

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

186,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



The Signaling Nature of Cellular Metabolism: The Hypoxia Signaling

Zsolt Fabian

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/intechopen.79952>

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-1 α and HIF-1 β , respectively) both of which are ubiquitously expressed in human tissues, whereas HIF-2 α and HIF-3 α 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-1 β 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.

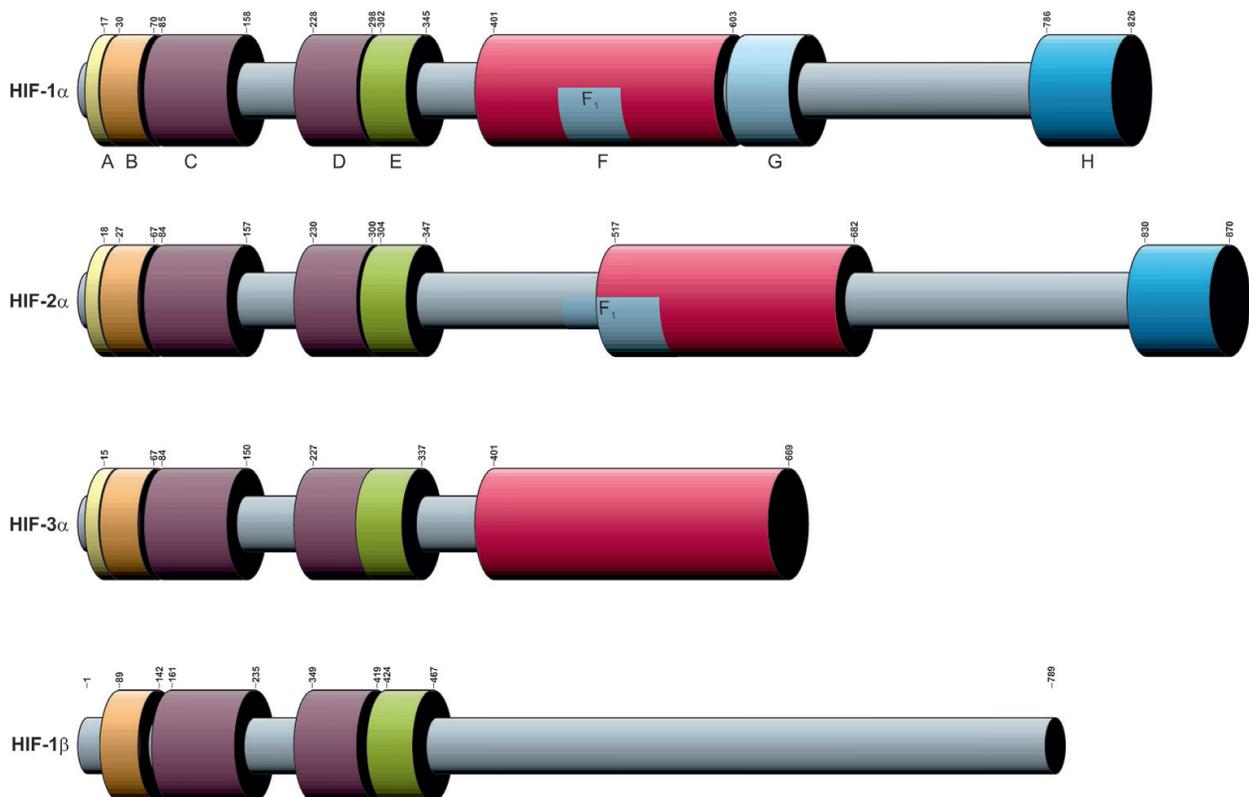


Figure 1. Domain structure of HIF polypeptides A: DNA-binding domain; B: basic helix-loop-helix domain; C: Per/Arnt/Sim (PAS) A domain; D: PAS B domain; E: PAC motif; F: oxygen-dependent degradation domain; F₁: N-terminal transactivation domain; G: ERK target domain; H: C-terminal transactivation domain. 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-1 α , 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-1 α) 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

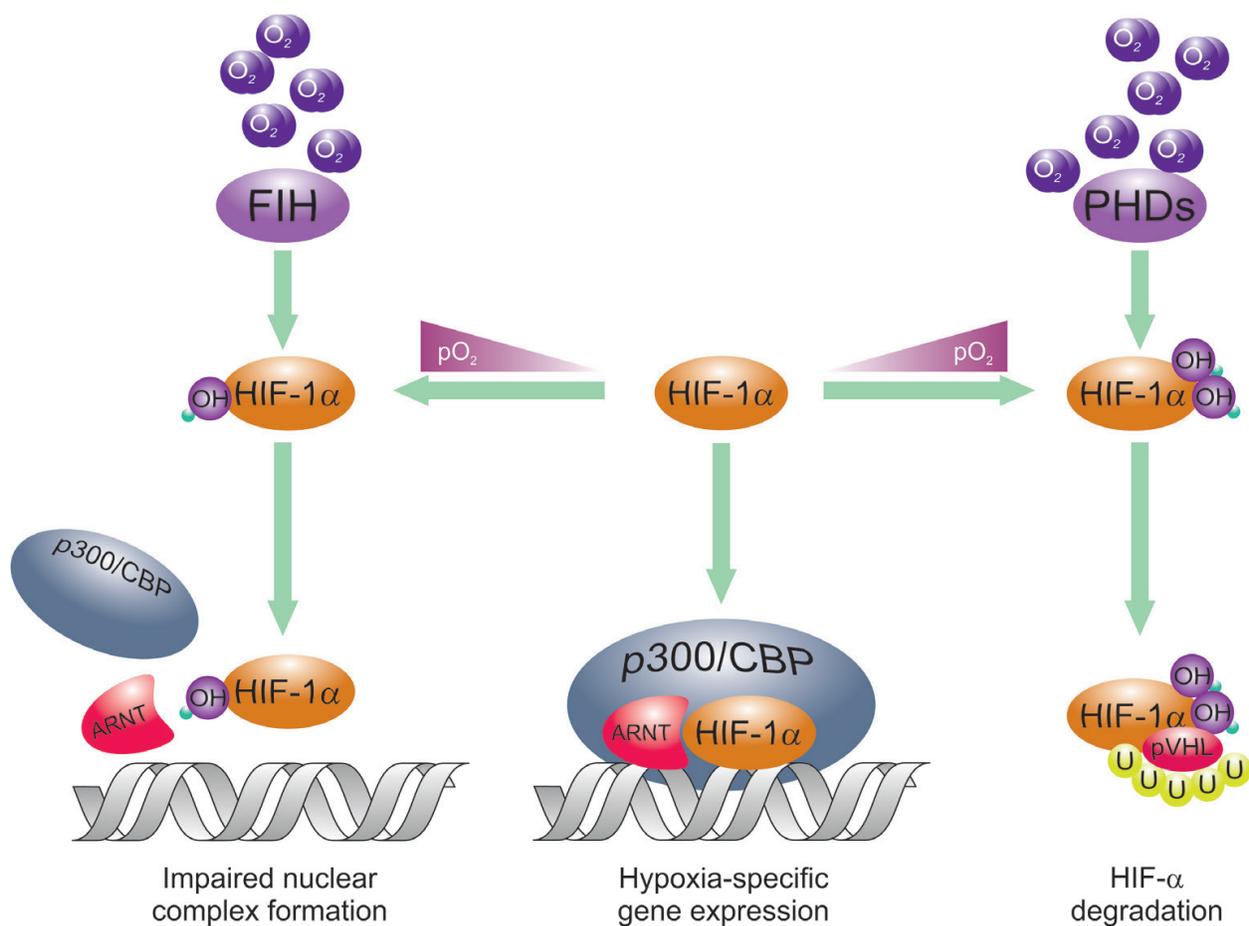


Figure 2. Oxygen sensing by hydroxylases. Abbreviations: FIH: factor inhibiting HIF; PHDs: prolyl-4-hydroxylases; HIF: hypoxia-inducible factor; ARNT: aryl hydrocarbon receptor nuclear translocator; pVHL: von Hippel-Lindau ubiquitin ligase; OH: hydroxylation; U: ubiquitylation; p300/CBP: transcriptional 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-1 α 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 O₂ 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].

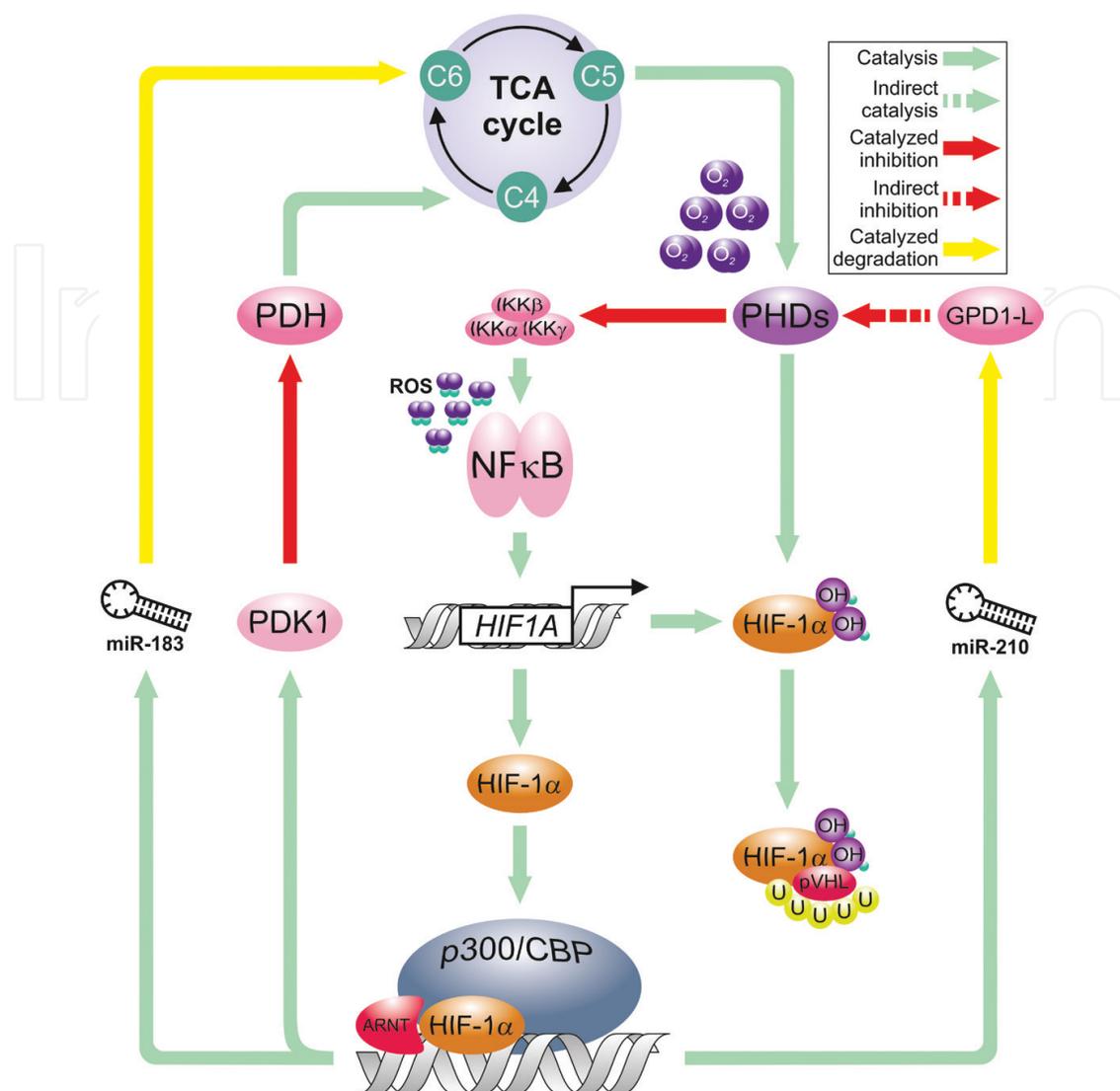


Figure 3. Interplay of the hypoxia and metabolic signaling. Abbreviations: TCA cycle: tricarboxylic acid cycle; C4, C5, and C6 represent the 4, 5, and 6 carbon metabolites of the TCA cycle, respectively; IKK α , IKK β , and IKK γ are the Inhibitory kappa-B kinase alpha, beta, and gamma subunits, respectively; PDH: pyruvate dehydrogenase; PDK1: PDH kinase 1; GPD1-L: Glycerol-3-phosphate dehydrogenase 1-like protein; PHDs: prolyl-4-hydroxylases; NF- κ B: 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 *PHDs* 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

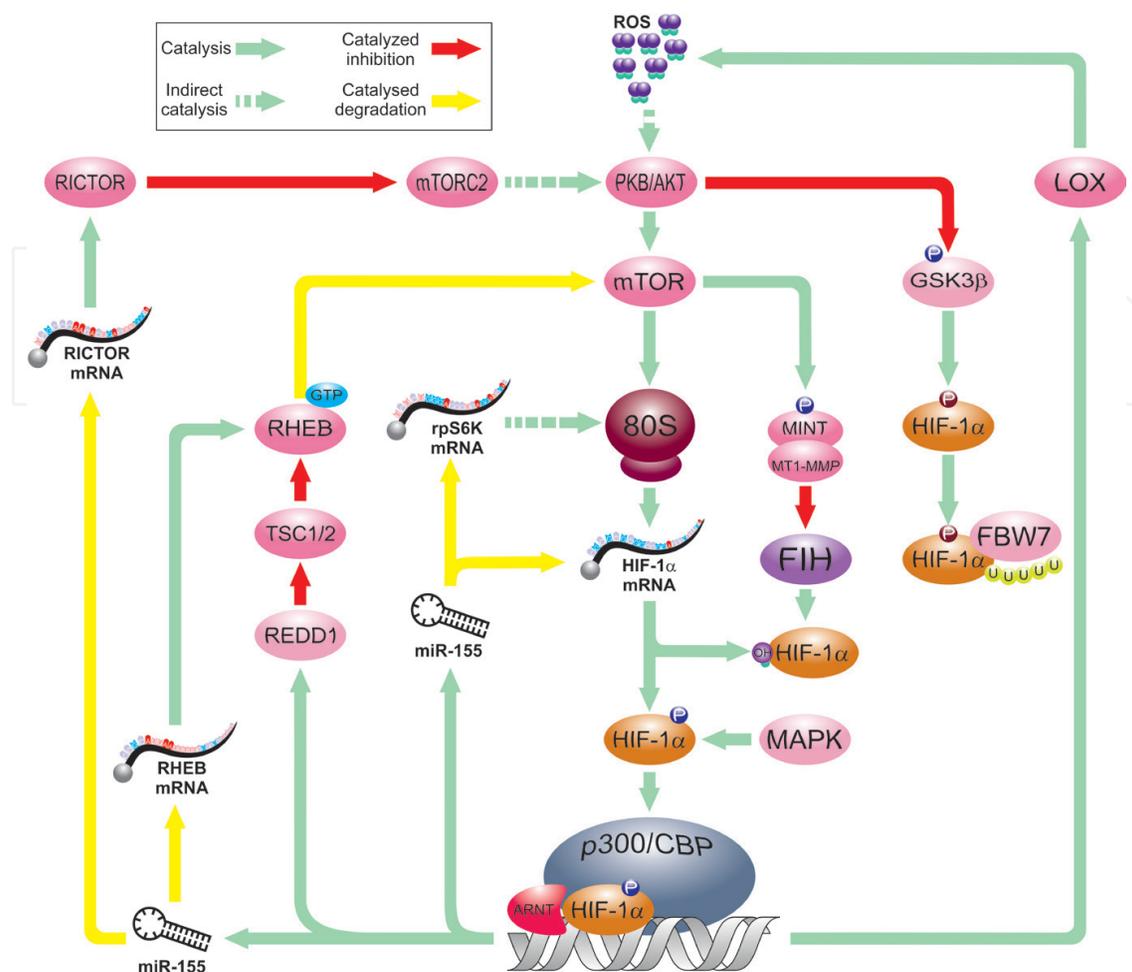


Figure 4. Cross talk between the hypoxia, anabolic, mitogen, and inflammatory signaling pathways. Abbreviations: ROS: reactive oxygen species; RICTOR: rapamycin-insensitive companion of mTOR; mTORC2: mammalian target of rapamycin complex 2; PKB/AKT: protein kinase B; mTOR: mammalian target of rapamycin; LOX: Lysyl oxidase; GSK3 β : 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 *PHDs* 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-1 α directly by interfering with the VHL-dependent degradation of HIF-1 α , 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-1 α 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 pO₂ 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 -2 α (**Figure 1**) [48]. As such, it functions as a dominant negative regulator of HIFs by competing with HIF-1 β [49]. The IPAS-specific splicing product is hypoxia-inducible and, at least 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-1 β

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-1 β , 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-1 β [54]. The class II bHLH/PAS HIF-1 β 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-1 β 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-1 β or HIF-1 α /HIF-1 β heterodimers, in the presence of hypoxia or AhR ligands, respectively, indicates that, in human cells, HIF-1 β acts as the limiting factor of the dimer formation [60]. Experimental data also indicates that HIF-1 α has higher affinity to HIF-1 β 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-2 α 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 (H₂O₂). Current data indicate that, following its HIF-dependent upregulation in hypoxia, LOX-generated H₂O₂ activates the PKB/AKT-mTOR axis resulting in the upregulation of HIF-1 α 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-1 α translation [74].

PKB/AKT has also been shown to affect the proteasomal degradation of HIF-1 α through glycogen synthase kinase 3 β (GSK3 β) [75]. GSK3 β -mediated phosphorylation of HIF-1 α facilitates its binding to FBW7, an E3 ubiquitin ligase, which recognizes GSK3 β -phosphorylated proteins and targets them for proteasomal degradation [76]. Since inactivating phosphorylation of GSK3 β is primarily mediated by PKB/AKT, activation of the PKB/AKT pathway not only influences the translational rate of HIF-1 α via mTOR but also mimics the effect of hypoxia via inhibition of proteasomal degradation of the HIF-1 α 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-1 α via the mTOR complex 1 member RAPTOR and a putative TOR motif within the HIF-1 α polypeptide [66]. Since mTOR is a serine/threonine kinase and one of the known posttranslational modifications that favors HIF-1 α 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 inflammatory 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-1 α mRNA, indicating its pivotal role in the regulation of the HIF pathway. Sequence analyses revealed that, besides the HIF responsive element, NF- κ B consensus sequences are also present in the miR-155 promoter indicating the capacity of NF- κ B-mediated stimuli to influence the HIF pathway via miR-155 [47]. Studies on the proposed link between hypoxia and inflammation revealed that NF- κ B 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-1 α in the absence of hypoxia, current data suggest that the canonical NF- κ B pathway is rather for pre-setting the HIF-1 α 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- κ B activity can be both up- and down-regulated upon inhibition of prolyl-4-hydroxylases depending on the NF- κ B stimulus received [89].

Intriguingly, besides *HIF1A*, NF- κ B transactivates *ARNT* as well, leading to enhanced formation of HIF-1 β :HIF-2 α that attenuates the proteasomal degradation of the latter. Considering the cell-type specific expression pattern of HIF-2 α , the NF- κ B-mediated induction of *ARNT* may represent a tissue-specific arm of the NF- κ B-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 (I κ B) from NF- κ B [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 I κ B and release of sequestered NF- κ B subunits. The consequent formation of active NF- κ B heterodimers culminates in moderate upregulation of the basal NF- κ B activity that is believed to potentiate NF- κ B 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- κ B 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-1 α 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-1 α 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 depicted 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.

Acknowledgements

The Open Access fee of this chapter was funded by the Munchausen fund provided by the Department of Medical Chemistry, Molecular Biology, and Pathobiochemistry, Faculty of Medicine, Semmelweis University.

Conflict of interest

The author declares no conflict of interest.

Author details

Zsolt Fabian

Address all correspondence to: fabian.zsolt@med.semmelweis-univ.hu

Department of Medical Chemistry, Molecular Biology and Pathobiochemistry, Faculty of Medicine, Semmelweis University, Budapest, Hungary

References

- [1] Conway Morris S. Darwin's dilemma: The realities of the Cambrian 'explosion'. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*. 2006;**361**(1470):1069-1083
- [2] Semenza GL. Hypoxia-inducible factor 1: Control of oxygen homeostasis in health and disease. *Pediatric Research*. 2001;**49**(5):614-617
- [3] Semenza GL et al. Hypoxia-inducible nuclear factors bind to an enhancer element located 3' to the human erythropoietin gene. *Proceedings of the National Academy of Sciences of the United States of America*. 1991;**88**(13):5680-5684
- [4] Talks KL et al. The expression and distribution of the hypoxia-inducible factors HIF-1 alpha and HIF-2 alpha in normal human tissues, cancers, and tumor-associated macrophages. *The American Journal of Pathology*. 2000;**157**(2):411-421
- [5] Jain S et al. Expression of ARNT, ARNT2, HIF1 alpha, HIF2 alpha and Ah receptor mRNAs in the developing mouse. *Mechanisms of Development*. 1998;**73**(1):117-123
- [6] Jaakkola P et al. Targeting of HIF-alpha to the von Hippel-Lindau ubiquitylation complex by O₂-regulated prolyl hydroxylation. *Science*. 2001;**292**(5516):468-472
- [7] Bruick RK, McKnight SL. A conserved family of prolyl-4-hydroxylases that modify HIF. *Science*. 2001;**294**(5545):1337-1340
- [8] Willam C et al. The prolyl hydroxylase enzymes that act as oxygen sensors regulating destruction of hypoxia-inducible factor alpha. *Advances in Enzyme Regulation*. 2004;**44**:75-92
- [9] Hirsila M et al. Characterization of the human prolyl 4-hydroxylases that modify the hypoxia-inducible factor. *The Journal of Biological Chemistry*. 2003;**278**(33):30772-30780
- [10] Maxwell PH et al. The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. *Nature*. 1999;**399**(6733):271-275
- [11] Lando D et al. Asparagine hydroxylation of the HIF transactivation domain a hypoxic switch. *Science*. 2002;**295**(5556):858-861
- [12] Li SH et al. A novel mode of action of YC-1 in HIF inhibition: Stimulation of FIH-dependent p300 dissociation from HIF-1 {alpha}. *Molecular Cancer Therapeutics*. 2008;**7**(12):3729-3738
- [13] McNeill LA et al. Hypoxia-inducible factor asparaginyl hydroxylase (FIH-1) catalyses hydroxylation at the beta-carbon of asparagine-803. *The Biochemical Journal*. 2002;**367**(Pt 3):571-575
- [14] Semenza GL, Wang GL. A nuclear factor induced by hypoxia via de novo protein synthesis binds to the human erythropoietin gene enhancer at a site required for transcriptional activation. *Molecular and Cellular Biology*. 1992;**12**(12):5447-5454

- [15] Wang GL, Semenza GL. Desferrioxamine induces erythropoietin gene expression and hypoxia-inducible factor 1 DNA-binding activity: Implications for models of hypoxia signal transduction. *Blood*. 1993;**82**(12):3610-3615
- [16] Nemeth E et al. Heparin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. *Science*. 2004;**306**(5704):2090-2093
- [17] Ravasi G et al. Circulating factors are involved in hypoxia-induced hepcidin suppression. *Blood Cells, Molecules and Diseases*. 2014;**53**(4):204-210
- [18] Mastrogiannaki M et al. Hepatic hypoxia-inducible factor-2 down-regulates hepcidin expression in mice through an erythropoietin-mediated increase in erythropoiesis. *Haematologica*. 2012;**97**(6):827-834
- [19] Hintze KJ, McClung JP. Heparin: A critical regulator of iron metabolism during hypoxia. *Advances in Hematology*. 2011;**2011**:510304
- [20] Ravasi G et al. Heparin regulation in a mouse model of acute hypoxia. *European Journal of Haematology*. 2018;**100**(6):636-643
- [21] Leung PS et al. Increased duodenal iron uptake and transfer in a rat model of chronic hypoxia is accompanied by reduced hepcidin expression. *Gut*. 2005;**54**(10):1391-1395
- [22] Shweiki D et al. Vascular endothelial growth factor induced by hypoxia may mediate hypoxia-initiated angiogenesis. *Nature*. 1992;**359**(6398):843-845
- [23] Takeda N et al. Endothelial PAS domain protein 1 gene promotes angiogenesis through the transactivation of both vascular endothelial growth factor and its receptor, Flt-1. *Circulation Research*. 2004;**95**(2):146-153
- [24] De Bock K et al. Role of PFKFB3-driven glycolysis in vessel sprouting. *Cell*. 2013;**154**(3):651-663
- [25] Xu Y et al. Endothelial PFKFB3 plays a critical role in angiogenesis. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2014;**34**(6):1231-1239
- [26] McNeill LA et al. Hypoxia-inducible factor prolyl hydroxylase 2 has a high affinity for ferrous iron and 2-oxoglutarate. *Molecular BioSystems*. 2005;**1**(4):321-324
- [27] Benita Y et al. An integrative genomics approach identifies Hypoxia Inducible Factor-1 (HIF-1)-target genes that form the core response to hypoxia. *Nucleic Acids Research*. 2009;**37**(14):4587-4602
- [28] Linn TC, Pettit FH, Reed LJ. Alpha-keto acid dehydrogenase complexes. X. Regulation of the activity of the pyruvate dehydrogenase complex from beef kidney mitochondria by phosphorylation and dephosphorylation. *Proceedings of the National Academy of Sciences of the United States of America*. 1969;**62**(1):234-241
- [29] Seifert F et al. Phosphorylation of serine 264 impedes active site accessibility in the E1 component of the human pyruvate dehydrogenase multienzyme complex. *Biochemistry*. 2007;**46**(21):6277-6287

- [30] Pan Y et al. Multiple factors affecting cellular redox status and energy metabolism modulate hypoxia-inducible factor prolyl hydroxylase activity in vivo and in vitro. *Molecular and Cellular Biology*. 2007;**27**(3):912-925
- [31] Selak MA et al. Succinate links TCA cycle dysfunction to oncogenesis by inhibiting HIF- α prolyl hydroxylase. *Cancer Cell*. 2005;**7**(1):77-85
- [32] Tannahill GM et al. Succinate is an inflammatory signal that induces IL-1 β through HIF-1 α . *Nature*. 2013;**496**(7444):238-242
- [33] Mracek T, Drahotka Z, Houstek J. The function and the role of the mitochondrial glycerol-3-phosphate dehydrogenase in mammalian tissues. *Biochimica et Biophysica Acta*. 2013;**1827**(3):401-410
- [34] Kelly TJ et al. A hypoxia-induced positive feedback loop promotes hypoxia-inducible factor 1 α stability through miR-210 suppression of glycerol-3-phosphate dehydrogenase 1-like. *Molecular and Cellular Biology*. 2011;**31**(13):2696-2706
- [35] Hagen T et al. Redistribution of intracellular oxygen in hypoxia by nitric oxide: effect on HIF1 α . *Science*. 2003;**302**(5652):1975-1978
- [36] Tanaka H et al. MicroRNA-183 upregulates HIF-1 α by targeting isocitrate dehydrogenase 2 (IDH2) in glioma cells. *Journal of Neuro-Oncology*. 2013;**111**(3):273-283
- [37] Giannakakis A et al. miR-210 links hypoxia with cell cycle regulation and is deleted in human epithelial ovarian cancer. *Cancer Biology and Therapy*. 2008;**7**(2):255-264
- [38] Marxsen JH et al. Hypoxia-inducible factor-1 (HIF-1) promotes its degradation by induction of HIF- α -prolyl-4-hydroxylases. *The Biochemical Journal*. 2004;**381**(Pt 3):761-767
- [39] Stiehl DP et al. Increased prolyl 4-hydroxylase domain proteins compensate for decreased oxygen levels. Evidence for an autoregulatory oxygen-sensing system. *Journal of Biological Chemistry*. 2006;**281**(33):23482-23491
- [40] Zhang Z et al. MicroRNA miR-210 modulates cellular response to hypoxia through the MYC antagonist MNT. *Cell Cycle*. 2009;**8**(17):2756-2768
- [41] Kim JW et al. Hypoxia-inducible factor 1 and dysregulated c-Myc cooperatively induce vascular endothelial growth factor and metabolic switches hexokinase 2 and pyruvate dehydrogenase kinase 1. *Molecular and Cellular Biology*. 2007;**27**(21):7381-7393
- [42] Doe MR et al. Myc posttranscriptionally induces HIF1 protein and target gene expression in normal and cancer cells. *Cancer Research*. 2012;**72**(4):949-957
- [43] Zhang H et al. HIF-1 inhibits mitochondrial biogenesis and cellular respiration in VHL-deficient renal cell carcinoma by repression of C-MYC activity. *Cancer Cell*. 2007;**11**(5):407-420
- [44] Koshiji M et al. HIF-1 α induces cell cycle arrest by functionally counteracting Myc. *The EMBO Journal*. 2004;**23**(9):1949-1956

- [45] Yoshioka Y et al. Micromanaging iron homeostasis: Hypoxia-inducible micro-RNA-210 suppresses iron homeostasis-related proteins. *The Journal of Biological Chemistry*. 2012;**287**(41):34110-34119
- [46] Wang H et al. Negative regulation of Hif1a expression and TH17 differentiation by the hypoxia-regulated microRNA miR-210. *Nature Immunology*. 2014;**15**(4):393-401
- [47] Bruning U et al. MicroRNA-155 promotes resolution of hypoxia-inducible factor 1 alpha activity during prolonged hypoxia. *Molecular and Cellular Biology*. 2011;**31**(19):4087-4096
- [48] Makino Y et al. Inhibitory PAS domain protein (IPAS) is a hypoxia-inducible splicing variant of the hypoxia-inducible factor-3 alpha locus. *The Journal of Biological Chemistry*. 2002;**277**(36):32405-32408
- [49] Makino Y et al. Inhibitory PAS domain protein is a negative regulator of hypoxia-inducible gene expression. *Nature*. 2001;**414**(6863):550-554
- [50] Makino Y et al. Transcriptional up-regulation of inhibitory PAS domain protein gene expression by hypoxia-inducible factor 1 (HIF-1): A negative feedback regulatory circuit in HIF-1-mediated signaling in hypoxic cells. *The Journal of Biological Chemistry*. 2007;**282**(19):14073-14082
- [51] Reyes H, Reisz-Porszasz S, Hankinson O. Identification of the Ah receptor nuclear translocator protein (Arnt) as a component of the DNA binding form of the Ah receptor. *Science*. 1992;**256**(5060):1193-1195
- [52] Poland A, Glover E, Kende AS. Stereospecific, high affinity binding of 2,3,7,8-tetrachlorodibenzo-p-dioxin by hepatic cytosol. Evidence that the binding species is receptor for induction of aryl hydrocarbon hydroxylase. *The Journal of Biological Chemistry*. 1976;**251**(16):4936-4946
- [53] Perdew GH. Association of the Ah receptor with the 90-kDa heat shock protein. *The Journal of Biological Chemistry*. 1988;**263**(27):13802-13805
- [54] Hoffman EC et al. Cloning of a factor required for activity of the Ah (dioxin) receptor. *Science*. 1991;**252**(5008):954-958
- [55] Tomita S et al. Conditional disruption of the aryl hydrocarbon receptor nuclear translocator (Arnt) gene leads to loss of target gene induction by the aryl hydrocarbon receptor and hypoxia-inducible factor 1 alpha. *Molecular Endocrinology*. 2000;**14**(10):1674-1681
- [56] Nukaya M et al. Aryl hydrocarbon receptor nuclear translocator in hepatocytes is required for aryl hydrocarbon receptor-mediated adaptive and toxic responses in liver. *Toxicological Sciences*. 2010;**118**(2):554-563
- [57] Whitlock JP Jr et al. Cytochromes P450 5: Induction of cytochrome P4501A1: A model for analyzing mammalian gene transcription. *The FASEB Journal*. 1996;**10**(8):809-818
- [58] Munzel PA et al. Aryl hydrocarbon receptor-inducible or constitutive expression of human UDP glucuronosyltransferase UGT1A6. *Archives of Biochemistry and Biophysics*. 1998;**350**(1):72-78

- [59] Nie M, Blankenship AL, Giesy JP. Interactions between aryl hydrocarbon receptor (AhR) and hypoxia signaling pathways. *Environmental Toxicology and Pharmacology*. 2001;**10**(1-2):17-27
- [60] Schults MA et al. Diminished carcinogen detoxification is a novel mechanism for hypoxia-inducible factor 1-mediated genetic instability. *The Journal of Biological Chemistry*. 2010;**285**(19):14558-14564
- [61] Gradin K et al. Functional interference between hypoxia and dioxin signal transduction pathways: Competition for recruitment of the Arnt transcription factor. *Molecular and Cellular Biology*. 1996;**16**(10):5221-5231
- [62] Chan WK et al. Cross-talk between the aryl hydrocarbon receptor and hypoxia inducible factor signaling pathways. Demonstration of competition and compensation. *Journal of Biological Chemistry*. 1999;**274**(17):12115-12123
- [63] Mayerhofer M et al. BCR/ABL induces expression of vascular endothelial growth factor and its transcriptional activator, hypoxia inducible factor-1 alpha, through a pathway involving phosphoinositide 3-kinase and the mammalian target of rapamycin. *Blood*. 2002;**100**(10):3767-3775
- [64] Treins C et al. Insulin stimulates hypoxia-inducible factor 1 through a phosphatidylinositol 3-kinase/target of rapamycin-dependent signaling pathway. *The Journal of Biological Chemistry*. 2002;**277**(31):27975-27981
- [65] Humar R et al. Hypoxia enhances vascular cell proliferation and angiogenesis in vitro via rapamycin (mTOR)-dependent signaling. *The FASEB Journal*. 2002;**16**(8):771-780
- [66] Land SC, Tee AR. Hypoxia-inducible factor 1 alpha is regulated by the mammalian target of rapamycin (mTOR) via an mTOR signaling motif. *Journal of Biological Chemistry*. 2007;**282**(28):20534-20543
- [67] Pez F et al. The HIF-1-inducible lysyl oxidase activates HIF-1 via the Akt pathway in a positive regulation loop and synergizes with HIF-1 in promoting tumor cell growth. *Cancer Research*. 2011;**71**(5):1647-1657
- [68] Heesom KJ, Denton RM. Dissociation of the eukaryotic initiation factor-4E/4E-BP1 complex involves phosphorylation of 4E-BP1 by an mTOR-associated kinase. *FEBS Letters*. 1999;**457**(3):489-493
- [69] Magagnin MG et al. The mTOR target 4E-BP1 contributes to differential protein expression during normoxia and hypoxia through changes in mRNA translation efficiency. *Proteomics*. 2008;**8**(5):1019-1028
- [70] Nayak BK et al. Stabilization of HIF-2 alpha through redox regulation of mTORC2 activation and initiation of mRNA translation. *Oncogene*. 2013;**32**(26):3147-3155
- [71] Moon EJ et al. NADPH oxidase-mediated reactive oxygen species production activates hypoxia-inducible factor-1 (HIF-1) via the ERK pathway after hyperthermia treatment.

Proceedings of the National Academy of Sciences of the United States of America. 2010;**107**(47):20477-20482

- [72] Bonello S et al. Reactive oxygen species activate the HIF-1 alpha promoter via a functional NFkappaB site. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2007;**27**(4):755-761
- [73] Simon MC. Mitochondrial reactive oxygen species are required for hypoxic HIF alpha stabilization. *Advances in Experimental Medicine and Biology*. 2006;**588**:165-170
- [74] Demidenko ZN, Blagosklonny MV. The purpose of the HIF-1/PHD feedback loop: To limit mTOR-induced HIF-1 alpha. *Cell Cycle*. 2011;**10**(10):1557-1562
- [75] Cassavaugh JM et al. Negative regulation of HIF-1 alpha by an FBW7-mediated degradation pathway during hypoxia. *Journal of Cellular Biochemistry*. 2011;**112**(12):3882-3890
- [76] Welcker M, Clurman BE. FBW7 ubiquitin ligase: A tumour suppressor at the crossroads of cell division, growth and differentiation. *Nature Reviews. Cancer*. 2008;**8**(2):83-93
- [77] Sakamoto T et al. Hypoxia-inducible factor 1 regulation through cross talk between mTOR and MT1-MMP. *Molecular and Cellular Biology*. 2014;**34**(1):30-42
- [78] Lando D et al. FIH-1 is an asparaginyl hydroxylase enzyme that regulates the transcriptional activity of hypoxia-inducible factor. *Genes and Development*. 2002;**16**(12):1466-1471
- [79] Petrella BL, Lohi J, Brinckerhoff CE. Identification of membrane type-1 matrix metalloproteinase as a target of hypoxia-inducible factor-2 alpha in von Hippel-Lindau renal cell carcinoma. *Oncogene*. 2005;**24**(6):1043-1052
- [80] Brown EJ et al. Control of p70 s6 kinase by kinase activity of FRAP in vivo. *Nature*. 1995;**377**(6548):441-446
- [81] Brugarolas J et al. Regulation of mTOR function in response to hypoxia by REDD1 and the TSC1/TSC2 tumor suppressor complex. *Genes and Development*. 2004;**18**(23):2893-2904
- [82] Inoki K et al. Rheb GTPase is a direct target of TSC2 GAP activity and regulates mTOR signaling. *Genes and Development*. 2003;**17**(15):1829-1834
- [83] Li Y et al. Bnip3 mediates the hypoxia-induced inhibition on mammalian target of rapamycin by interacting with Rheb. *The Journal of Biological Chemistry*. 2007;**282**(49):35803-35813
- [84] Wan G et al. Hypoxia-induced MIR155 is a potent autophagy inducer by targeting multiple players in the MTOR pathway. *Autophagy*. 2014;**10**(1):70-79
- [85] Rius J et al. NF-kappaB links innate immunity to the hypoxic response through transcriptional regulation of HIF-1 alpha. *Nature*. 2008;**453**(7196):807-811
- [86] Minet E et al. HIF1A gene transcription is dependent on a core promoter sequence encompassing activating and inhibiting sequences located upstream from the transcription initiation site and cis elements located within the 5' UTR. *Biochemical and Biophysical Research Communications*. 1999;**261**(2):534-540

- [87] Fitzpatrick SF et al. An intact canonical NF-kappaB pathway is required for inflammatory gene expression in response to hypoxia. *Journal of Immunology*. 2011;**186**(2):1091-1096
- [88] Cummins EP et al. Prolyl hydroxylase-1 negatively regulates IkappaB kinase-beta, giving insight into hypoxia-induced NFkappaB activity. *Proceedings of the National Academy of Sciences of the United States of America*. 2006;**103**(48):18154-18159
- [89] Scholz CC et al. Regulation of IL-1beta-induced NF-kappaB by hydroxylases links key hypoxic and inflammatory signaling pathways. *Proceedings of the National Academy of Sciences of the United States of America*. 2013;**110**(46):18490-18495
- [90] van Uden P et al. Evolutionary conserved regulation of HIF-1beta by NF-kappaB. *PLoS Genetics*. 2011;**7**(1):e1001285
- [91] Fitzpatrick SF et al. Prolyl hydroxylase-1 regulates hepatocyte apoptosis in an NF-kappaB-dependent manner. *Biochemical and Biophysical Research Communications*. 2016;**474**(3):579-586
- [92] Oliver KM et al. Hypoxia activates NF-kappaB-dependent gene expression through the canonical signaling pathway. *Antioxidants and Redox Signaling*. 2009;**11**(9):2057-2064
- [93] Schmedtje JFJ et al. Hypoxia induces cyclooxygenase-2 via the NF-kappaB p65 transcription factor in human vascular endothelial cells. *The Journal of Biological Chemistry*. 1997;**272**(1):601-608
- [94] Figueroa YG et al. NF-kappaB plays a key role in hypoxia-inducible factor-1-regulated erythropoietin gene expression. *Experimental Hematology*. 2002;**30**(12):1419-1427
- [95] Bandarra D et al. HIF-1 alpha restricts NF-kappaB-dependent gene expression to control innate immunity signals. *Disease Models and Mechanisms*. 2015;**8**(2):169-181
- [96] Richard DE et al. p42/p44 mitogen-activated protein kinases phosphorylate hypoxia-inducible factor 1 alpha (HIF-1 alpha) and enhance the transcriptional activity of HIF-1. *The Journal of Biological Chemistry*. 1999;**274**(46):32631-32637
- [97] Minet E et al. ERK activation upon hypoxia: Involvement in HIF-1 activation. *FEBS Letters*. 2000;**468**(1):53-58
- [98] Sodhi A et al. The Kaposi's sarcoma-associated herpes virus G protein-coupled receptor up-regulates vascular endothelial growth factor expression and secretion through mitogen-activated protein kinase and p38 pathways acting on hypoxia-inducible factor 1 alpha. *Cancer Research*. 2000;**60**(17):4873-4880
- [99] Shi YH et al. In vitro study of HIF-1 activation and VEGF release by bFGF in the T47D breast cancer cell line under normoxic conditions: Involvement of PI-3K/Akt and MEK1/ERK pathways. *The Journal of Pathology*. 2005;**205**(4):530-536
- [100] Dimova EY, Kietzmann T. The MAPK pathway and HIF-1 are involved in the induction of the human PAI-1 gene expression by insulin in the human hepatoma cell line HepG2. *Annals of the New York Academy of Sciences*. 2006;**1090**:355-367

- [101] Mylonis I et al. Identification of MAPK phosphorylation sites and their role in the localization and activity of hypoxia-inducible factor-1 alpha. *The Journal of Biological Chemistry*. 2006;**281**(44):33095-33106
- [102] Mylonis I et al. Atypical CRM1-dependent nuclear export signal mediates regulation of hypoxia-inducible factor-1 alpha by MAPK. *The Journal of Biological Chemistry*. 2008;**283**(41):27620-27627
- [103] Fabian Z et al. Basic fibroblast growth factor modifies the hypoxic response of human bone marrow stromal cells by ERK-mediated enhancement of HIF-1 alpha activity. *Stem Cell Research*. 2014;**12**(3):646-658

IntechOpen