium dies unless reperfused; however, reperfusion post-ischemia causes ischemia-reperfusion injury [78] (Fig. 3b). In seeking to mitigate this, investigations identified ischemic preconditioning (IPC) as an endogenous cardioprotective mechanism, whereby alternating ischemia and reperfusion episodes protect against subsequent lethal ischemia [79]. Intermittent hypoxia, mimicking IPC, fails to protect murine hearts heterozygous for HIF-1α [80].

$CoCl_2$ [81] and desferroxamine [82] both HIF-α stabilizers, confer cardioprotection or mitigate reperfusion-induced endothelial dysfunction [83], as do PHD inhibitors [84]. Recent findings suggest that HIF-1α [85, 86] and HIF-2α [87] are both central to IPC (Fig. 3b), suggesting that there are emerging ways to mimic the salutary effects of IPC.

Skeletal muscle gene therapy with HIF-1α protects murine hearts, with associated higher $[bilirubin]_{serum}$ [88]. This protection is mimicked with remote HMOX-1 treatment, an enzyme producing carbon monoxide and ultimately bilirubin, both of which reduce H_2O_2-induced cell death in HL-1 cardiomyocytes [88]. Media from HIF-1α-transfected cells or serum from HIF-1α-pretreated mice applied on naïve HL-1 cells reduces H_2O_2-induced cell death [89]. The observation that cardioprotective factors are secreted from the site of treatment to the site of effect may be exploited therapeutically.

Pulmonary hypertension

Pulmonary arterioles constrict in response to hypoxia thereby shunting away blood from less ventilated areas towards functional alveoli. In extensive lung disease, the ensuing increased tone of pulmonary arterioles causes pulmonary hypertension. Mice heterozygous for either HIF-1α [90] or HIF-2α [91] are immune to development of hypoxia-induced pulmonary hypertension (Fig. 4a). In pulmonary arterial smooth muscle cells (PASMCs) subjected to chronic hypoxia, HIF-1α-dependent upregulation of the sodium–hydrogen exchanger NHE1 [92], transient receptor potential calcium channels TRPC1 and TRPC6

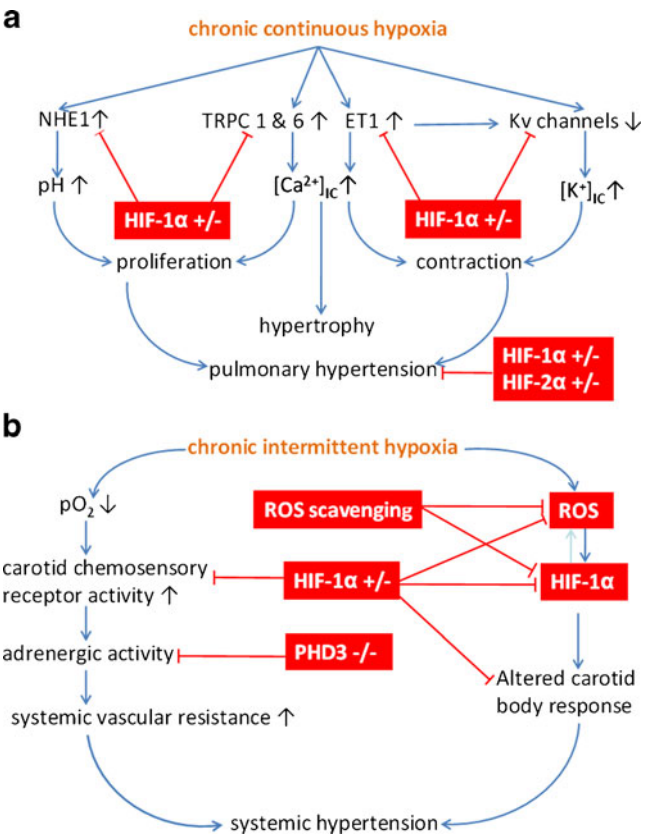

Fig. 4 Contribution of the hypoxia-inducible factor (HIF) pathway to pulmonary (**a**) and systemic hypertension (**b**). **a** Chronic continuous hypoxia activates HIF-1α that upregulates NHE1, TRPC1, TRPC6, and endothelin-1 (ET-1) and downregulates K_v channels. Increased pH_{IC} and $[Ca^{2+}]_{IC}$ in pulmonary arterial smooth muscle cells (PASMCs) promote cell proliferation, elevated $[K^+]_{IC}$ along with $[Ca^{2+}]_{IC}$ increase vascular tone, while elevated $[Ca^{2+}]_{IC}$ alone contributes to PASMC hypertrophy. **b** Chronic intermittent hypoxia evokes carotid chemosensory receptor activity. Adrenergic activity leads to increased systemic vascular resistance (SVR) and systemic hypertension. Intermittent hypoxia generates reactive oxygen species (ROS) that induces HIF-1α. HIF-1α aids generation of more ROS forming a positive feedback loop. Changes in gene expression profile alter the carotid body reflex, ultimately leading to systemic hypertension. Adapted from Semenza with permission [8]

with increased $[Ca^{2+}]_{IC}$ [93], and downregulation of voltage-gated potassium (K_v) channels [63] occur. Increased pH_{IC} and $[Ca^{2+}]_{IC}$ in PASMCs promote cell proliferation. PASMC hypertrophy, constriction, and proliferation all lead to pulmonary hypertension (Fig. 4a).

Patients with Chuvash congenital polycythemia, carrying a missense mutation in pVHL impairing binding to hydroxylated HIF-1α, have increased pulmonary vascular tone, basal ventilation, and enhanced pulmonary vasoconstrictive and cardiorespiratory responses to acute hypoxia [94].

Systemic hypertension

Obstructive sleep apnea may contribute to 30% of essential hypertension cases [95]. Airway occlusion and cessation of respiration (Fig. 4b) induces chronic intermittent hypoxia (CIH). CIH is sensed by chemosensory receptors of the carotid body that activate the sympathetic nervous system. Enhanced adrenergic activity is responsible for increased systemic vessel tone [95, 96]. Correspondingly, exposure to CIH results in increased plasma noradrenalin and elevated systolic and diastolic blood pressures in wild-type mice, but not in HIF-1α heterozygous littermates [97]. CIH-induced ROS production [96], HIF-1α expression [97], and hypertension are prevented by ROS scavenging (Fig. 4b). In HIF-1α heterozygous mice, such ROS production is lost [97] (Fig. 4b). ROS and HIF-1α thus collaborate in a feed-forward mechanism to contribute to the pathogenesis of hypertension (Fig. 4b). Disruption of this vicious circle may hold therapeutic promise.

Pathological cardiac hypertrophy

Hypertrophied hearts demonstrate increased reliance on glucose as a fuel (Fig. 5a), reverting to the fetal metabolic profile [98]. HIF-1α promotes cardiac hypertrophy by reprogramming myocardial metabolism [99] (Fig. 5b). HIF-1α facilitates glycolytic flux, lipid anabolism, glucose-to-lipid conversion, apoptosis, and contractile dysfunction. Hypertrophic stimuli induce HIF-1α-dependent myocardial lipid accumulation and contractile dysfunction [99].

Altered mitochondrial biology contributes to metabolic reprogramming (Fig. 5b). Besides activation of glucose transporters and glycolytic enzymes (Fig. 5b), HIF-1α induces pyruvate dehydrogenase kinase 1 (PDK1) [100] and PDK3 [101], thereby inhibiting the entry of carbohydrate-derived pyruvate to the Krebs cycle (Fig. 5b). In hypoxia, HIF-1-mediated COX subunit switch optimizes the electron-transfer efficiency to O_2 [102]. Cardiomyocytes transfected with HIF-1α exhibit reduced

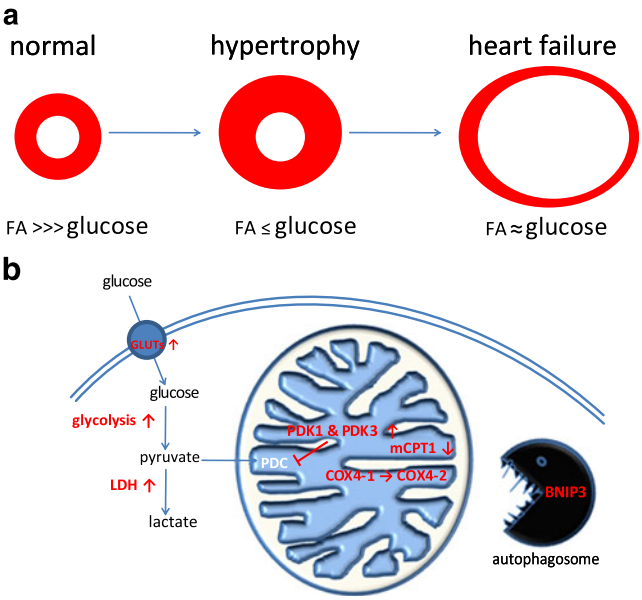

Fig. 5 Metabolic changes occurring during development of hypertrophy and heart failure. **a** Despite being a metabolic omnivore, the healthy heart prefers fatty acids (FA) as fuel. In compensated hypertrophy, FA utilization diminishes with reciprocal increases in glucose use as an energy source. In heart failure, both FA and glucose utilization are decreased leading to energy starvation. **b** Hypoxia-inducible factor-1α induces glucose transporters GLUT1 and -3, glycolytic enzymes, lactate dehydrogenase (LDH), pyruvate dehydrogenase kinase 1 (PDK1) and 3 (PDK3; that inhibit pyruvate entry to the Krebs cycle), while downregulating muscle carnitine palmitoyltransferase I (mCPT1) essential for mitochondrial free FA uptake, induces an isoform shift of COX4-1 to COX4-2, and upregulates BNIP3 that elicits mitochondrial autophagy

muscle carnitine palmitoyltransferase I expression (Fig. 5b), mitochondrial fatty acid oxidation, and DNA-binding activity of PPARα/RXR [103]. HIF-1 inhibits mitochondrial biogenesis and cellular respiration in VHL-deficient renal cell carcinoma [104]. Pressure overload elicits a robust autophagic response in cardiomyocytes [105]. Mitochondrial autophagy occurs via the expression of HIF-1-responsive BNIP3 in hypoxia [106] and in cardiac pVHL knockout mice [68] (Fig. 5b). To what extent individual mechanisms occur in pathological cardiac hypertrophy, particularly hypoxia independently, remains to be elucidated.

Cardiomyopathy and heart failure

Pressure overload initially promotes vascular growth through HIF-1α activation [107]. p53, an HIF-1α inhibitor, accumulates during hypertrophy. Blocking p53 restores cardiac function and inhibits the hypertrophy-to-heart failure transition [107].

Somatic deletion of PHD2 causes polycythemia, congestive heart disease, and premature death [108]. Additional PHD3 loss creates an exacerbated phenocopy resembling more that of cardiac pVHL deletion [68, 109].

The failing heart has been termed "an engine out of fuel." The reprogrammed metabolism of failing hearts leads to a progressive loss of phosphocreatine/ATP [110] (Fig. 5a). Fatty acid oxidation is further decreased, so is glucose utilization (Fig. 5a).

Myocardial cobalt toxicity, also termed beer cardiomyopathy, consists of reversible pericardial effusion, low cardiac output and, frequently, polycythemia [111]. Histology and electromicroscopy reveal mitochondrial damage with lipid deposition and reduced ability to oxidize octanoate or pyruvate [112]. The striking symptomatic resemblance between hearts genetically overexpressing HIF-1α and beer cardiomyopathy, along with the fact that cobalt is a HIF-α stabilizer, makes it conceivable that the HIF pathway is chronically activated, which metabolically reprograms the heart [99].

HIF-α activation: adaptive or maladaptive?

Activation of the HIF system represents adaptation in ischemic disorders; however, it also occurs in disorders where the primary stimulus is typically not hypoxic in nature.

Chronic activation of HIF-1α is enough to drive myocardial metabolic reprogramming. Indeed, HIF-1α is expressed in pathological hypertrophy [113]. What induces HIF-1α in cardiac hypertrophy remains unknown, but catecholamines [113, 114] and increased $[Ca^{2+}]_{IC}$ [42, 115] are plausible candidates. Independently of the nature of activation, HIF-1α may be part of a complex machinery driving healthy myocardium to a state of pathological hypertrophy.

In summary, the HIF system can take on both adaptive and maladaptive roles in the cardiovascular system depending on the context.

Future perspectives

Involvement of the HIF system in the pathogenesis of a range of cardiovascular pathologies makes its elements emerging therapeutic targets.

Beyond existing PHD modulators, development of PHD isoform-specific agents may have additional advantages. The identification of non-HIF PHD targets will raise the question of what action such drugs have on HIFs and on non-HIF targets.

As with all candidate "molecular" therapy, the specificity of an agent for the target tissue, undesired sequelae arising from "off-target" effects, including those on non-cardiovascular tissue, and duration of effect (especially if unduly prolonged) are vital considerations in determining overall efficacy. With regard to HIF system modulators, promotion of cancerogenesis is a particular concern.

HIF-α transcription factors operate through their target genes. Some target genes have cell-autonomous effects (i.e., metabolic enzymes), while others exert paracrine/endocrine influence (i.e., VEGF and EPO). Thus, simply controlling HIF-α stability does not guarantee control of the tissue distribution of translated target gene subpopulations.

If these problems were to be circumvented, we may anticipate a new generation of therapeutic interventions, based on highly selective manipulation of the HIF pathway for the treatment of cardiovascular disease.

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