Provisional chapter

The Signaling Nature of Cellular Metabolism: The Hypoxia Signaling

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Additional information is available at the end of the chapter

Abstract

Identification of the hypoxia-inducible factors (HIFs) as core players of the transcriptional response to hypoxia transformed our understanding of the mechanism underpinning the hypoxic response at the molecular level and led to discoveries on the role of metabolism in cell signaling alike. It has now become clear that HIFs act in the heart of a pathway where oxygen may be considered as a signaling entity recognized by molecular sensors conveying the oxygen signal to the transcriptional regulator HIFs as distal effectors. The pathway is under multiple levels of regulatory control shaping the cellular response to hypoxia and gives hypoxia signaling an intricate and dynamic activity profile. These include regulatory mechanisms within the HIF pathway as well as diverse interplay with other metabolic and signaling pathways of critical cellular functions. The emerging model reflects a multi-level regulatory network that apparently affects all aspects of cell physiology.

Keywords: hypoxia, hypoxia-inducible factors, prolyl hydroxylases

1. Introduction

The development of molecular machineries capable of utilizing atmospheric oxygen for bioenergetic purposes was a key event in the evolution of life on Earth. This, along with other processes like compartmentalization, allowed eukaryotic organisms to substantially enhance metabolic efficiency. The accompanying development of a range of biochemical processes provided the bioenergetic capacity to permit the evolution of more complex life forms of metazoans [1]. In parallel, the high degree of dependence of a constant oxygen supply to maintain metabolic homeostasis provoked the evolution of counter measures termed the hypoxia pathway [2].

2. The hypoxia pathway

The critical dependence on oxygen for metabolic homeostasis and survival led to the early evolution of a molecular mechanism that enabled cells, tissues, and organisms to adapt to hypoxia. This adaptive response is primarily orchestrated by a family of transcription factors termed hypoxia-inducible factors (HIFs) [3]. In mammals, three members of the helix-loop-helix-type HIF family have been identified to date (HIF-1, HIF-2, and HIF-3) of which the prototype is HIF-1 (**Figure 1**). The active HIF-1 is composed of discrete alpha and beta subunits (HIF-1α and HIF-1β, respectively) both of which are ubiquitously expressed in human tissues, whereas HIF-2α and HIF-3α are selectively expressed in certain cell types [4, 5]. Unlike HIF-β that is stably expressed in the cells, HIF-α subunits are continuously degraded by the 26S proteasome under normoxic conditions. This mechanism prevents the formation and activity of HIF heterodimers in sufficiently oxygenized cells and the launch of their hypoxia-adaptive genetic program. In hypoxia, however, HIF-α subunits escape from the constitutive degradation, become stabilized in the cytoplasm, dimerize with HIF-1β and the nuclear heterodimers rearrange gene expression pattern of the hypoxic cell. This primarily, but not exclusively, includes induction of genes that mediate the switch from oxygen-dependent to anaerobic metabolism.

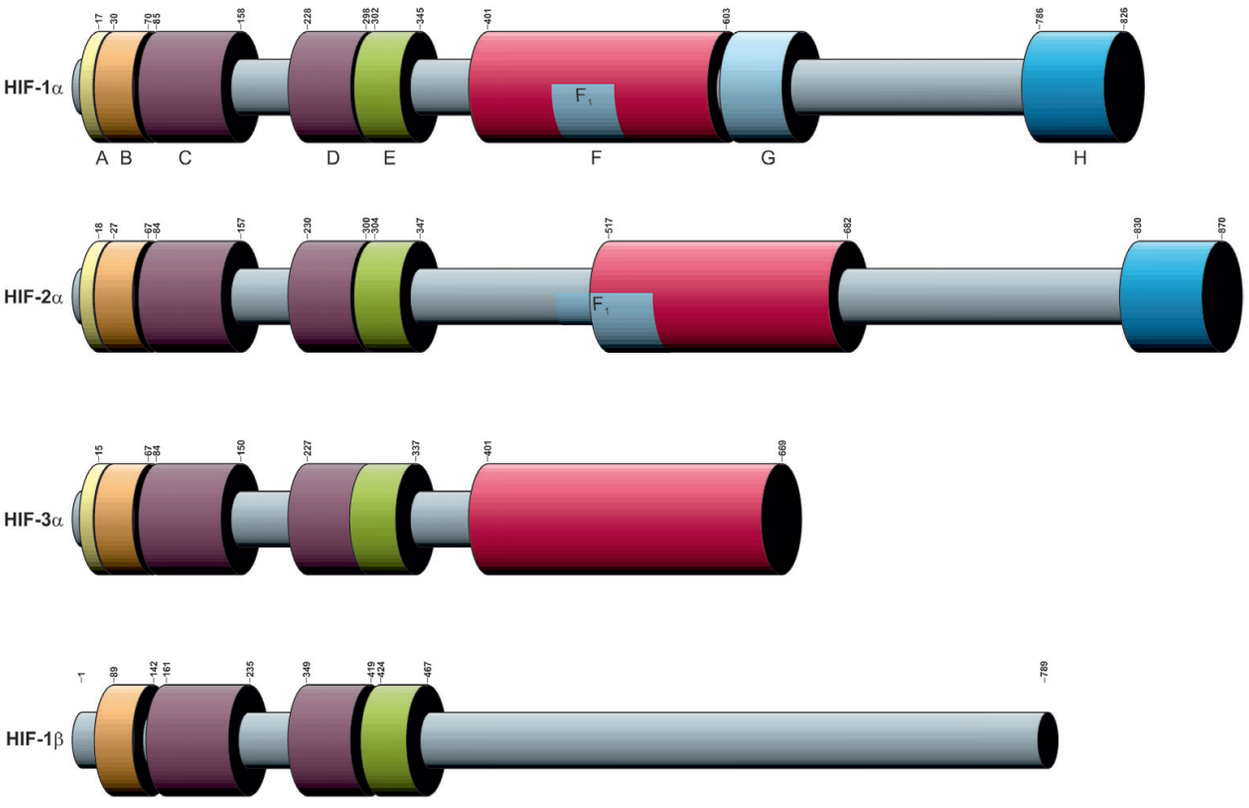

Figure 1. Domain structure of HIF polypeptides A: DNA-binding domain; B: basic helix-loop-helix domain; C: Per/Arnt/Sim (PAS) A domain; D: PAS B domain; E: PAC motif; F: oxygen-dependent degradation domain; $F_{1:}$ N-terminal transactivation domain; G: ERK target domain; H: C-terminal transactivation domain. Amino acid positions indicated are based on current Uniprot.

The molecular background of normoxic degradation of HIF-α was first elucidated in 2001 [6, 7] (**Figure 2**). It turned out that its continuous proteasomal elimination is triggered by the oxygen-dependent hydroxylation mediated by a family of prolyl-4-hydroxylases reminiscent of procollagen prolyl hydroxylases that had long been known at the time. To date, three HIF-regulating prolyl-4-hydroxylases (also known as PHD1, PHD2, and PHD3) have been identified in mammalian cells [8]. They utilize molecular oxygen, ascorbic acid, iron, and the tricarboxylic acid (TCA) cycle intermediate α-ketoglutarate as co-factors and co-substrates to hydroxylate the HIF-α subunits at conserved prolyl residues [6, 9]. In HIF-1α, these are proline 402 and 564 and their hydroxylation increases the affinity of the polypeptide to the von Hippel-Lindau protein (pVHL), the substrate recognition component of the E3 ubiquitin ligase complex of Elongin-B and -C, Cul2, and Rbx1 [10]. This leads to pVHL-mediated ubiquitylation of lysine residues (lysine 532, 538, and 547 in case of HIF-1α) within the so-called oxygen-dependent degradation domain that renders the polypeptide for constitutive proteasomal degradation. In hypoxia, this hydroxylation activity is reduced due to the lack of available oxygen, resulting in stabilization of the HIF-α subunits. In addition to the PHD-mediated post-translational modifications, a second level of hydroxylation-dependent regulation of HIF-α has also been

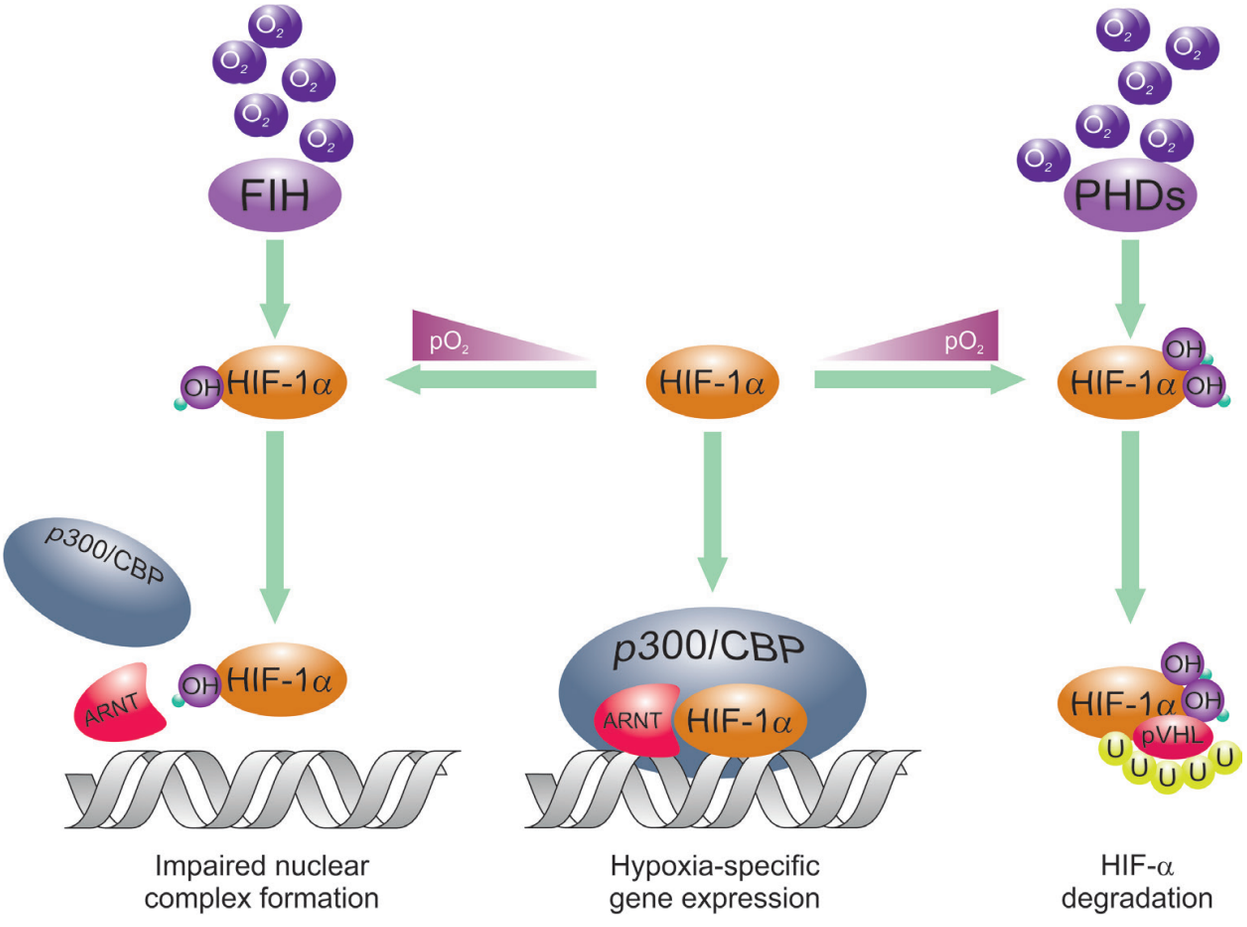

Figure 2. Oxygen sensing by hydroxylases. Abbreviations: FIH: factor inhibiting HIF; PHDs: prolyl-4-hydroxylases; HIF: hypoxia-inducible factor; ARNT: aryl hydrocarbon receptor nuclear translocator; pVHL: von Hippel-Lindau ubiquitin ligase; OH: hydroxylation; U: ubiquitylation; p300/CBP: transcriptional co-activators of HIF heterodimers.

discovered. This post-translational modification is mediated by the asparagine hydroxylase termed factor inhibiting HIF (FIH). Unlike PHDs, however, the FIH-mediated hydroxylation is believed to primarily prevent HIF's interaction with transcriptional co-activators like the p300/CBP (**Figure 2**) [11–13].

In the hypoxia pathway, oxygen may be considered the "ligand" for oxygen sensor prolyl-4-hydroxylases that principally govern HIF activity. This, eventually, culminates in the launch of a complex adaptive program that fundamentally affects cellular homeostasis via metabolic switch from the oxygen-dependent oxidative phosphorylation to glycolysis, increased angiogenesis and enhanced erythropoiesis. Thus, it is not surprising that a range of additional inputs, including feedback loops and multiple cross talks with other signaling pathways, shape the spatial and temporal nature of the ultimate response to oxygen depletion. These interactions form a metabolic signaling network that confers a dynamic profile and a high degree of complexity upon the hypoxic response.

3. Regulatory measures in hypoxia signaling

3.1. Supracellular signaling

In principle, adaptation to hypoxia may involve two directions of counter measures; reduction of oxygen consumption and increase of oxygen supply. In multicellular organisms, the latter one requires coordinated supracellular, multi-organ measures governed by HIF-inducible genes including elevated erythropoiesis and angiogenesis. At a systemic level, hypoxia-activated HIFs induce erythropoietin (EPO) expression in liver and interstitial kidney cells that, subsequently, triggers erythropoiesis in the bone marrow [14, 15]. This elevated red blood cell production, however, requires increased iron supply of bone marrow erythroblasts regulated by, at least in part, the hepatocyte-specific iron homeostasis regulator hepcidin. This short peptide is believed to be responsible for inhibiting the iron release and absorption from macrophages and intestinal epithelial cells, respectively, by binding the only known cellular iron exporter ferroportin [16]. Upregulated HIF-driven erythropoiesis provokes repression of the hepatic hepcidin-encoding gene, although the identity of the soluble mediator of this effect is yet to be confirmed [17–20]. The drop of serum hepcidin upon hypoxia, eventually, results in elevated iron release from the intestinal epithelium supplying the increased iron demand of expanded erythropoiesis [21].

When hypoxia develops locally, sheer increase of the oxygen transport capacity may not be sufficient to elevate the oxygen supply of hypoxic tissues. In these conditions, hypoxia is accompanied by angiogenesis representing another tissue-level negative feedback loop of hypoxia signaling. This arm of the regulation is mediated by the key angiogenesis-regulating growth factor termed vascular endothelial growth factor A (VEGF-A). Similar to *EPO*, *VEGFA*, the key determinant of survival and proliferation of endothelial cells upon embryonic vasculogenesis, is another common target of HIF-1 and -2 [22, 23]. In addition, high levels of VEGF-A expressed by hypoxic stromal or tumor cells regulate endothelial cells metabolism

by biasing it toward glycolysis via induction of isoform 3 of 6-phospho-fructo-2-kinase/fructose-2,6-biphosphatase [24]. Increased glycolysis not only supports endothelial cell survival under hypoxic conditions but also triggers vessel sprouting, further representing a complementary mechanism of VEGF-mediated angiogenesis [25]. Elevated oxygen and iron levels, consequently, provide prolyl-4-hydroxylases increased supply of their co-substrates completing the supracellular regulatory loop of hypoxia signaling [26].

3.2. Intracellular metabolic signaling in hypoxia

At the cellular level, HIFs reprogram metabolism directly targeting a cluster of metabolic enzyme-coding genes [27]. Their prototype is pyruvate dehydrogenase kinase-1 (PDK-1) which phosphorylates pyruvate dehydrogenase (PDH), the enzyme that supplies TCA cycle with acetyl-coenzyme A [28]. PDK-1 mediates inactivating phosphorylation of PDH that shuts down the TCA cycle due to shortage of acetyl-coenzyme A. This leads to fundamental changes in mitochondrial functions including the accumulation of TCA cycle intermediates [29]. Since HIF-regulating prolyl-4-hydroxylases utilize α-ketoglutarate and produce succinate during their catalytic activity, one can speculate that accumulation of the latter one blocks catalytic activity of prolyl-4-hydroxylases [30]. Indeed, it was found that loss-of-function mutations of succinate dehydrogenase, the TCA cycle enzyme that converts succinate into fumarate, block PHDs and, consequently, stabilize HIF-α subunits [31]. In addition, it has also been demonstrated that this effect is, primarily, mediated by the accumulation of succinate (**Figure 3**) [32].

Besides their direct metabolic target genes, HIFs also regulate the hypoxia pathway sensor PHDs indirectly through the HIF-inducible microRNA-210 (miR-210)-mediated silencing of the glycerol-3-phosphate dehydrogenase 1 like protein (GPD1-L). GPD1-L has a similar enzymatic activity to that of the mitochondrial glycerol-3-phosphate dehydrogenase and catalyzes the redox conversion of glycerol-3-phosphate (G3P) to dihydroxyacetone-phosphate [33]. Although the mechanism behind the connection is still not clear, the miR-210-mediated downregulation of GPD1-L is accompanied by increased PHD-mediated HIF-1α degradation [34]. Since decreased enzymatic GPD1-L activity results in increased G3P levels, upregulated glycolysis may contribute to the redistribution of available oxygen from mitochondria to PHDs and, thus, represents the link between miR-210 and the restoration of PHD activity. This hypothesis is further supported by the observation that inhibition of the mitochondrial respiration by nitric oxide is followed by redistribution of O_2 and inactivation of HIFs [35].

The concept of metabolite-mediated regulation of PHDs is further supported by the observation that, in hypoxia, another microRNA, miR-183, targets isocitrate dehydrogenase, the TCA cycle mediator that produces α-ketoglutarate from isocitrate. Although the mechanism of its hypoxic upregulation is yet to be determined, the miR-183-mediated blockade of α-ketoglutarate production exploits the α-ketoglutarate-dependent nature of prolyl-4-hydroxylases and promotes stabilization of HIF-α via inhibition of PHDs [36]. Thus, PHDs not only act as oxygen sensors but can also integrate metabolic stimuli forming synergistic metabolic positive feedback loops within hypoxia signaling [37].

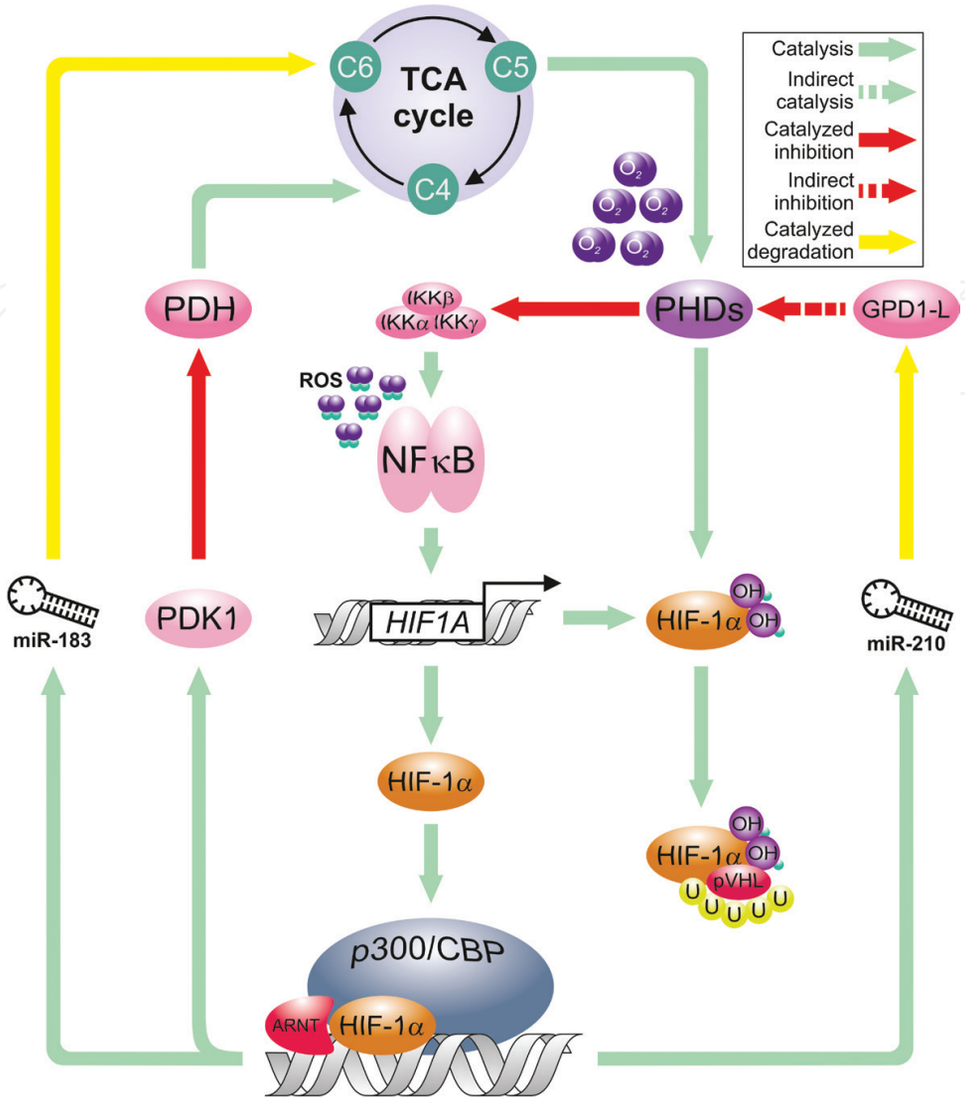

Figure 3. Interplay of the hypoxia and metabolic signaling. Abbreviations: TCA cycle: tricarboxylic acid cycle; C4, C5, and C6 represent the 4, 5, and 6 carbon metabolites of the TCA cycle, respectively; IKKα, IKKβ, and IKKγ are the Inhibitory kappa-B kinase alpha, beta, and gamma subunits, respectively; PDH: pyruvate dehydrogenase; PDK1: PDH kinase 1; GPD1-L: Glycerol-3-phosphate dehydrogenase 1-like protein; PHDs: prolyl-4-hydroxylases; NF-κB: nuclear factor kappa-B; HIF: hypoxia-inducible factor; ARNT: aryl hydrocarbon receptor nuclear translocator; pVHL: von Hippel–Lindau ubiquitin ligase; OH: hydroxylation; U: ubiquitylation; p300/CBP: transcriptional coactivators of HIF heterodimers; miR-183 and miR-210 are microRNA-183 and microRNA-210, respectively.

3.3. Transcriptional feedbacks

HIFs directly induce genes with wide range of functions including both *PHD2* and *PHD3* (**Figure 4**). Although HIF-mediated transactivation of *PHD*s resembles a canonical, direct negative feedback arm within the PHD-HIF axis, it may have more complex functions [38, 39]. HIFs can only transactivate their targets upon oxygen depletion so one can speculate that, induction of the oxygen-dependent PHDs is useless under hypoxia. In fact, however, despite their oxygen dependency, which prevents them from functioning under hypoxic conditions, experimental data indicate that enzymatic activity of both PHD2 and PHD3 remains detectable in hypoxic

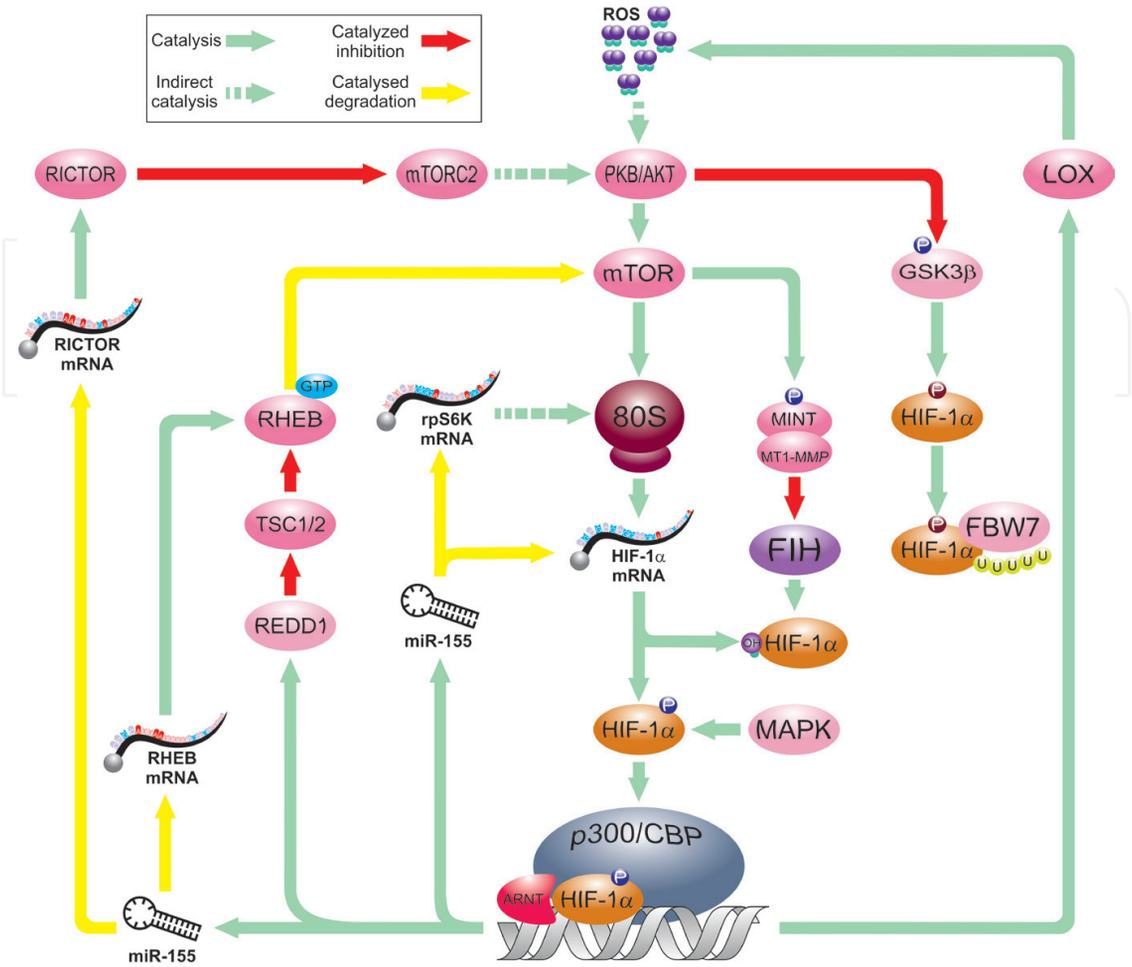

Figure 4. Cross talk between the hypoxia, anabolic, mitogen, and inflammatory signaling pathways. Abbreviations: ROS: reactive oxygen species; RICTOR: rapamycin-insensitive companion of mTOR; mTORC2: mammalian target of rapamycin complex 2; PKB/AKT: protein kinase B; mTOR: mammalian target of rapamycin; LOX: Lysyl oxidase; GSK3β: glycogen synthase kinase-3 beta; RHEB: Ras homolog mTOR complex 1-binding protein; 80S: mature eukaryotic ribosome; MINT: Amyloid beta precursor protein binding family A, member 3; MT1-MMP: membrane-type 1 matrix metalloproteinase; TSC1/2: Tuberous Sclerosis complex 1/2; REDD1: DNA damage-inducible transcript 4; PHDs: prolyl-4-hydroxylases; FIH: factor inhibiting HIF; MAPK: mitogen activated protein kinase; HIF: hypoxia-inducible factor; ARNT: aryl hydrocarbon receptor nuclear translocator; FBW7: F-Box and WD-40 domain-containing protein 7; OH: hydroxylation; U: ubiquitylation; p300/CBP: transcriptional coactivators of HIF heterodimers; miR-155: microRNA-155.

cells maintaining reactivity of the HIF system for further hypoxic insults [39]. Thus, HIF-mediated induction of *PHD*s in conjunction with their metabolic effects, possibly, functions as a mechanism responsible for resetting hypoxia signaling to a new steady state at lower oxygen levels.

The HIF-target miR-210 also plays multiple, apparently opposing, roles in the regulation of the hypoxia pathway. It not only indirectly facilitates HIF activity by targeting GPD1-L but also silences MYC antagonist MNT, a member of the MYC/MAD/MYX transcription factor family [40]. This downregulation deliberates MYC-mediated induction of genes like those involved in the resolution of HIF-induced cell cycle arrest or metabolic switch via PDK1 illustrating the complexity of the HIF-provoked signaling responses. While, through the

former action, MYC counteracts hypoxia signaling, MYC-mediated induction of PDK1 synergizes with HIF activity [41]. Since MYC has also been reported to support HIF-1α directly by interfering with the VHL-dependent degradation of HIF-1α, data strongly suggest the existence of a MYC-mediated feedforward loop in the HIF pathway [42]. In return, HIF counteracts MYC by various underlying mechanisms including the induction of MXI1, another MYC antagonist, competition with MYC for promoter binding or promoting its proteasomal degradation [43, 44]. Since the opposing effects of the HIF/MYC interaction in the regulation of cell cycle and metabolism may reflect differences of the experimental models used, the biological relevance of the hypoxia-inducible miR-210-promoted MYC functions requires further investigations. Additional targets of miR-210 like the mitochondrial iron-sulfur cluster scaffold protein or transferrin suggest the potential signal integration role of miR-210 in hypoxia signaling [45, 46].

Besides miR-210, the HIF-inducible miR-155 represents another level of microRNA-mediated transcriptional regulation of the hypoxia pathway. Upon a hypoxic insult, it shapes dynamics of the HIF-response by facilitating the RISC-mediated degradation of the HIF-1α transcript [47]. Intriguingly, the miR-155-mediated direct silencing of HIF-α expression not only illustrates an isoform-specific resolution of hypoxia signaling upon prolonged hypoxia but also resembles the HIF-mediated induction of PHDs and might ensure the cellular reactivity to hypoxia at lower pO_2 levels.

An additional form of the transcriptional regulation of hypoxia signaling is mediated by the *HIF3A-encoded* isoform termed inhibitory PAS domain protein (IPAS). IPAS is an alternative splicing product of the *HIF3A* locus and generates a polypeptide that lacks the C-terminal transactivation domains of HIF-1 and -2α (**Figure 1**) [48]. As such, it functions as a dominant negative regulator of HIFs by competing with HIF-1β [49]. The IPAS-specific splicing product is hypoxia-inducible and, at last in part, is under the control of a HIF-1-specific hypoxia-response element representing one of the classic negative feedback loops of the hypoxia pathway [48, 50]. Interestingly, the IPAS-specific mRNA splicing also takes place in the absence of the HIF-1-binding site of the IPAS promoter suggesting the existence of HIF-independent factors involved in the expression of IPAS [50]. The uncoupled nature of IPAS expression and IPAS mRNA splicing underpins the presence of an additional control layer in the IPAS-mediated HIF regulation. Indeed, since normoxic expression of IPAS is, apparently, restricted to corneal epithelial cells and some neuronal elements in mice, the HIF-independent control mechanisms may contribute to the tissue-specific nature of the IPAS-mediated regulation of hypoxia signaling.

4. Cross talks

4.1. Cross talk through HIF-1β

Due to the fundamental role of oxygen in the cellular homeostasis, the hypoxic insult requires counter measures that rely on tight coordination of the full spectrum of cellular functions. As part of this, extensive interplays exist between the primary hypoxia sensing PHD-HIF axis

and distinct molecular machineries involved in cellular functions like catabolism, cell cycle, or cellular defense mechanisms. One of the mediators of these interactions is the β subunit of HIF heterodimers also known as the aryl hydrocarbon nuclear translocator (ARNT).

Besides its critical role in the formation of active HIFs, the constitutively expressed HIF-1β, as its alias indicates, is also the partner of the aryl hydrocarbon receptor (AhR), a transcription factor that targets genes involved in the biotransformation of xenobiotics [51, 52]. The class I bHLH/PAS protein family member AhR is ubiquitously expressed and activated by various endo- and exogenous ligands. In its inactive state, it forms heterodimers with repressor proteins, like the heat shock protein 90, in the cytoplasm [53]. Upon ligand binding, its nuclear localization signal becomes exposed and, following the consequent nuclear translocation, AhR dimerizes with HIF-1β [54]. The class II bHLH/PAS HIF-1β is essential for the AhR-mediated induction of genes with 5′-TNGCGTG-3′ sequences (also known as xenobiotic response element [XRE]) present in their promoters [55–57]. These include phase I and II detoxifying enzymes like the cytochrome P450 (*CYP1A1*) and UDP-glucuronosyltransferase 1 isoforms, respectively [58].

Due to their shared partner, it was proposed that activation of hypoxia signaling affects AhR-mediated responses and *vice versa*, AhR-mediated engagement of HIF-1β attenuates hypoxia responses. Indeed, in hypoxic cells exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), the prototype ligand for AhR, the AhR-mediated *CYP1A1* expression was found reduced. In contrast, TCDD treatment of cells inhibited HIF-mediated transcriptional responses indicating the cross talk between the AhR and hypoxia signaling [59]. Compromised formation of the AhR/HIF-1β or HIF-1α/HIF-1β heterodimers, in the presence of hypoxia or AhR ligands, respectively, indicates that, in human cells, HIF-1β acts as the limiting factor of the dimer formation [60]. Experimental data also indicates that HIF-1α has higher affinity to HIF-1β than that of the AhR, suggesting that human cells experience the oxygen depleted milieu as a potentially more harmful environmental stimulus than that of the presence of xenobiotics [61]. In the case of genes under the regulation of both HRE and XRE sequences, cross talk between the hypoxia and AhR pathways is even more complex as simultaneous presence of AhR ligands and hypoxia was found to be rather additive, reflecting the cell-type or stimulus-dependent nature of these responses [62].

4.2. Cross talk between the hypoxic and anabolic signaling

Hypoxia is one of the strongest stimuli of autophagy that is considered as an indicator of depleted ATP pools of hypoxic cells. In concert, the HIF-orchestrated adaptive program involves downregulation of catabolic pathways like the one regulated by the mammalian target of rapamycin (mTOR) (**Figure 4**). The involvement of mTOR in the regulation of HIF-1 was first suggested by independent studies on the oncogene-related activation of VEGF [63–66]. Unlike PHDs, mTOR enhances HIF-1-mediated transcriptional activity without affecting its degradation rate [64, 67]. mTOR alters the protein expression pattern of hypoxic cells, at least in part, via phosphorylation of the eukaryotic initiation factor 4E-binding protein 1 (eIF4E-BP1), a suppressor of the 5′ CAP-dependent translation [68]. These observations together with findings on the negative effects of rapamycin on HIF-1 suggest that mTOR enhances HIF-α

mRNA translation [69]. This concept is also supported by the observation that downregulation of the mTOR complex 2 (mTORC2), a redox-sensitive activator of the PKB/AKT pathway, leads to decreased abundance of the HIF-2α transcripts in the polysomal fractions [70]. Current data indicate that the dominant upstream regulator of the hypoxia-related mTOR activity is protein kinase B (PKB/AKT) [67]. Hypoxic activation of PKB/AKT that, at least in part, depends on reactive oxygen species (ROS), was shown to regulate PHD activity and promote stabilization of HIFs [71–73]. Intriguingly, HIF is actively involved in the generation of ROS under hypoxia by inducing lysyl oxidase (LOX) [67]. *LOX* encodes a copper-dependent amine oxidase that catalyzes cross-linking of collagen and elastin in the extracellular matrix while producing hydrogen peroxide (H_2O_2). Current data indicate that, following its HIF-dependent upregulation in hypoxia, LOX-generated H_2O_2 activates the PKB/AKT-mTOR axis resulting in the upregulation of HIF-1α translation illustrating a positive feedback loop between mTOR and HIF [67]. It is noteworthy that HIF-mediated induction of PHDs, thus, may not only play a role in resetting the hypoxia pathway at a lower oxygen tension but also represents a limiting step of the mTOR-mediated enhancement of HIF-1α translation [74].

PKB/AKT has also been shown to affect the proteasomal degradation of HIF-1α through glycogen synthase kinase 3β (GSK3β) [75]. GSK3β-mediated phosphorylation of HIF-1α facilitates its binding to FBW7, an E3 ubiquitin ligase, which recognizes GSK3β-phosphorylated proteins and targets them for proteasomal degradation [76]. Since inactivating phosphorylation of GSK3β is primarily mediated by PKB/AKT, activation of the PKB/AKT pathway not only influences the translational rate of HIF-1α via mTOR but also mimics the effect of hypoxia via inhibition of proteasomal degradation of the HIF-1α polypeptide.

To make the picture even more colorful, mTOR also phosphorylates MINT3, a membrane-type matrix metalloproteinase (MT-MMPs) regulator, at its threonine 5/serine 7 residues [77]. This modification promotes binding of MINT3 to FIH-1 leading to the inactivation of the latter [78]. By sequestering the HIF-1 suppressor FIH-1 to the Golgi membrane in cooperation with the MT1-MMP, the mTOR/MINT3/MT1-MMP axis can also support transcriptional activity of HIF-1 independently of its translational rate. Interestingly, in renal cell carcinoma, MT1-MMP has been found to be a target gene for HIF-2 raising the question if the mTOR-regulated MINT3/MT1-MMP/FIH-1-mediated positive feedback loop is a general mechanism in the regulation of HIFs [79]. Although its biological relevance is yet to be determined, it is noteworthy that mTOR has also been reported to associate with HIF-1α via the mTOR complex 1 member RAPTOR and a putative TOR motif within the HIF-1α polypeptide [66]. Since mTOR is a serine/threonine kinase and one of the known posttranslational modifications that favors HIF-1α transcriptional activity is phosphorylation, the possibility that this co-localization also supports the effect of mTOR on HIF-1 via direct phosphorylation cannot be excluded but is yet to be confirmed [66, 80].

Eventually, rapamycin-sensitive upregulation of HIF-1 supports the induction of a wide range of HIF-1 targets, of which many have been found to form feedback loops via regulation of the hypoxia-related activity of mTOR. These include REDD1 that activates the tuberous sclerosis complex 1/2 (TSC1/2) [81]. The TSC1/2 possesses GTPase-activating function that renders the mTOR activator RHEB inactive [82]. BNIP3, another known HIF-1 target, also facilitates the accumulation of the GDP-bound form of RHEB and the consequent downregulation of mTOR

in hypoxia [83]. In addition, the HIF-1-inducible miR-155 also targets elements of the mTOR pathway including RHEB, the mTORC2 member RICTOR and the mTOR effector ribosomal protein S6KB2 [84]. Downregulation of these targets, seemingly, complements the effect of REDD1 and BNIP3 and may contribute to the limitation of mTOR signaling in hypoxia.

4.3. Interplay with the imflammatory and mitogen signaling pathways

Besides its role in the mTOR-mediated HIF regulation discussed above, hypoxia-inducible miR-155 is one of the identified measures directly targeting HIF-1α mRNA, indicating its pivotal role in the regulation of the HIF pathway. Sequence analyses revealed that, besides the HIF responsive element, NF-κB consensus sequences are also present in the miR-155 promoter indicating the capacity of NF-κB-mediated stimuli to influence the HIF pathway via miR-155 [47]. Studies on the proposed link between hypoxia and inflammation revealed that NF-κB can also induce *HIF1A* via evolutionary conserved consensus binding sites identified in the *HIF1A* promoter [72, 85–87]. Since this induction is not sufficient for the accumulation of HIF-1α in the absence of hypoxia, current data suggest that the canonical NF-κB pathway is rather for pre-setting the HIF-1α mRNA level according to the redox state and inflammatory cytokine composition of the extracellular milieu [88]. This concept is further supported by the observation that NF-κB activity can be both up- and downregulated upon inhibition of prolyl-4-hydroxylases depending on the NF-κB stimulus received [89].

Intriguingly, besides *HIF1A*, NF-κB transactivates *ARNT* as well, leading to enhanced formation of HIF-1β:HIF-2α that attenuates the proteasomal degradation of the latter. Considering the cell-type specific expression pattern of HIF-2α, the NF-κB-mediated induction of *ARNT* may represent a tissue-specific arm of the NF-κB-governed regulation of hypoxia signaling [90]. The interplay, however, is apparently bidirectional and the hypoxic signal can also be conveyed to the inflammatory pathway. Under normoxic conditions, PHDs inhibit the I kappa B kinase (IKK) complex attenuating the dissociation of inhibitory kappa B (IκB) from NF-κB [85, 88, 91]. In the absence of oxygen, however, the PHD-mediated blockade of IKK is resolved, the IKK complex becomes active leading to the phosphorylation of IκB and release of sequestered NF-κB subunits. The consequent formation of active NF-κB heterodimers culminates in moderate upregulation of the basal NF-κB activity that is believed to potentiate NF-κB responsiveness to cytokines like the tumor necrosis factor-alpha (TNF-α) or reactive oxygen species, stimuli typically accompanying inflammatory conditions [72, 92–95]. Thus, the interaction between the NF-κB and HIF pathways well illustrates the close pathophysiologic connection between hypoxia and inflammation and allows the cell to integrate inflammatory stimuli in the adaptive response under hypoxic conditions.

Anabolic extracellular signals that activate the mTOR pathway often diverge and activate the ERK signaling cascade as well, raising the question if ERK and hypoxia signaling interplay. Experimental data indicate that the HIF-1α polypeptide can be phosphorylated by p42/44 MAP kinases both under hypoxic conditions and in response to receptor-mediated ERK-activating stimuli [96–98]. The ERK-mediated phosphorylation was found to enhance the transcriptional activity of HIF-1 in various model systems, although the exact mechanism is still not clear [99, 100]. On one hand, it was proposed that phosphorylation of HIF-1α at positions 641 and 643 supports the transcriptional activity by attenuating its nuclear export

[101, 102]. On the other, it was also demonstrated that ERK activity fundamentally alters the predicted composition of HIF-1-containing nuclear complexes suggesting multiple effects of ERK activity on hypoxia signaling [103]. Independently of the mechanistic details, current data suggest that ERK-mediated upregulation of the HIF pathway differs from the mTOR-mediated effect and, primarily, acts on the transactivation function of HIFs, possibly, complementing the mTOR-mediated effects (**Figure 4**).

5. Conclusion

Extensive experimental work over the past three decades deciphered the molecular background of the cellular response to oxygen depletion, one of the fundamental physiologic processes. To date, these efforts depictured an intricate molecular network that bridges, apparently, every aspect of cellular physiology. Within this network, the PHD-HIF axis plays an integrative role of various signals that allows the hypoxic cell to shape dynamics of the adaptive response according to the actual endogenous metabolic state and surrounding microenvironment alike. Deeper understanding of these molecular machineries gives the opportunity to develop more efficient medical modalities for pathologies like chronic inflammation, ischemia or neoplasms.

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Conflict of interest

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