© 2021. Published by The Company of Biologists Ltd | Development (2021) 148, dev200030. doi:10.1242/dev.200030

PRIMER

Oxygen-sensing mechanisms in development and tissue repair

Yida Jiang[1], Li-Juan Duan[1] and Guo-Hua Fong[1,2,*]

ABSTRACT

Under normoxia, hypoxia inducible factor (HIF) α subunits are hydroxylated by PHDs (prolyl hydroxylase domain proteins) and subsequently undergo polyubiquitylation and degradation. Normal embryogenesis occurs under hypoxia, which suppresses PHD activities and allows HIFα to stabilize and regulate development. In this Primer, we explain molecular mechanisms of the oxygen-sensing pathway, summarize HIF-regulated downstream events, discuss loss-of-function phenotypes primarily in mouse development, and highlight clinical relevance to angiogenesis and tissue repair.

KEY WORDS: Oxygen sensing, Prolyl hydroxylase, Hypoxia inducible factor, Development, Tissue repair

Introduction

Before the onset of vasculogenesis, normal embryonic tissues are under developmental hypoxia, with oxygen partial pressure (pO_2) averaging ~23 mm Hg (~3% of sea level air) (Fathollahipour et al., 2018). Although the development of the cardiovascular systems brings in additional oxygen, the upper limit of the embryonic oxygenation level is dictated by placental pO_2 at 60 mm Hg (7.9%), which is substantially lower than the 10-13.1% found in adult arterial blood (Rodesch et al., 1992). In addition, rapid tissue growth is energetically demanding and consumes oxygen at high rates. For these reasons, embryonic tissues remain more hypoxic throughout development relative to adult tissues (Rieger et al., 1997; Siggaard-Andersen and Huch, 1995).

Hypoxia in normal embryos serves as a physiological signal that regulates diverse developmental processes. A key mechanism mediating hypoxia signaling is oxygen-dependent degradation of HIFα proteins. Under normoxia, prolyl hydroxylase domain (PHD) proteins split O_2 to hydroxylate specific proline residues in hypoxia inducible factor (HIF) α proteins, labeling them for polyubiquitylation and proteasomal degradation (Fig. 1A). Under hypoxia, hydroxylation is blocked; HIFα proteins translocate into nuclei where they heterodimerize with HIFβ proteins to activate the expression of their target genes (Fig. 1B) (Bruick and McKnight, 2001; Epstein et al., 2001; Hu et al., 2006; Ivan et al., 2002; Maxwell et al., 1999; Wang et al., 1995). For their discovery of the oxygen-sensing mechanism, William Kaelin Jr, Sir Peter Ratcliffe and Gregg Semenza were awarded the 2019 Nobel Prize in Physiology or Medicine (NobelPrize.Org, 2019).

In this Primer, we provide an overview of molecular and cellular mechanisms of oxygen sensing and hypoxia signaling, and summarize loss-of-function phenotypes in development, mostly in mice. We also highlight clinical relevance, focusing on angiogenesis and tissue ischemia (lack of perfusion). A discussion of other pathological processes in which hypoxia is also a component, such as tumorigenesis, diabetes and inflammation, is beyond the scope of this Primer. However, interested readers may consult several recent reviews on these subjects (Choueiri and Kaelin, 2020; Hayes et al., 2020; Semenza, 2019; Stothers et al., 2018).

Molecular mechanisms of the oxygen-sensing pathway

PHDs and other HIF-hydroxylases

Structure and intracellular localization of PHDs

The first PHD family member to be discovered was the EGLN1 protein encoded by the egg laying 9 (*egl-9*) gene in *C. elegans* (Epstein et al., 2001). In mammals, the PHD family has three members: EGLN1 (PHD2), EGLN2 (PHD1) and EGLN3 (PHD3, also known as SM-20 in rats) (Bruick and McKnight, 2001; Epstein et al., 2001; Ivan et al., 2002). Structural features of PHD proteins are shown schematically in Fig. 2A. Notably, PHD3 has a shortened sequence, containing only the catalytic domain, whereas PHD2 is unique in that it contains a MYND domain, which mediates specific protein interaction (Barth et al., 2009; Shao et al., 2009; Song et al., 2013). PHD1, PHD2 and PHD3 are all soluble enzymes: PHD1 is exclusively located in the nucleus; PHD2 is predominantly cytoplasmic, but also shuttles into the nucleus; PHD3 is evenly distributed between both the nucleus and the cytoplasm (Metzen et al., 2003; Steinhoff et al., 2009). Functionally, PHDs are iron (Fe^{2+}) and 2-oxoglutarate (2-OG)-dependent dioxygenases, which split O_2 to hydroxylate their substrates (Epstein et al., 2001). In addition, PHDs have various oxygen-independent functions (Box 1) and substrates (Box 2).

Prolyl 4-hydroxylase transmembrane (P4H-TM, PHD4 or EGLN4) is anchored in the endoplasmic reticulum (ER) membrane with its catalytic domain protruding into the ER lumen, and has an EF domain (Fig. 2A), which may potentially bind Ca^{2+} (Koivunen et al., 2007; Myllykoski et al., 2021). The P4H-TM protein sequence is more closely related to collagen hydroxylase than to PHDs, except that the substrate-binding region is more conserved with PHDs (Koivunen et al., 2007). Although P4H-TM has been shown to carry out prolyl hydroxylation in both HIF1α and HIF2α (Koivunen et al., 2007), it is unclear how it overcomes the topological barrier imposed by the ER membrane, or whether there are physiologically relevant levels of HIFα proteins within ER lumens. Finally, factor inhibiting HIF (FIH) is an oxygen-dependent asparagine hydroxylase but its domain organization has not been clearly defined (Koivunen et al., 2004; Mahon et al., 2001). FIH mostly hydroxylates a C-terminal asparaginyl residue on HIF1α and, to a much less extent, HIF2α (Koivunen et al., 2004).

Regulation of PHD expression and degradation under hypoxia

Among the different PHD family members, PHD2 is most broadly expressed and plays a major role in oxygen sensing (Berra et al., 2003). The expression of PHDs, especially PHD2 and PHD3, is upregulated by HIF1α (Fig. 2B), presumably as a feedback

[1]Center for Vascular Biology, University of Connecticut Health Center, Farmington, CT 06030, USA. [2]Department of Cell Biology, University of Connecticut Health Center, Farmington, CT 06030, USA.

*Author for correspondence (fong@uchc.edu)

G.-H.F., 0000-0001-5792-6486

Development (2021) 148, dev200030. doi:10.1242/dev.200030

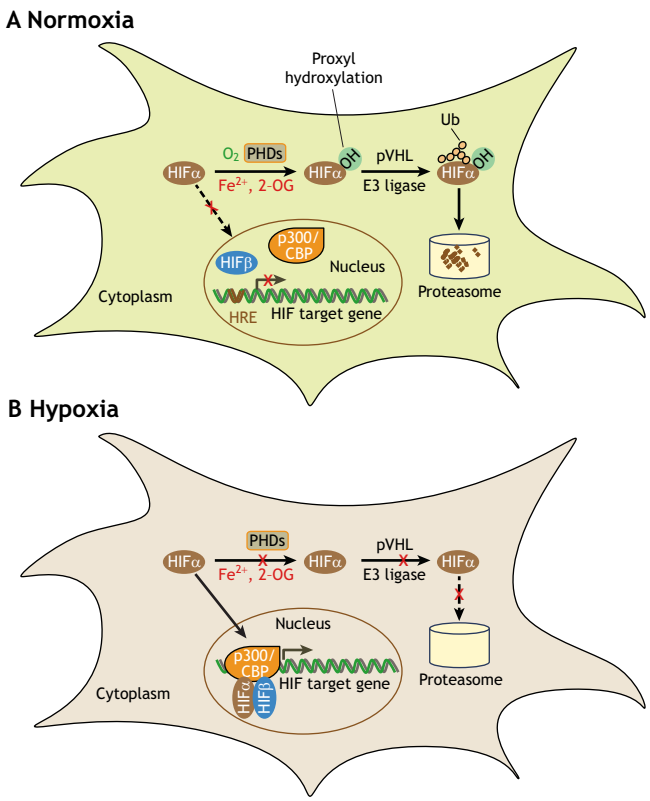

Fig. 1. Schematic showing oxygen-dependent regulation of HIFα stability by prolyl hydroxylase domain proteins (PHDs). (A) In normoxia, and in the presence of adequate levels of Fe^{2+} and 2-oxoglutarate (2-OG), PHDs split O_2 to hydroxylate (OH) HIF1α and HIF2α (indicated as HIFα), which are then recruited into E3 ubiquitin ligase complexes by pVHL (von Hippel Lindau protein), polyubiquitylated (Ub) and degraded by the proteasome. As a result, HIFα fail to enter nucleus. (B) In hypoxia, the hydroxylation reaction is mostly blocked (red crosses). HIFα proteins are stable, and translocate into the nucleus where they heterodimerize with HIFβ. HIFα also recruits p300/CBP to activate the transcription of target genes.

mechanism to prevent uncontrolled HIFα accumulation under hypoxia (Bagnall et al., 2014; Fujita et al., 2012). On the other hand, the levels of PHD1 and PHD3, but not PHD2, are further fine-tuned by Siah1a/2-mediated ubiquitylation and proteasomal degradation under hypoxia (Fig. 2B) (Nakayama et al., 2004). It should be noted that in cells under physiological hypoxia, oxygen-dependent prolyl hydroxylation is still an ongoing process (Fig. 2B), although at a significantly slower pace than under normoxia. Once hydroxylated, degradation proceeds independently of oxygen concentration.

Oxygen-independent regulation of PHD prolyl hydroxylase activity

As mentioned above, the catalytic activity of PHDs depends on not only oxygen, but also their essential co-factors Fe^{2+} and 2-OG (Fig. 2C) (Epstein et al., 2001; Gerald et al., 2004; Hewitson et al., 2007). The availability of Fe^{2+} is significantly influenced by cellular redox states. Reactive oxygen species (ROS) lead to the oxidation of Fe^{2+} to Fe^{3+}, the latter of which strongly suppresses PHD activities (Briggs et al., 2016; Epstein et al., 2001; Gerald et al., 2004). Several Krebs cycle intermediates, including fumarate, succinate and isocitrate, are structural analogs to 2-OG. In wild-type cells, these intermediates are mostly confined to mitochondria, but in cells with dysfunctional mitochondria, they can accumulate at high levels and leak into the cytoplasm to competitively inhibit PHDs (Hewitson et al., 2007).

Prolyl hydroxylation efficiency of HIF1α is enhanced by recruiting PHD2 to HSP90/HIF1α complex (Fig. 2D), via the interaction between MYND domain in PHD2 and co-chaperonin proteins of HSP90, including p23 and possibly also FKBP38 (Barth et al., 2009; Shao et al., 2009; Song et al., 2013). As a result, PHD2 is brought into close proximity of HIF1α. Furthermore, interaction between PHD2 and a protein called OS-9, also thought to work by bringing PHD2 closer to HIF1α, can also enhance hydroxylation, but specific domains involved in the interaction have not been characterized (Baek et al., 2005).

Hypoxia-induced factors

Structural organization and heterodimerization of HIF proteins

As depicted in Fig. 3, both HIFα and β subunits are members of the bHLH-PAS family of transcription factors. The N-terminal basic-helix-loop-helix (bHLH) motif (for DNA binding) and central PAS domain (mediating protein-protein interaction) are well conserved among different subunits, but the length and location of catalytic domains display substantial variability, except between HIF1α and HIF2α (Rieger et al., 1997; Tian et al., 1997; Wang et al., 1995).

HIF3α exists in three isoforms due to alternative splicing (Huang et al., 2013; Makino et al., 2001; Yamashita et al., 2008). HIF3α isoforms have very weak or no transcriptional activity, due to the lack of C-TAD or the entire catalytic domain, depending on the isoform (Huang et al., 2013; Makino et al., 2001; Yamashita et al., 2008). Therefore, HIF3α isoforms, especially inhibitory PAS domain protein (IPAS), play inhibitory roles by trapping HIF1β and competing for binding to hypoxia response elements (HREs) (Huang et al., 2013; Makino et al., 2001). In addition, IPAS also directly binds to HIF1α to inhibit its activity (Huang et al., 2013; Makino et al., 2001).

HIF1β, also known as ARNT or ARNT1 [aryl hydrocarbon receptor (AhR) nuclear translocator], heterodimerizes with HIF1α and HIF2α proteins to form HIF1 and HIF2 heterodimers, respectively (Motta et al., 2018; Wang et al., 1995; Wu et al., 2015). ARNT also heterodimerizes with AhR to mediate xenobiotic detoxification response, a HIF-independent function (Reisz-Porszasz et al., 1994).

ARNT2 is similar to ARNT in structure, although its expression pattern is much more restricted (Hirose et al., 1996; Keith et al., 2001; Mandl et al., 2016). Within the HIF family, studies on ARNT2 are mostly limited to its interaction with HIF1α (Hirose et al., 1996; Keith et al., 2001; Mandl et al., 2016). However, ARNT2 (but not ARNT) is known to heterodimerize with SIM1, an oxygen-independent member of the bHLH-PAS family of transcription factors involved in hypothalamus development (Michaud et al., 2000).

Expression patterns of HIFα and β subunits

In mouse embryos, HIF1α is broadly expressed, whereas HIF2α has a more restricted expression pattern (Jain et al., 1998). HIF2α was first thought to be specifically expressed in the vascular endothelium (hence, also named endothelial PAS protein 1 or EPAS1) (Tian et al., 1997), but was later found to be expressed in additional cell types, such as kidney interstitial fibroblasts, neural crest cells and retinal astrocytes (Mowat et al., 2010; Niklasson et al., 2021; Paliege et al., 2010; Tian et al., 1998). In adult mice and cultured cells, the expression of both HIF1α and HIF2α is induced by growth factors and cytokines, independently of oxygenation conditions, but it is unclear whether similar induction also occurs in embryos (Dutta et al., 2008; Feldhoff et al., 2017; Wang et al., 2018).

Development (2021) 148, dev200030. doi:10.1242/dev.200030

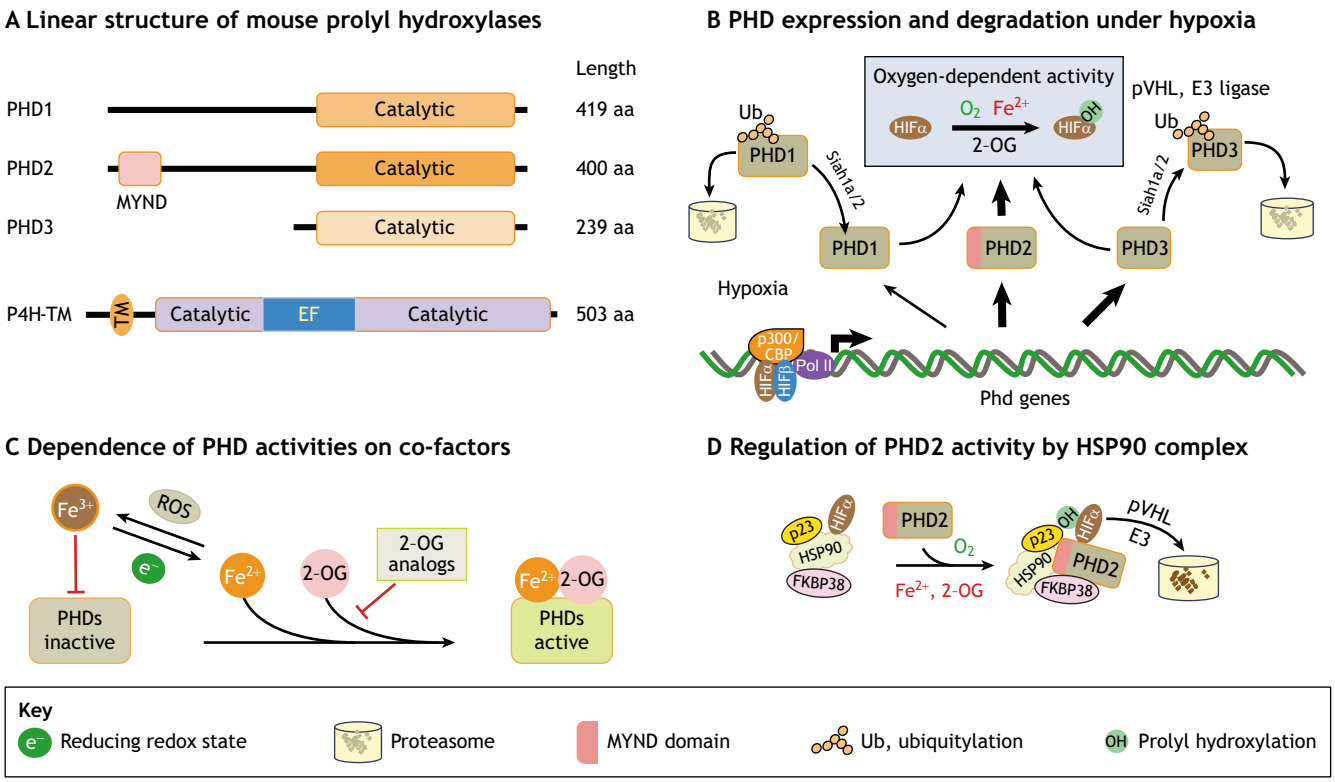

Fig. 2. Regulation of prolyl hydroxylase domain protein (PHD) expression, stability and activity. (A) Linear structure of prolyl hydroxylases. MYND in PHD2 interacts with p23 and FKBP38; EF hand (EF) domain is believed to bind to Ca^{2+}. (B) Induction of PHD expression and degradation of PHD1 and PHD3 under hypoxia. PHD2 and PHD3 are more strongly expressed in response to hypoxia than PHD1 (indicated by arrow thickness). Siah1a/2 (a ubiquitin ligase) polyubiquitylates PHD1 and PHD3 (but not PHD2) under hypoxia. PHD1 and PHD3 are then degraded in proteasomes. In hypoxia, oxygen-dependent prolyl hydroxylation is inefficient, but is not completely blocked (blue box), leading to the slow degradation of HIF1α and HIF2α (indicated as HIFα). (C) Regulation of PHD activities by modulating co-factor availability and interaction. Reduced redox state (e^-) may facilitate PHD activation by promoting the regeneration of Fe^{2+} from Fe^{3+}, whereas reactive oxygen species (ROS) do the reverse. Several Krebs cycle intermediates (fumerate, succinate and isocitrate – not included in the figure) are structural analogs of 2-OG and competitively inhibit PHDs. (D) A complex containing HSP90, HIF1α and co-chaperonin proteins (p23 and possibly also FKBP38) can interact with the MYND domain in PHD2. As a result, PHD2 is brought into close proximity of HIF1α, enhancing prolyl hydroxylation efficiency of HIF1α and leading to its degradation. aa, amino acids; P4H-TM, prolyl 4-hydroxylase transmembrane; TM, transmembrane domain.

HIF3α expression varies greatly among different isoforms. NEPAS expression is strong in multiple embryonic tissues but undetectable in adults (Yamashita et al., 2008). The expression of the prototypical HIF3α isoform is weak in embryos, but is moderately increased in adult tissues (Yamashita et al., 2008). IPAS is undetectable in embryos but is strongly expressed in Purkinje neurons and cornea epithelium in adult mice (Makino et al., 2001; Yamashita et al., 2008).

ARNT is broadly expressed in mouse embryos. In contrast, ARNT2 is strongly expressed only in developing neural and kidney tissues, but its expression is weak in most other embryonic tissues (Jain et al., 1998).

Regulation of HIFα stability by oxygen and PHD-dependent mechanisms

HIF1α and HIF2α are both hydroxylated by PHDs at two proline residues in the conserved LXXLAP motif, each located within a separate oxygen-dependent degradation (ODD) domain (Fig. 3) (Hirsila et al., 2003). Hydroxylation occurs efficiently under normoxia, and hydroxylated HIFα proteins are recruited into E3 ubiquitin ligase complexes by pVHL (von Hippel Lindau protein), polyubiquitylated, and then routed to proteasomes for degradation (Fig. 1A) (Epstein et al., 2001; Ivan et al., 2002; Maxwell et al., 1999; Min et al., 2002). The entire process is very rapid; the half-life of HIF1α protein is only 5-8 min in cells cultured under normoxia (Berra et al., 2001). In a hypoxic tissue environment, such as in developing embryos, the intracellular oxygen concentration is below K_m values (Hirsila et al., 2003), thus allowing much of HIFα proteins to escape hydroxylation and accumulate at relatively high levels.

Regulation of HIF1α protein stability by oxygen-independent mechanisms

In addition to oxygen-dependent degradation, HIFα stability is further fine-tuned by oxygen-independent mechanisms (Fig. 4A). HIF1α is methylated by lysine-specific methyltransferase SET7/9, but demethylated by LSD1, a lysine-specific demethylase (Kim et al., 2016; Liu et al., 2015). Lysine methylated HIF1α is subject to E3 ligase-dependent polyubiquitylation and proteasomal degradation (Kim et al., 2016). Oxygen-independent HIF1α ubiquitylation in hypoxia is suppressed by two mechanisms, including interaction with the molecular chaperone HSP90 (Liu et al., 2007) and phosphorylation by protein kinase A (PKA) (Bullen et al., 2016).

HIFα is also modified by acetylation and SUMOylation under hypoxia, leading to stabilization or degradation by the same modification in a context-dependent manner (Bilton et al., 2005; Carbia-Nagashima et al., 2007; Cheng et al., 2007; Jeong et al., 2002). In addition, HIF1α has been shown to be stabilized or destabilized by p53 in different studies (Madan et al., 2019; Ravi et al., 2000; Sano et al., 2007), but p53 itself is consistently stabilized by HIF1α (An et al., 1998; Chen et al., 2003). Although

Development (2021) 148, dev200030. doi:10.1242/dev.200030

Box 1. Oxygen-independent functions of PHDs

Besides hydroxylating HIFα and non-HIFα substrates, there have been several reports that PHD1 or PHD3 also stabilize certain proteins by hydroxylase-independent mechanisms. For example, PHD3 stabilizes occludin and inhibitor of nuclear factor kappa-B kinase subunit γ (IKKγ) by physically blocking ubiquitylation of these proteins (Chen et al., 2015; Fu and Taubman, 2013). One of these proteins, occluding (Ocln), is developmentally important, with *Ocln*$^{-/-}$ mice exhibiting growth retardation (Saitou et al., 2000).

HIF2α also interacts with p53, the effect on HIF2α is unknown; however, HIF2α suppresses p53 expression and/or activity (Bertout et al., 2009; Das et al., 2012; Roberts et al., 2009).

Regulation of HIF complex assembly by PHD-independent mechanisms

Although the stability of HIFα is mostly regulated by prolyl hydroxylation, the assembly of HIFα into transcriptionally active HIF complexes is regulated by PHD-independent mechanisms (e.g. Fig. 4B). Specifically, HIF1α phosphorylation by p42/p44 MAPK indirectly promotes heterodimerization by promoting HIF1α nuclear localization (Mylonis et al., 2006). On the other hand, HIF3α, especially its IPAS isoform, inhibits HIF heterodimerization by trapping HIF1β (Makino et al., 2001; Yamashita et al., 2008). HIF activity also depends on the recruitment of p300/CBP, which is a major co-activator for HIFα (Ema et al., 1999; Kasper et al., 2005). Oxygen-dependent (but PHD-independent) HIF1α asparaginyl hydroxylation, mediated by FIH, inhibits the interaction between HIF1α and p300/CBP, thus suppressing HIF1α transcriptional activity (Koivunen et al., 2004; Mahon et al., 2001; Chen et al., 2014; Kasper et al., 2005; Lando et al., 2002).

Summary

HIFα prolyl hydroxylation by PHDs plays a major role in oxygen sensing. However, a variety of other regulatory mechanisms are also highly relevant. Both PHD expression and activity are subject to regulation. PHD genes themselves are HIF targets, a feedback loop exists to prevent excessive HIFα accumulation. The hydroxylase activity of PHDs is regulated by a variety of conditions that influence the intracellular levels and distribution of different co-factors of PHDs. At the functional level, PHDs not only hydroxylate HIFα proteins, but also several other proteins (Box 2). Conversely, HIFα proteins are not only hydroxylated by PHDs, but also by P4H-TM and FIH. Furthermore, HIFα stability and activity are also regulated by several mechanisms that are independent of oxygen and hydroxylases.

HIF-regulated downstream events

Regulation of target gene expression

Target gene specificity

HIF1 and HIF2 activate the expression of their target genes by interacting with HRE or HRE-like elements located in the enhancer sequences (Coulet et al., 2003; Kappel et al., 1999). Some of the target genes are common to HIF1 and HIF2, such as VEGF-A and Glut1, but others are preferably activated by either of them (Chen et al., 2001; Downes et al., 2018; Forsythe et al., 1996; Hu et al., 2003). Genes encoding several glycolytic enzymes, such as 6-phosphofructo-2-kinase (PFKFB3) (Obach et al., 2004), phosphoglycerate kinase 1 (PGK1) (Li et al., 1996) and pyruvate dehydrogenase kinase 3 (PDK3) (Lu et al., 2008) are HIF1-specific target genes. Conversely, endothelial expression of several angiogenesis regulators, including eNOS (Coulet et al., 2003), Flk1 (VEGFR2) (Elvert et al., 2003; Kappel et al., 1999), Flt1 (VEGFR1) (Dutta et al., 2008) and *Tie2* (also known as *Tek*) (Jiang et al., 2019), are upregulated by HIF2α. HIF2α also activates the expression of the pluripotency genes *Nanog*, *Pou5f1* (encoding Oct4), *Sox2* and *Erk1* (also known as *Mapk3*) in stem or progenitor cells, such as neural stem cells, myoblast progenitors and mesenchymal stem cells (Gustafsson et al., 2005; Zhu et al., 2016).

In vivo regulation of erythropoietin (EPO) expression represents an interesting example where the expression patterns of HIF1α and HIF2α play a decisive role. EPO is strongly upregulated by HIF1α *in vitro* (Wang and Semenza, 1995). However, *in vivo* studies have demonstrated that EPO expression is dependent on HIF2α in mouse liver, kidney and cortical astrocytes, whereas HIF1α is dispensable (Chavez et al., 2006; Kapitsinou et al., 2010; Rankin et al., 2007). There is evidence that HIF2α, but not HIF1α, is expressed in the cortex interstitial cells, the main cell type responsible for EPO production (Paliege et al., 2010). However, HIF1α overexpression in brain tissues activates local EPO expression, conferring neural protection in the cerebral ischemia-reperfusion model (Li et al., 2020).

Interestingly, HIF2α promotes translation, in addition to transcription, under hypoxia (Uniacke et al., 2012). In hypoxic cells, the normal translational initiation mechanism is suppressed. However, HIF2α interacts with polysomes to facilitate the translation of mRNAs important for cellular functions under hypoxia (Uniacke et al., 2012).

Cooperation between HIFα and other transcription factors

Besides HIFβ and p300/CBP, HIFα proteins also directly bind to a number of other transcription factors. In a mouse myogenic cell line, HIF1α interacts with Notch intracellular domain (NICD), leading to the upregulation of NICD target genes that are important for the maintenance of an undifferentiated state (Gustafsson et al., 2005). HIF2α-Ets interaction enhances the expression of *Flt1* and *Flk1* *in vitro* and/or in mice (Dutta et al., 2008; Elvert et al., 2003). HIF1α stabilizes p53, activating the expression of p53-dependent genes

Box 2. Prolyl hydroxylation of non-HIF protein substrates by PHDs

At least 20 non-HIF proteins have been reported to be hydroxylated by PHDs via oxygen-dependent mechanisms; controversies of between some *in vitro* hydroxylation studies are likely due to differences in experimental conditions (Anderson et al., 2011; Cockman et al., 2019; Cummins et al., 2006; Guo et al., 2016; Luo et al., 2014; Moser et al., 2013; Rodriguez et al., 2018; Zheng et al., 2014). Prolyl hydroxylation of different non-HIF proteins has different consequences, including degradation, e.g. ATF4 (Goda et al., 2003), or functional inactivation, e.g. Akt1 (Guo et al., 2016).

Some non-HIF substrates have known developmental roles. For example, *Atf4*$^{-/-}$ mice exhibit delayed bone development (Yoshizawa et al., 2009), whereas Akt1 deficiency inhibits neonatal retinal angiogenesis (Lee et al., 2014). As the phenotypes in these mice are associated with the loss of the entire protein instead of specific mutation of the prolyl hydroxylation sites, it remains to be determined whether the developmental defects are specifically due to loss of oxygen-dependent regulation. It should be noted, however, that no studies have reported specific mutations in the prolyl hydroxylation sites in the endogenous HIFα loci in animal models, although transgenic overexpression of cDNAs lacking these sites have been carried out (Covello et al., 2006; Elson et al., 2001).

Development (2021) 148, dev200030. doi:10.1242/dev.200030

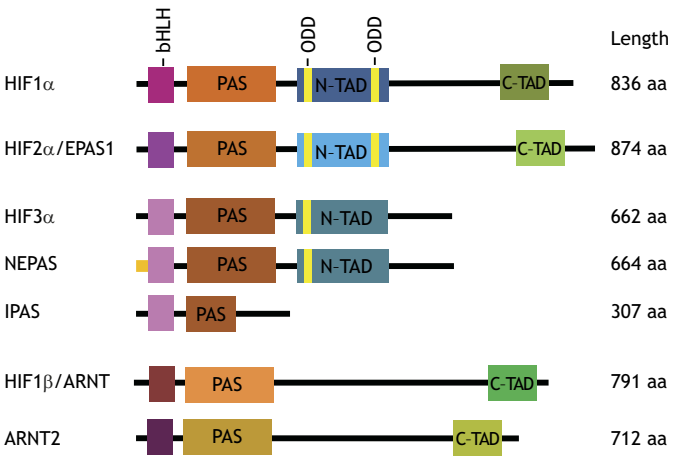

Fig. 3. Linear structure of mouse HIF subunits. Similar but different shades of colors represent related but different sequences. HIF1α and HIF2α have two oxygen-dependent degradation (ODD) domains each, but HIF3α has only one ODD domain. aa, amino acids; bHLH, basic helix loop helix domain; C-TAD, C-terminal transactivation domain; N-TAD, N-terminal transactivation domain; PAS, Per-Arnt-Sim domain.

(An et al., 1998; Carmeliet et al., 1998; Zhou et al., 2015). In *Hif1α*$^{-/-}$ mouse embryonic stem cells, p53 protein levels are significantly reduced and apoptosis is blocked under hypoxia (Carmeliet et al., 1998).

Cell-autonomous events regulated by HIF

HIF may regulate cell events in a cell-autonomous manner, in which HIF-activated gene products exert their functions in the same cells in which they are produced. An example of this type of regulation is metabolic reprogramming. HIF may also regulate cell functions in a non-autonomous manner. This may be best illustrated by VEGFA expression, which leads to endothelial cell differentiation, proliferation and migration. Here, we focus on cell-autonomous events.

Metabolism

Reprogramming of cellular metabolism by HIFα has been most extensively studied in cancer cells (reviewed by Elzakra and Kim, 2021; Semenza, 2011). More pertinent to this Primer is metabolic reprogramming in stem cells for tissue regeneration. In this regard, HIF1α promotes glycolysis in several types of stem cell, such as mouse embryonic stem cells (Carmeliet et al., 1998), neural stem cells (Lange et al., 2016), hematopoietic stem cells (Kocabas et al., 2015) and human mesenchymal stem cells (Palomaki et al., 2013). In diabetic mice, HIF1α overexpression confers protection against myocardial injury by promoting glycolysis and angiogenesis (Xue et al., 2010).

A major role of HIF2α in metabolism is to promote oxidative phosphorylation in mitochondria (Oktay et al., 2007; Scortegagna et al., 2003). Mechanistically, HIF2α activates the expression of SOD2 (mitochondrial superoxide dismutase) and frataxin, a chaperonin protein that enhances activity of aconitase in the Krebs cycle. Although HIF2α has not been found to upregulate glycolysis in most studies, one publication shows HIF2α-dependent upregulation of glycolysis during iPSC induction from human fibroblasts (Mathieu et al., 2014). At present, there is no explanation for this exception. However, we speculate that in most cells there might be a factor(s) that prevents HIF2α from interacting with the genes encoding glycolytic enzymes. Occasionally, the expression or function of such a factor(s) might be lost for different reasons, such as mutation or culture conditions, thus allowing HIF2α to activate the expression of glycolytic enzymes.

Other cell-autonomous events regulated by HIFα

HIF1α and HIF2α also regulate various other cellular events. HIF1α promotes autophagy (Cosin-Roger et al., 2017), alveolar epithelial cell differentiation (Saini et al., 2008) and apoptosis in mice (Carmeliet et al., 1998), but suppresses ER stress in rat nucleus pulposus cells and proliferation of mouse embryonic mesenchymal cells (Carmeliet et al., 1998; Novais et al., 2021). HIF2α is required for neural crest cell migration in chick embryos (Niklasson et al., 2021), EPO synthesis in kidney interstitial fibroblasts in rats (Paliege et al., 2010), iron transport in intestinal epithelial cells in a humanized mouse model of sickle cell disease (Das et al., 2015), suppression of lipid accumulation in adipocytes (Feng et al., 2018) and production of catecholamine by the organ of Zuckerkandl in mouse embryos (Tian et al., 1998). These observations indicate that, despite biochemical similarities between HIF1α and HIF2α, their biological functions are often distinct. To some extent, the differential functions probably reflect differences in their expression profiles.

Summary

HIF1α and HIF2α share overlapping but distinct sets of target genes. Activation of target gene expression *in vivo* depends on not only molecular specificity of HIF1α and HIF2α, but also on their expression profiles. In addition, interaction between HIFα and other transcription factors may also influence the expression of target genes. HIFα target genes regulate various cellular functions, such as metabolism, autophagy and apoptosis.

Loss-of-function phenotypes in development

Phenotypes associated with loss of HIF hydroxylases

Here, we discuss major phenotypes related to embryonic and neonatal development in mice. Adult phenotypes are also briefly mentioned if they share common elements with development, such as tissue growth and organization. A more complete list of phenotypes is included in Table 1. However, studies strictly focused on specialized topics, such as tumorigenesis and erythrocytosis, are not discussed; however, interested readers may consult several reviews on these subjects (Chan and Giaccia, 2010; Haase, 2017).

Germline Phd knockout

Phd2$^{-/-}$ (*Egln1*$^{-/-}$) embryos die at mid-gestation stages, but *Phd1*$^{-/-}$ (*Egln2*$^{-/-}$), *Phd3*$^{-/-}$ (*Egln3*$^{-/-}$) and *Phd1*$^{-/-}$/*Phd3*$^{-/-}$ are viable (Takeda et al., 2006). In *Phd2*$^{-/-}$ mice, HIF1α and HIF2α protein levels are significantly elevated in both the placenta and embryo proper, except in the heart. However, the myocardium thickness and trabeculation are dramatically reduced in *Phd2*$^{-/-}$ embryos, potentially secondary to poor nutrition resulting from defective placental development (Takeda et al., 2006). PHD2-deficient placentas display reduced labyrinthine branching morphogenesis, abnormal migration of spongiotrophoblasts and giant cells, and reduced angiogenesis. The placental defects in *Phd2*$^{-/-}$ embryos partially resemble those in *Vhl*$^{-/-}$ mouse embryos, which also display vascular deficiency in the poorly developed labyrinth (Gnarra et al., 1997). The exact reason for reduced angiogenesis is unknown, but poor labyrinthine development and cellular defects in the placenta could have some negative impacts.

Tissue-specific PHD deficiency

PHD2 deficiency in retinal astrocytes decelerates their differentiation from proliferative immature states towards non-

Development (2021) 148, dev200030. doi:10.1242/dev.200030

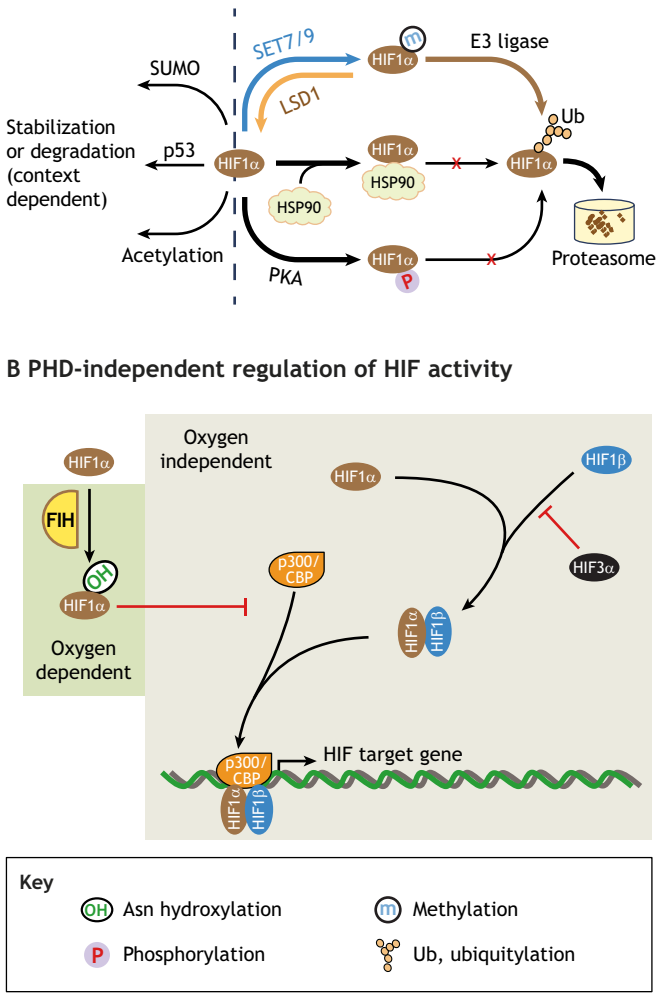

Fig. 4. PHD-independent regulation of HIF1α stability and activity. (A) Oxygen-independent regulation of HIF1α protein stability. The left one-third of the panel shows regulatory events with undefined (context-dependent) outcomes, including both stabilization and degradation for the same type of post-translational modification (e.g. SUMOylation or acetylation) or interaction with p53. The right two-thirds of the panel shows events with well-defined outcomes: methylation of HIF1α by SET7/9, a lysine-specific methyltransferase, leads to ubiquitylation (Ub) and degradation by the proteasome. The actions of SET7/9 can be reversed by the activity of lysine-specific demethylase (LSD1). Conversely, interaction of HIF1α with heat shock protein 90 (HSP90) or phosphorylation of HIF1α by protein kinase A (PKA) prevents HIF1α degradation. (B) Regulation of the assembly of the transcriptionally active HIF complex by PHD-independent mechanisms. Events on the right are oxygen independent. HIF3α inhibits heterodimerization between HIF1α and HIF1β (by competing for HIF1β). Interaction between HIF1α and p300/CBP is important for HIF transcriptional activity, but this interaction is suppressed by factor-inhibiting hypoxia-inducible factor (FIH) through oxygen-dependent asparaginyl hydroxylation (OH) of HIF1α. Although HIF2α and Arnt2 are expected to be regulated by similar mechanisms to HIF1α and HIF1β, they are not included in the diagram because direct data are still lacking for some of the steps.

proliferative mature states, leading to prolonged expansion of the astrocyte population (Duan and Fong, 2019). Increased abundance of immature astrocytes blocks retinal vascular pruning in neonatal mice (Duan and Fong, 2019).

Neural-specific *Phd2* deletion promotes brain vascularization due to HIF1α stabilization and VEGFA overexpression (Nasyrov et al., 2019). In kidneys, PHD2 and PHD3 double deficiency in stromal cells suppresses kidney development, manifested as reduced number of glomeruli and smaller kidney size (Kobayashi et al., 2017). In the lung, endothelial and hematopoietic PHD2 deficiency leads to obliterative lung vascular remodeling and pulmonary hypertension (Dai et al., 2016). Mechanistically, upregulated HIF2α in PHD2-deficient endothelium promotes CXCL12 expression, which in turn stimulates the proliferation of associated vascular smooth muscle cells (Dai et al., 2016).

FIH deficiency in zebrafish and mice

FIH knockdown in zebrafish by morpholino oligonucleotides (MO) promotes angiogenesis of intersomitic vessels (So et al., 2014). However, *Fih*$^{-/-}$ mice and zebrafish both develop normally. In adults, *Fih*$^{-/-}$ mice display elevated metabolism, whereas *Fih*$^{-/-}$ mice and zebrafish both have improved tolerance to hypoxia (Cai et al., 2018; Zhang et al., 2010). Discrepancies between MO and gene targeting studies are most likely due to MO off target effects (Kok et al., 2015).

HIF mutant phenotypes in development

Most mutant phenotypes discussed below are associated with loss-of-function mutations in mice, but some data are based on knock-in studies. A more complete list of phenotypes is presented in Table 2.

Germline knockout of HIFα genes

Consistent with its broad expression pattern and important roles in a variety of cellular processes, HIF1α is required in multiple embryonic tissues. *Hif1a*$^{-/-}$ embryos are grossly abnormal, displaying a variety of developmental defects, including poor angiogenesis, failure of neural tube closure, branchial arch hypoplasia, pericardial effusion, brain tissue deficiency and cardiac hyperplasia. *Hif1a*$^{-/-}$ embryos suffer from growth retardation and die *in utero* between E9.5 and E10.5 (Carmeliet et al., 1998; Iyer et al., 1998; Ryan et al., 1998).

Hif2a$^{-/-}$ embryonic defects are milder, in part due to the more restricted expression pattern of HIF2α. In some background strains, *Hif2a*$^{-/-}$ embryos exhibit vascular disorganization, hemorrhage (Peng et al., 2000), bradycardia and die *in utero* at mid-gestational stages (Peng et al., 2000; Tian et al., 1998). In other cases, *Hif2a*$^{-/-}$ mice are born live, but exhibit growth retardation, reduced body weight and a high mortality rate at juvenile ages (Peng et al., 2000; Scortegagna et al., 2003). Mechanistically, SOD2 expression and aconitase activity are both reduced in *Hif2a*$^{-/-}$ mice, leading to oxidative stress in the mitochondria and compromised activity of the Krebs cycle (Oktay et al., 2007; Scortegagna et al., 2003). *Hif2a*$^{-/-}$ mice delivered by Cesarean section die within a few hours due to respiratory distress syndrome (RDS). The development of RDS is due to surfactant deficiency, which in turn arises from diminished VEGFA expression in epithelial cells during alveolarization (Compernolle et al., 2002).

HIF1α and HIF2α have both overlapping and unique roles in development. *Hif1a*$^{-/-}$/*Hif2a*$^{-/-}$ embryos die before E8.5, substantially earlier than either *Hif1a*$^{-/-}$ or *Hif2a*$^{-/-}$ embryos (Cowden Dahl et al., 2005). In addition, *Hif1a*$^{-/-}$/*Hif2a*$^{-/-}$ embryos completely lack placental vasculogenesis. Conversely, substituting HIF1α with HIF2α by HIF2α cDNA knock-in at the *Hif1a* locus leads to elevated Oct4 (encoded by *Pou5f1*) expression and embryonic death before E7.5 (Covello et al., 2006).

In *Hif3a*$^{-/-}$ (also known as *NEPAS*$^{-/-}$) neonatal mice, HIF2α activity is upregulated, presumably due to the relief of HIF1β

Development (2021) 148, dev200030. doi:10.1242/dev.200030

Table 1. Partial list of phenotypes resulting from genetic manipulations of PHD expression

Manipulations	Species	Main phenotype	Reference
Egln1$^{-/-}$ (*Phd2*$^{-/-}$)	*C. elegans*	Egg laying defects	Epstein et al. (2001)
Phd1$^{-/-}$	Mouse	Viable, increased glycolysis	Takeda et al. (2006); Aragones et al. (2008)
Phd2$^{-/-}$	Mouse	Death at mid-gestation, defective placental labyrinthine and myocardial development	Takeda et al. (2006)
Phd3$^{-/-}$	Mouse	Viable, failure of intestinal epithelial barrier function and sympathoadrenal hypofunction	Takeda et al. (2006); Bishop et al. (2008); Chen et al. (2015)
Phd2 hypomorphic	Mouse	Viable, improved hindlimb muscle regeneration and protection against I/R injury	Hyvarinen et al. (2010a); Settelmeier et al. (2020)
Myocardial-specific KO of *Phd2**	Mouse	Protection from acute myocardial ischemic injury	Holscher et al. (2011)
Phd2$^{+/-}$	Mouse	Improved arteriogenesis and expansion of M2 macrophage population	Takeda et al. (2011)
Phd2 KO in skeletal muscles	Mouse	Protection of cardiomyocytes against I/R injury	Olenchock et al. (2016)
Phd2 KO at P1‡	Mouse	Resistance of retinal blood vessels to oxygen	Duan et al. (2011)
Phd2 KO in astrocyte progenitors	Mouse	Increased retinal astrocyte progenitors due to delayed maturation; blocking of retinal vascular pruning due to extra astrocytes	Duan and Fong (2019)
Phd2 KO in keratinocytes or hepatocytes	Mouse	Improved would healing and arteriogenesis	Takeda et al. (2014); Zimmermann et al. (2014)
Phd2 KO after 6 weeks of age‡	Mouse	Polycythemia, increased vascular growth	Takeda et al. (2008); Takeda et al. (2007)
Phd2 somatic KO‡	Mouse	Polycythemia, congestive heart failure	Minamishima et al. (2008)
Phd1$^{-/-}$/*Phd3*$^{-/-}$	Mouse	Erythrocytosis	Takeda et al. (2008)
Kidney stroma DKO of *Phd2*/*Phd3*	Mouse	Reduced number of glomeruli; small kidneys	Kobayashi et al. (2017)
Neural tissue specific *Phd2 KO*	Mouse	Increased brain vascularization	Nasyrov et al. (2019)
Keratinocyte-specific KO of *Phd2*, *Phd2*/*Phd3* and *Phd1*/*Phd2*/*Phd3*	Mouse	Increased angiogenesis (moderate in Phd2 KO alone, most robust in triple KO)	Takeda et al. (2014)
Hepatocyte-specific KO of *Phd1*/*Phd2*/*Phd3*	Mouse	Loss of liver tissue, fusion of vascular lumens, fatty liver and erythrocytosis	Duan et al. (2014a)
P4H-TM morpholino KD	Zebrafish	Head defects and pericardial edema	Hyvarinen et al. (2010b)
P4h-tm$^{-/-}$	Mouse	Defective calcium signaling in astrocytes	Byts et al. (2021)
fih morpholino KD	Zebrafish	Increased angiogenesis of intersomitic vessels	So et al. (2014)
fih overexpression	Zebrafish	Reduced intersomitic vessel angiogenesis	So et al. (2014)
fih$^{-/-}$	Zebrafish	Reduced apoptosis	Cai et al. (2018)
Fih$^{-/-}$	Mouse	Increased metabolic rate and reduced body weight.	Zhang et al. (2010)
Myocardial-specific KD of *Phd2*/*Fih*§	Mouse	Improved myocardial repair, stem cell recruitment and angiogenesis	Huang et al. (2011)
Vhl$^{-/-}$	Mouse	Death at E9.5, lack of placental vessels	Gnarra et al. (1997)
Hepatocyte-specific KO of *Vhl*	Mouse	Polycythemia, steatosis and vascular tumor	Haase et al. (2001)

Pathological phenotypes that are not directly related to non-malignant tissue growth are not included.
*Knockout of floxed Phd2 by myocardial-specific Cre. Although Cre-mediated deletion is generally less than 100% efficient, the manipulation is referred to as KO to avoid confusion with siRNA-mediated knockdown;.
‡KO in these mice was mediated by Rosa26CreERT2 or chicken β-actin-CreERT.
§KD, knockdown by intramyocardial injection of shRNA minicircle vectors.
I/R, ischemia-reperfusion; KD, knockdown; KO, knockout.

sequestration. Elevated HIF2α activity promotes the expression of its target gene endothelin 1 (*Edn1*), which leads to incomplete lung alveolarization (Yamashita et al., 2008).

Germline knockout of *Arnt* (*Hifb*) genes

Arnt$^{-/-}$ (*Hif1b*$^{-/-}$) embryos essentially phenocopy the developmental defects of *Hif1a*$^{-/-}$ mice and die by E10.5, which is slightly later than embryonic lethality at E9.5-E10.5 in *Hif1a*$^{-/-}$ mice (Maltepe et al., 1997). The phenotypic similarity between *Hif1a*$^{-/-}$ and *Arnt*$^{-/-}$ embryos is consistent with HIF1α and ARNT functioning as a heterodimer. *Arnt2*$^{-/-}$ mice die at perinatal stages due to hypothalamus defects, but *Arnt*$^{-/-}$/*Arnt2*$^{-/-}$ embryos die before E8.5 (Keith et al., 2001). The earlier embryonic lethality in *Arnt*$^{-/-}$/*Arnt2*$^{-/-}$ is suggestive of functional redundancy between ARNT and ARNT2.

Tissue-specific HIFα deficiency

Owing to embryonic lethality in germline knockout mice, tissue-specific approaches have been taken to determine HIFα functions. Cardiomyocyte-specific deletion of *Hif1a* in mice causes failure in heart-tube looping (Krishnan et al., 2008), while *Hif1a* knockout in the second heart field results in decreased sympathetic innervation in the heart (Bohuslavova et al., 2019). Specific loss of *Hif-1a* in neural cells or lung epithelium causes defective brain development (Tomita et al., 2003) or RDS-like phenotypes (Saini et al., 2015), respectively.

Neuro-specific disruption of HIF2α leads to altered expression of genes involved in neurogenesis. In these mice, migration of HIF2α-deficient neural stem cells is defective, leading to pyramidal neuron deficiency and cognitive abnormalities (Kleszka et al., 2020). Endothelial cell-specific HIF2α deficiency reduces Tie2 expression in airway endothelial cells (ECs) and hence causes apoptosis (Jiang et al., 2019). In the same mice, pericyte coverage is also reduced, presumably secondary to EC apoptosis. As pericytes normally provide a major source of angiopoietin 1, a Tie2 ligand, their deficiency further compromises Tie 2 signaling in airway ECs (Jiang et al., 2019). In neonatal retinas, astrocyte-specific *Hif2a* deficiency greatly accelerates the differentiation of proliferative astrocyte progenitors (proangiogenic) into non-proliferative mature

Development (2021) 148, dev200030. doi:10.1242/dev.200030

Table 2. Partial list of phenotypes associated with genetic manipulation of HIF components

Manipulations	Species	Main phenotypes	Molecular changes	Reference
Hif1a$^{-/-}$	Mouse	Lethal before E9.5; defects in metabolism, angiogenesis, proliferation and apoptosis	VEGFA, PGK1, p53, p21, Bcl2 and Glut1/3 decreased	Carmeliet et al. (1998); Iyer et al. (1998); Ryan et al. (1998)
Hif1a KO in CNS	Mouse	Defective brain development	Glut1 and VEGFA decreased	Tomita et al. (2003)
Hif1a KO in second heart field SNS	Mouse	Defective innervation of heart	Not determined	Bohuslavova et al. (2019)
Hif1a KO in lung epithelium	Mouse	Neonatal respiratory distress syndrome-like phenotypes	HIF2α, β-catenin and VEGFA decreased	Saini et al. (2008)
Hif1a KO growth plate (GF) chondrocytes	Mouse	Chondrocyte death and overproliferation in different GP regions; perinatal death due to tracheal defects	p57, PGK and VEGFA decreased; VEGFA increased (secondary effect)	Schipani et al. (2001)
Hif1a KO in ECs and myeloid cells	Mouse	Cardioprotection	Not determined	Sarkar et al. (2012)
Hif1a/*Hif2a* DKO, lung epithelium	Mouse	Rescued lung development	NF-κB and Myc decreased	Saini et al. (2015)
Hif1a KO in cardiomyocytes	Mouse	Aborted heart development (failure in heart looping); death between E11 and E12	Mef2c, Tbx5 and Titin decreased	Krishnan et al. (2008)
Hif1a morpholino KD	*Xenopus*	Failed cardiogenesis.	Nkx2.5 decreased	Nagao et al. (2008)
Epidermal *Hif1a* overexpression	Mouse	Skin hypervascularization by non-leaky blood vessels	VEGFA and Glut1 decreased	Elson et al. (2001)
Cardiac *Hif1a* OE in diabetic mice	Mouse	Prevention of cardiac remodeling.	Glycolytic pathway increased	Xue et al. (2010)
Hif1a OE in transplanted rabbit bone marrow cells	Rabbit	Improved bone regeneration and increased bone angiogenesis.	VEGFA increased	Ding et al. (2013)
Hif2a$^{-/-}$	Mouse	Vascular leakage, neonatal RDS-like phenotypes, mitochondrial disease and bradycardia.	VEGFA, surfactants, Sod2 and catecholamines decreased	Compernolle et al. (2002); Peng et al. (2000); Scortegagna et al. (2003); Tian et al. (1998)
Hypomorphic *Hif2a*$^{kd/kd}$	Mouse	Reduced neovascularization in ischemic retinas	EPO decreased	Morita et al. (2003)
Hif2a KO in neurons	Mouse	Pyramidal neurons deficiency	*Wnt7b* increased; *Pou4f1* and *Nrp2* decreased	Kleszka et al. (2020)
Hif2a KO in astrocytes and their progenitors	Mouse	Depletion of the retinal astrocyte progenitor pool; poor retinal astrocyte proliferation; diminished retinal vascular development	VEGFA decreased	Duan et al. (2014b); Perelli et al. (2021)
Hif2a KO in astrocytes	Mouse	Reduced neovascularization in ischemic retinas	VEGFA decreased	Weidemann et al. (2010)
Hif2a KO in ECs	Mouse	Loss of airway ECs and pericytes; failure to protect ischemic renal tissues; poor arteriogenesis in ischemic hindlimb muscles	Angpt1/Tie2 and delta-like 4 decreased	Jiang et al. (2019); Kapitsinou et al. (2014); Skuli et al. (2012)
Hif2a KO in cardiomyocytes	Mouse	Increased myocardial infarct size in the ischemia/reperfusion model	Epithelial growth factor amphiregulin decreased	Koeppen et al. (2018)
Hif2a KO (CRISPR) and OE	Chicken	Defective neural crest cell migration	Not determined	Niklasson et al. (2021)
Hif2a KD by shRNA	Human fibroblast	Reduced iPSC induction	Glycolysis decreased	Mathieu et al. (2014)
Hif2a KD by shRNA	Human HSCs in mice	Increased apoptosis	Mitochondrial ROS and ER stress decreased	Rouault-Pierre et al. (2013)
Hif2a cDNA KI/KI at the *Hif1a* locus[11]	Mouse	Death before E7.5 and abnormal gastrulation	Oct4, VEGFA and TGFα increased	Covello et al. (2006)
Hif1a$^{-/-}$/*Hif2a*$^{-/-}$	Mouse	Death before E8.5, defective trophoblast differentiation and placental vascularization.	Mash2 decreased	Cowden Dahl et al. (2005)
Hif1a/*Hif2a* KO in myoblasts	Mouse	Defective muscle regeneration in postnatal/adult mice	Notch signaling decreased	Yang et al. (2017)
NEPAS$^{-/-}$ (*Hif3a*$^{-/-}$)	Mouse	Enlarged right ventricle; impaired alveolarization	HIF2α activity and endothelin 1 increased	Yamashita et al. (2008)
Hif3a OE in airway epithelium	Mouse	Impaired alveolarization; fewer lung epithelial and Clara cells	Sox2 increased; HIF2α decreased	Huang et al. (2013)
IPAS antisense oligonucleotides	Mouse	Angiogenesis in the cornea	HIF1α activity decreased	Makino et al. (2001)
Arnt$^{-/-}$	Mouse	Death at E9.5; defective angiogenesis, metabolism and placental development, and yok sac hematopoiesis	VEGFA, PGK1 and Alda decreased	Adelman et al. (2000); Adelman et al. (1999); Maltepe et al. (1997)
Arnt2$^{-/-}$	Mouse	Perinatal death and defective hypothalamus development	PGK, VEGFA and Glut3 decreased	Keith et al. (2001)
Arnt$^{-/-}$/*Arnt2*$^{-/-}$	Mouse	Embryo resorption before E8.5	Not determined	Keith et al. (2001)

Where standard genotype symbols are too lengthy, they are replaced by unconventional descriptions. For example, *Hif1a*/*Hif2a* double KO in lung epithelium is used for SPC-rtTA-tg/(tetO)7-CMV-Cretg/tg/*Hif-1α*flox/flox/*Hif-2α*flox/flox. Regulation is at the level of expression, unless indicated as 'activity'.
CNS, sympathetic nervous system; DKO, double knockout; ER, endoplasmic reticulum; HSCs, hematopoietic stem cells; iPSC, induced pluripotent stem cell; KD, knockdown; KI, knock-in; KO refers to Cre mediated disruption of floxed allele; whereas –/– is reserved for germline mutation; OE, overexpression; RDS, respiratory distress syndrome; ROS, reactive oxygen species; SNS, sympathetic nervous system.

astrocytes (non-angiogenic) (Duan et al., 2014b; Perelli et al., 2021). As a result, the pool of proangiogenic astrocyte progenitors is rapidly depleted and retinal angiogenesis is severely suppressed (Duan et al., 2014b; Perelli et al., 2021).

Summary

Phd1$^{-/-}$ mice exhibit defective placentation and heart development, and die in mid-gestational stages, whereas *Phd1*$^{-/-}$, *Phd3*$^{-/-}$ and *Phd1*$^{-/-}$/*Phd3*$^{-/-}$ are viable. Cre-mediated *Phd2* knockout has also revealed important roles in several neonatal tissues. HIF1α and HIF2α play both overlapping and unique roles in development. While *Hif1a*$^{-/-}$ embryonic defects are more severe and widespread than in *Hif2a*$^{-/-}$ mice, *Hif1a*$^{-/-}$/*Hif2a*$^{-/-}$ embryos display the most severe defects, suggesting partially overlapping functions. On the other hand, HIF1α cannot be replaced by HIF2α, suggesting that at least some roles are unique. Tissue-specific gene-targeting studies

Development (2021) 148, dev200030. doi:10.1242/dev.200030

also revealed developmental defects specifically associated with HIF1α or HIF2α deficiency. ARNT and ANRT2 also display partially overlapping roles, with ARNT being the major isoform required for development.

Clinical relevance to the repair of ischemic tissues

Among different human diseases, tissue ischemia shares many molecular and cellular features related to embryonic development. In both cases, cells are under hypoxia and their growth/repair depends on angiogenesis. As tissue repair is generally promoted by HIFα, but suppressed by HIFα deficiency, discussion in this section is focused on approaches that enhance HIFα activity, such as tissue-specific PHD knockout, delivery of PHD inhibitors and delivery of HIFα overexpression vectors. More information is included in Table 1 for PHD-related studies and in Table 2 for HIFα-related studies.

Angiogenesis and tissue repair by PHD targeting or inhibition

Beneficial effects of PHD deficiency on tissue repair

In several models, tissue-specific PHD2 deficiency is beneficial for the survival and repair of ischemic tissues. Cardiomyocyte-specific *Phd2* disruption in mice protects mice against acute myocardial ischemic injury (Holscher et al., 2011). The specific mechanism underlying protection has not yet been investigated, but HIF1α protein level is increased in these mice, suggesting a role of the canonical PHD2-HIF1α axis in this model (Holscher et al., 2011).

Keratinocyte-specific PHD2 deficiency promotes dermal angiogenesis moderately, but loss of additional PHD proteins yields much more robust effects (Takeda et al., 2014). Molecularly, dermal levels of HIF1α and VEGFA are increased in a dose-dependent manner – most strongly upregulated in mice deficient in all three PHDs in their skin tissues (Takeda et al., 2014). In a wound-healing model, dermal PHD2 deletion promotes angiogenesis and wound healing (Zimmermann et al., 2014). In the mouse model of hindlimb ischemia, shRNA-mediated knockdown of all three PHDs promotes vascularization by HIF-dependent mechanisms (Loinard et al., 2009).

PHD deficiency may also confer protective effects by HIF-independent mechanisms. In *Phd1*$^{-/-}$ mice, brain tissues are better protected against ischemia injury than wild-type mice, mostly due to NFκB-dependent metabolic reprogramming and redox balancing (Quaegebeur et al., 2016). In the mouse model of hindlimb ischemia, *Phd2* haploinsufficiency accelerates arteriogenesis through NFκB-dependent macrophage repolarization (to a M2 phenotype), which is proangiogenic (Takeda et al., 2011). Molecularly, the partial loss of PHD2 promotes NFκB assembly by reducing prolyl hydroxylation of IKKβ, a non-HIF substrate protein (Cummins et al., 2006), promoting NFκB assembly.

Interestingly, PHD2 deficiency in one tissue may also protect or accelerate repair in a remote tissue. PHD2 deficiency in keratinocytes or hepatocytes promotes arteriogenesis in ischemic hindlimb muscles (Takeda et al., 2014). Similarly, skeletal muscle-specific *Phd2* disruption protects the heart against ischemia-reperfusion (I/R) injury (Olenchock et al., 2016). PHD2 deficiency in skeletal muscles leads to the accumulation of 2-OG, which enters the circulation and promotes the secretion of kynurenic acid (KYNA) from the liver.

Harmful effects of PHD deficiency

Several human diseases are linked to abnormalities in oxygen sensing. For example, heterozygous loss-of-function mutations in *PHD2* and gain-of-function mutations in *HIF2A* are both associated with erythrocytosis: a high concentration of red blood cells (Percy et al., 2012, 2006). In the *VHL* locus, thousands of heterozygous germline and somatic mutations have been identified, many of which underlie the development of erythrocytosis and/or a variety of malignant tumors (Kaelin, 2007).

In mice, similar pathological disorders may develop due to defective oxygen sensing. For example, Cre-dependent global *Phd2* deletion in postnatal or adult mice leads to severe erythrocytosis, cardiac congestion and death within several months of *Phd2* deletion (Minamishima et al., 2008; Takeda et al., 2008). Hepatocyte-specific deficiency of all three PHDs or VHL also leads to robust erythrocytosis and major defects in the liver, including severe steatosis (fatty liver) and tissue loss in PHD-deficient mice (Duan et al., 2014a), and vascular tumors in VHL deficient mice (Haase et al., 2001). These findings call for caution when targeting the oxygen-sensing mechanisms for clinical benefits.

Promoting tissue repair by pharmacologically inhibiting PHD hydroxylase activities

The therapeutic potential of small molecule PHD hydroxylase inhibitors are being actively tested (Semenza, 2019). One study loaded a PHD hydroxylase inhibitor (1,4-dihydrophenonthrolin-4-one-3-carboxylic acid, 'DPCA') into a hydrogel nanofiber construct and then implanted it into wounded sites, where it upregulated HIF1α protein levels and improved tissue repair (Cheng et al., 2019). Some of the small molecule-based studies have advanced to early phase clinical trials; Daprodustat, another PHD hydroxylase inhibitor, has been tested in a Phase I trial. The inhibitor was found to be safe when applied topically, with very minimal systemic absorption (Olson et al., 2019). As systemic PHD deficiency could lead to undesirable consequences, being able to deliver PHD hydroxylase inhibitors locally is of practical importance.

Angiogenesis and tissue repair by HIFα overexpression

Increased angiogenesis and tissue repair by transgenic HIF1α overexpression

Transgenic mice overexpressing HIF-1α in specific tissues display improved protection and repair of ischemic tissues, whereas tissue specific HIFα deficiency has the opposite effect. Cardiomyocyte-specific HIF1α overexpression prevents cardiac remodeling in streptozotocin-induced diabetic mice, in part by promoting the glycolytic pathway under diabetic conditions (Xue et al., 2010). In a rabbit model of corticosteroid-induced osteonecrosis, HIF1α overexpression in bone marrow cells promotes bone tissue regeneration, along with increased VEGFA expression and bone angiogenesis (Ding et al., 2013). Keratinocyte-specific HIF1α overexpression leads to hypervascularization of dermal tissues with non-leaky blood vessels (Elson et al., 2001). In contrast to overexpression, tissue-specific HIFα deficiency interferes with repair of ischemic tissues (Koeppen et al., 2018; Sarkar et al., 2012; Skuli et al., 2012).

Promoting tissue repair by viral or plasmid vector-mediated HIFα overexpression

In addition to transgenic overexpression, vector-based approaches have also been taken to achieve HIFα overexpression by localized delivery. In the mouse model of hindlimb ischemia, intramuscular injection of adenoviral vector overexpressing HIF-1α accelerated the recovery of hindlimb perfusion and function, in part by promoting the recruitment of circulating angiogenic cells (Ibuki

et al., 2020). In a rat model of Alzheimer-like neurodegeneration, intracerebral injection of adeno-associated viral vector expressing HIF1α attenuated hippocampal neuronal apoptosis (Chai et al., 2014). In a rat stroke model, brain infarct volume was reduced by the transplantation of bone marrow-derived stem cells engineered to overexpress HIF1α (Lv et al., 2017). Finally, angiogenesis in wounded skin tissue is promoted by local injection of a plasmid vector overexpressing HIF2α (Chen et al., 2010).

Some of the tissue repair-related studies have moved on to clinical trials. For example, a Phase I trial has demonstrated the safety of intramuscular injection of an adenoviral vector expressing HIF1α lacking prolyl hydroxylation sites (Rajagopalan et al., 2007), although success in more advanced clinical trials remains to be demonstrated. Although not the focus of this Primer, HIF inhibitors are actively studied in both animal models and clinical trials, often for the purpose of suppressing neovascularization. Interested readers may consult a recent review focused on this important topic (Semenza, 2019).

Summary

In preclinical models for angiogenesis and tissue repair, HIFα, especially HIF1α, levels are boosted by different approaches, including Phd gene targeting, pharmacological inhibition of PHD activities, and HIFα overexpression. These studies overwhelmingly indicate that tissue-specific or localized upregulation HIFα levels is often beneficial for the protection and repair of ischemic tissues. On the other hand, systemic or excessive HIFα stabilization can be harmful, as shown in mice globally deficient for PHD2 or liver-specific loss of all three PHDs. Overall, pros and cons of targeting the oxygen-sensing mechanisms are context dependent, and each specific approach needs careful evaluation on a case by case basis.

Conclusions

Physiological hypoxia in embryonic tissues stabilizes HIFα proteins, allowing them to activate the transcription of developmentally important target genes. Embryonic phenotypes in *Hif1a*$^{-/-}$ and *Hif1b*$^{-/-}$ mice provide unequivocal evidence that HIF1 is broadly required in many cellular functions during embryogenesis. Although the role of HIF2α is more restricted during early embryogenesis, it is required in multiple processes in postnatal mice. PHD family knockout studies have provided evidence that oxygen-sensing mechanisms operate *in vivo*, essentially as predicted by *in vitro* molecular studies, involving both the canonical PHD-HIF axis and non-HIF PHD substrates. Furthermore, the embryonic lethality of *Hif1a*$^{-/-}$, *Hif1b*$^{-/-}$, *Hif2a*$^{-/-}$ and *Phd2*$^{-/-}$ mice suggests that embryogenesis requires an optimal level of HIFα activity. Embryonic development is perturbed by HIF levels above or below this level in either direction. On the other hand, adult mice are relatively tolerant to locally elevated HIFα levels in several types of tissues, such as myocardium, brain, skeletal muscles and dermal tissues, and can even benefit from such conditions during the repair process of ischemic tissues. Nonetheless, systemic PHD2 deficiency or tissue-specific deficiency of multiple PHD proteins may have deleterious consequences. Collectively, these studies have demonstrated the proof of principle that oxygen-sensing mechanisms may provide novel therapeutic targets to accelerate the repair of ischemia-injured tissues, whereas safety and efficacy-related issues need to be evaluated for each specific approach.

Competing interests

The authors declare no competing or financial interests.

Funding

The authors' research is supported by grants from the National Institutes of Health (2R01EY019721-06 and 1R01EY031593-01). Deposited in PMC for release after 12 months.

References

Adelman, D. M., Maltepe, E. and Simon, M. C. (1999). Multilineage embryonic hematopoiesis requires hypoxic ARNT activity. *Genes Dev.* **13**, 2478-2483. doi:10.1101/gad.13.19.2478

Adelman, D. M., Gertsenstein, M., Nagy, A., Simon, M. C. and Maltepe, E. (2000). Placental cell fates are regulated in vivo by HIF-mediated hypoxia responses. *Genes Dev.* **14**, 3191-3203. doi:10.1101/gad.853700

An, W. G., Kanekal, M., Simon, M. C., Maltepe, E., Blagosklonny, M. V. and Neckers, L. M. (1998). Stabilization of wild-type p53 by hypoxia-inducible factor 1alpha. *Nature* **392**, 405-408. doi:10.1038/32925

Anderson, K., Nordquist, K. A., Gao, X., Hicks, K. C., Zhai, B., Gygi, S. P. and Patel, T. B. (2011). Regulation of cellular levels of Sprouty2 protein by prolyl hydroxylase domain and von Hippel-Lindau proteins. *J. Biol. Chem.* **286**, 42027-42036. doi:10.1074/jbc.M111.303222

Aragones, J., Schneider, M., Van Geyte, K., Fraisl, P., Dresselaers, T., Mazzone, M., Dirkx, R., Zacchigna, S., Lemieux, H., Jeoung, N. H. et al. (2008). Deficiency or inhibition of oxygen sensor Phd1 induces hypoxia tolerance by reprogramming basal metabolism. *Nat. Genet.* **40**, 170-180. doi:10.1038/ng.2007.62

Baek, J. H., Mahon, P. C., Oh, J., Kelly, B., Krishnamachary, B., Pearson, M., Chan, D. A., Giaccia, A. J. and Semenza, G. L. (2005). OS-9 interacts with hypoxia-inducible factor 1alpha and prolyl hydroxylases to promote oxygen-dependent degradation of HIF-1alpha. *Mol. Cell* **17**, 503-512. doi:10.1016/j.molcel.2005.01.011

Bagnall, J., Leedale, J., Taylor, S. E., Spiller, D. G., White, M. R., Sharkey, K. J., Bearon, R. N. and See, V. (2014). Tight control of hypoxia-inducible factor-alpha transient dynamics is essential for cell survival in hypoxia. *J. Biol. Chem.* **289**, 5549-5564. doi:10.1074/jbc.M113.500405

Barth, S., Edlich, F., Berchner-Pfannschmidt, U., Gneuss, S., Jahreis, G., Hasgall, P. A., Fandrey, J., Wenger, R. H. and Camenisch, G. (2009). Hypoxia-inducible factor prolyl-4-hydroxylase PHD2 protein abundance depends on integral membrane anchoring of FKBP38. *J. Biol. Chem.* **284**, 23046-23058. doi:10.1074/jbc.M109.032631

Berra, E., Roux, D., Richard, D. E. and Pouyssegur, J. (2001). Hypoxia-inducible factor-1 alpha (HIF-1 alpha) escapes O(2)-driven proteasomal degradation irrespective of its subcellular localization: nucleus or cytoplasm. *EMBO Rep.* **2**, 615-620. doi:10.1093/embo-reports/kve130

Berra, E., Benizri, E., Ginouves, A., Volmat, V., Roux, D. and Pouyssegur, J. (2003). HIF prolyl-hydroxylase 2 is the key oxygen sensor setting low steady-state levels of HIF-1alpha in normoxia. *EMBO J.* **22**, 4082-4090. doi:10.1093/emboj/cdg392

Bertout, J. A., Majmundar, A. J., Gordan, J. D., Lam, J. C., Ditsworth, D., Keith, B., Brown, E. J., Nathanson, K. L. and Simon, M. C. (2009). HIF2alpha inhibition promotes p53 pathway activity, tumor cell death, and radiation responses. *Proc. Natl. Acad. Sci. USA* **106**, 14391-14396. doi:10.1073/pnas.0907357106

Bilton, R., Mazure, N., Trottier, E., Hattab, M., Dery, M. A., Richard, D. E., Pouyssegur, J. and Brahimi-Horn, M. C. (2005). Arrest-defective-1 protein, an acetyltransferase, does not alter stability of hypoxia-inducible factor (HIF)-1alpha and is not induced by hypoxia or HIF. *J. Biol. Chem.* **280**, 31132-31140. doi:10.1074/jbc.M504482200

Bishop, T., Gallagher, D., Pascual, A., Lygate, C. A., de Bono, J. P., Nicholls, L. G., Ortega-Saenz, P., Oster, H., Wijeyekoon, B., Sutherland, A. I. et al. (2008). Abnormal sympathoadrenal development and systemic hypotension in PHD3-/- mice. *Mol. Cell. Biol.* **28**, 3386-3400. doi:10.1128/MCB.02041-07

Bohuslavova, R., Cerychova, R., Papousek, F., Olejnickova, V., Bartos, M., Gorlach, A., Kolar, F., Sedmera, D., Semenza, G. L. and Pavlinkova, G. (2019). HIF-1alpha is required for development of the sympathetic nervous system. *Proc. Natl. Acad. Sci. USA* **116**, 13414-13423. doi:10.1073/pnas.1903510116

Briggs, K. J., Koivunen, P., Cao, S., Backus, K. M., Olenchock, B. A., Patel, H., Zhang, Q., Signoretti, S., Gerfen, G. J., Richardson, A. L. et al. (2016). Paracrine induction of HIF by glutamate in breast cancer: EglN1 senses cysteine. *Cell* **166**, 126-139. doi:10.1016/j.cell.2016.05.042

Bruick, R. K. and McKnight, S. L. (2001). A conserved family of prolyl-4-hydroxylases that modify HIF. *Science* **294**, 1337-1340. doi:10.1126/science.1066373

Bullen, J. W., Tchernyshyov, I., Holewinski, R. J., DeVine, L., Wu, F., Venkatraman, V., Kass, D. L., Cole, R. N., Van Eyk, J. and Semenza, G. L. (2016). Protein kinase A-dependent phosphorylation stimulates the transcriptional activity of hypoxia-inducible factor 1. *Sci. Signal.* **9**, ra56. doi:10.1126/scisignal.aaf0583

Byts, N., Sharma, S., Laurila, J., Paudel, P., Miinalainen, I., Ronkainen, V. P., Hinttala, R., Tornquist, K., Koivunen, P. and Myllyharju, J. (2021).

Transmembrane Prolyl 4-hydroxylase is a novel regulator of calcium signaling in astrocytes. *eNeuro* **8**, ENEURO.0253-20.2020. doi:10.1523/ENEURO.0253-20.2020

Cai, X., Zhang, D., Wang, J., Liu, X., Ouyang, G. and Xiao, W. (2018). Deletion of the fih gene encoding an inhibitor of hypoxia-inducible factors increases hypoxia tolerance in zebrafish. *J. Biol. Chem.* **293**, 15370-15380. doi:10.1074/jbc.RA118.003004

Carbia-Nagashima, A., Gerez, J., Perez-Castro, C., Paez-Pereda, M., Silberstein, S., Stalla, G. K., Holsboer, F. and Arzt, E. (2007). RSUME, a small RWD-containing protein, enhances SUMO conjugation and stabilizes HIF-1alpha during hypoxia. *Cell* **131**, 309-323. doi:10.1016/j.cell.2007.07.044

Carmeliet, P., Dor, Y., Herbert, J. M., Fukumura, D., Brusselmans, K., Dewerchin, M., Neeman, M., Bono, F., Abramovitch, R., Maxwell, P. et al. (1998). Role of HIF-1alpha in hypoxia-mediated apoptosis, cell proliferation and tumour angiogenesis. *Nature* **394**, 485-490. doi:10.1038/28867

Chai, X., Kong, W., Liu, L., Yu, W., Zhang, Z. and Sun, Y. (2014). A viral vector expressing hypoxia-inducible factor 1 alpha inhibits hippocampal neuronal apoptosis. *Neural Regen Res* **9**, 1145-1153. doi:10.4103/1673-5374.135317

Chan, D. A. and Giaccia, A. J. (2010). PHD2 in tumour angiogenesis. *Br. J. Cancer* **103**, 1-5. doi:10.1038/sj.bjc.6605682

Chavez, J. C., Baranova, O., Lin, J. and Pichiule, P. (2006). The transcriptional activator hypoxia inducible factor 2 (HIF-2/EPAS-1) regulates the oxygen-dependent expression of erythropoietin in cortical astrocytes. *J. Neurosci.* **26**, 9471-9481. doi:10.1523/JNEUROSCI.2838-06.2006

Chen, C., Pore, N., Behrooz, A., Ismail-Beigi, F. and Maity, A. (2001). Regulation of glut1 mRNA by hypoxia-inducible factor-1. Interaction between H-ras and hypoxia. *J. Biol. Chem.* **276**, 9519-9525. doi:10.1074/jbc.M010144200

Chen, D., Li, M., Luo, J. and Gu, W. (2003). Direct interactions between HIF-1 alpha and Mdm2 modulate p53 function. *J. Biol. Chem.* **278**, 13595-13598. doi:10.1074/jbc.C200694200

Chen, L., Endler, A., Uchida, K., Horiguchi, S., Morizane, Y., Iijima, O., Toi, M. and Shibasaki, F. (2010). Int6/eIF3e silencing promotes functional blood vessel outgrowth and enhances wound healing by upregulating hypoxia-induced factor 2alpha expression. *Circulation* **122**, 910-919. doi:10.1161/CIRCULATIONAHA.109.931931

Chen, Z., Liu, X., Mei, Z., Wang, Z. and Xiao, W. (2014). EAF2 suppresses hypoxia-induced factor 1alpha transcriptional activity by disrupting its interaction with coactivator CBP/p300. *Mol. Cell. Biol.* **34**, 1085-1099. doi:10.1128/MCB.00718-13

Chen, Y., Zhang, H. S., Fong, G. H., Xi, Q. L., Wu, G. H., Bai, C. G., Ling, Z. Q., Fan, L., Xu, Y. M., Qin, Y. Q. et al. (2015). PHD3 stabilizes the tight junction protein occludin and protects intestinal epithelial barrier function. *J. Biol. Chem.* **290**, 20580-20589. doi:10.1074/jbc.M115.653584

Cheng, J., Kang, X., Zhang, S. and Yeh, E. T. (2007). SUMO-specific protease 1 is essential for stabilization of HIF1alpha during hypoxia. *Cell* **131**, 584-595. doi:10.1016/j.cell.2007.08.045

Cheng, J., Amin, D., Latona, J., Heber-Katz, E. and Messersmith, P. B. (2019). Supramolecular Polymer Hydrogels for Drug-Induced Tissue Regeneration. *ACS Nano* **13**, 5493-5501. doi:10.1021/acsnano.9b00281

Choueiri, T. K. and Kaelin, W. G.Jr. (2020). Targeting the HIF2-VEGF axis in renal cell carcinoma. *Nat. Med.* **26**, 1519-1530. doi:10.1038/s41591-020-1093-z

Cockman, M. E., Lippl, K., Tian, Y. M., Pegg, H. B., Figg, W. D. J., Abboud, M. I., Heilig, R., Fischer, R., Myllyharju, J., Schofield, C. J. et al. (2019). Lack of activity of recombinant HIF prolyl hydroxylases (PHDs) on reported non-HIF substrates. *Elife* **8**, e46490. doi:10.7554/eLife.46490

Compernolle, V., Brusselmans, K., Acker, T., Hoet, P., Tjwa, M., Beck, H., Plaisance, S., Dor, Y., Keshet, E., Lupu, F. et al. (2002). Loss of HIF-2alpha and inhibition of VEGF impair fetal lung maturation, whereas treatment with VEGF prevents fatal respiratory distress in premature mice. *Nat. Med.* **8**, 702-710. doi:10.1038/nm721

Cosin-Roger, J., Simmen, S., Melhem, H., Atrott, K., Frey-Wagner, I., Hausmann, M., de Valliere, C., Spalinger, M. R., Spielmann, P., Wenger, R. H. et al. (2017). Hypoxia ameliorates intestinal inflammation through NLRP3/mTOR downregulation and autophagy activation. *Nat. Commun.* **8**, 98. doi:10.1038/s41467-017-00213-3

Coulet, F., Nadaud, S., Agrapart, M. and Soubrier, F. (2003). Identification of hypoxia-response element in the human endothelial nitric-oxide synthase gene promoter. *J. Biol. Chem.* **278**, 46230-46240. doi:10.1074/jbc.M305420200

Covello, K. L., Kehler, J., Yu, H., Gordan, J. D., Arsham, A. M., Hu, C. J., Labosky, P. A., Simon, M. C. and Keith, B. (2006). HIF-2alpha regulates Oct-4: effects of hypoxia on stem cell function, embryonic development, and tumor growth. *Genes Dev.* **20**, 557-570. doi:10.1101/gad.1399906

Cowden Dahl, K. D., Fryer, B. H., Mack, F. A., Compernolle, V., Maltepe, E., Adelman, D. M., Carmeliet, P. and Simon, M. C. (2005). Hypoxia-inducible factors 1alpha and 2alpha regulate trophoblast differentiation. *Mol. Cell. Biol.* **25**, 10479-10491. doi:10.1128/MCB.25.23.10479-10491.2005

Cummins, E. P., Berra, E., Comerford, K. M., Ginouves, A., Fitzgerald, K. T., Seeballuck, F., Godson, C., Nielsen, J. E., Moynagh, P., Pouyssegur, J. et al. (2006). Prolyl hydroxylase-1 negatively regulates IkappaB kinase-beta, giving insight into hypoxia-induced NFkappaB activity. *Proc. Natl. Acad. Sci. USA* **103**, 18154-18159. doi:10.1073/pnas.0602235103

Dai, Z., Li, M., Wharton, J., Zhu, M. M. and Zhao, Y. Y. (2016). Prolyl-4 hydroxylase 2 (PHD2) deficiency in endothelial cells and hematopoietic cells induces obliterative vascular remodeling and severe pulmonary arterial hypertension in mice and humans through hypoxia-inducible factor-2alpha. *Circulation* **133**, 2447-2458. doi:10.1161/CIRCULATIONAHA.116.021494

Das, B., Bayat-Mokhtari, R., Tsui, M., Lotfi, S., Tsuchida, R., Felsher, D. W. and Yeger, H. (2012). HIF-2alpha suppresses p53 to enhance the stemness and regenerative potential of human embryonic stem cells. *Stem Cells* **30**, 1685-1695. doi:10.1002/stem.1142

Das, N., Xie, L., Ramakrishnan, S. K., Campbell, A., Rivella, S. and Shah, Y. M. (2015). Intestine-specific Disruption of Hypoxia-inducible Factor (HIF)-2alpha Improves Anemia in Sickle Cell Disease. *J. Biol. Chem.* **290**, 23523-23527. doi:10.1074/jbc.C115.681643

Ding, H., Gao, Y. S., Hu, C., Wang, Y., Wang, C. G., Yin, J. M., Sun, Y. and Zhang, C. Q. (2013). HIF-1alpha transgenic bone marrow cells can promote tissue repair in cases of corticosteroid-induced osteonecrosis of the femoral head in rabbits. *PLoS ONE* **8**, e63628. doi:10.1371/journal.pone.0063628

Downes, N. L., Laham-Karam, N., Kaikkonen, M. U. and Yla-Herttuala, S. (2018). Differential but complementary HIF1alpha and HIF2alpha Transcriptional regulation. *Mol. Ther.* **26**, 1735-1745. doi:10.1016/j.ymthe.2018.05.004

Duan, L. J. and Fong, G. H. (2019). Developmental vascular pruning in neonatal mouse retinas is programmed by the astrocytic oxygen-sensing mechanism. *Development* **146**, dev175117. doi:10.1242/dev.175117

Duan, L. J., Takeda, K. and Fong, G. H. (2011). Prolyl hydroxylase domain protein 2 (PHD2) mediates oxygen-induced retinopathy in neonatal mice. *Am. J. Pathol.* **178**, 1881-1890. doi:10.1016/j.ajpath.2010.12.016

Duan, L. J., Takeda, K. and Fong, G. H. (2014a). Hematological, hepatic, and retinal phenotypes in mice deficient for prolyl hydroxylase domain proteins in the liver. *Am. J. Pathol.* **184**, 1240-1250. doi:10.1016/j.ajpath.2013.12.014

Duan, L. J., Takeda, K. and Fong, G. H. (2014b). Hypoxia inducible factor-2alpha regulates the development of retinal astrocytic network by maintaining adequate supply of astrocyte progenitors. *PLoS ONE* **9**, e84736. doi:10.1371/journal.pone.0084736

Dutta, D., Ray, S., Vivian, J. L. and Paul, S. (2008). Activation of the VEGFR1 chromatin domain: an angiogenic signal-ETS1/HIF-2alpha regulatory axis. *J. Biol. Chem.* **283**, 25404-25413. doi:10.1074/jbc.M804349200

Elson, D. A., Thurston, G., Huang, L. E., Ginzinger, D. G., McDonald, D. M., Johnson, R. S. and Arbeit, J. M. (2001). Induction of hypervascularity without leakage or inflammation in transgenic mice overexpressing hypoxia-inducible factor-1alpha. *Genes Dev.* **15**, 2520-2532. doi:10.1101/gad.914801

Elvert, G., Kappel, A., Heidenreich, R., Englmeier, U., Lanz, S., Acker, T., Rauter, M., Plate, K., Sieweke, M., Breier, G. et al. (2003). Cooperative interaction of hypoxia-inducible factor-2alpha (HIF-2alpha) and Ets-1 in the transcriptional activation of vascular endothelial growth factor receptor-2 (Flk-1). *J. Biol. Chem.* **278**, 7520-7530. doi:10.1074/jbc.M211298200

Elzakra, N. and Kim, Y. (2021). HIF-1alpha Metabolic Pathways in Human Cancer. *Adv. Exp. Med. Biol.* **1280**, 243-260. doi:10.1007/978-3-030-51652-9_17

Ema, M., Hirota, K., Mimura, J., Abe, H., Yodoi, J., Sogawa, K., Poellinger, L. and Fujii-Kuriyama, Y. (1999). Molecular mechanisms of transcription activation by HLF and HIF1alpha in response to hypoxia: their stabilization and redox signal-induced interaction with CBP/p300. *EMBO J.* **18**, 1905-1914.

Epstein, A. C., Gleadle, J. M., McNeill, L. A., Hewitson, K. S., O'Rourke, J., Mole, D. R., Mukherji, M., Metzen, E., Wilson, M. I., Dhanda, A. et al. (2001). C. elegans EGL-9 and mammalian homologs define a family of dioxygenases that regulate HIF by prolyl hydroxylation. *Cell* **107**, 43-54. doi:10.1016/S0092-8674(01)00507-4

Fathollahipour, S., Patil, P. S. and Leipzig, N. D. (2018). Oxygen regulation in development: lessons from embryogenesis towards tissue engineering. *Cells Tissues Organs* **205**, 350-371. doi:10.1159/000493162

Feldhoff, L. M., Rueda, C. M., Moreno-Fernandez, M. E., Sauer, J., Jackson, C. M., Chougnet, C. A. and Rupp, J. (2017). IL-1beta induced HIF-1alpha inhibits the differentiation of human FOXP3(+) T cells. *Sci. Rep.* **7**, 465. doi:10.1038/s41598-017-00508-x

Feng, Z., Zou, X., Chen, Y., Wang, H., Duan, Y. and Bruick, R. K. (2018). Modulation of HIF-2alpha PAS-B domain contributes to physiological responses. *Proc. Natl. Acad. Sci. USA* **115**, 13240-13245. doi:10.1073/pnas.1810897115

Forsythe, J. A., Jiang, B. H., Iyer, N. V., Agani, F., Leung, S. W., Koos, R. D. and Semenza, G. L. (1996). Activation of vascular endothelial growth factor gene transcription by hypoxia-inducible factor 1. *Mol. Cell. Biol.* **16**, 4604-4613. doi:10.1128/MCB.16.9.4604

Fu, J. and Taubman, M. B. (2013). EGLN3 inhibition of NF-kappaB is mediated by prolyl hydroxylase-independent inhibition of IkappaB kinase gamma ubiquitination. *Mol. Cell. Biol.* **33**, 3050-3061. doi:10.1128/MCB.00273-13

Fujita, N., Markova, D., Anderson, D. G., Chiba, K., Toyama, Y., Shapiro, I. M. and Risbud, M. V. (2012). Expression of prolyl hydroxylases (PHDs) is selectively controlled by HIF-1 and HIF-2 proteins in nucleus pulposus cells of the intervertebral disc: distinct roles of PHD2 and PHD3 proteins in controlling

HIF-1alpha activity in hypoxia. *J. Biol. Chem.* **287**, 16975-16986. doi:10.1074/jbc.M111.334466

Gerald, D., Berra, E., Frapart, Y. M., Chan, D. A., Giaccia, A. J., Mansuy, D., Pouyssegur, J., Yaniv, M. and Mechta-Grigoriou, F. (2004). JunD reduces tumor angiogenesis by protecting cells from oxidative stress. *Cell* **118**, 781-794. doi:10.1016/j.cell.2004.08.025

Gnarra, J. R., Ward, J. M., Porter, F. D., Wagner, J. R., Devor, D. E., Grinberg, A., Emmert-Buck, M. R., Westphal, H., Klausner, R. D. and Linehan, W. M. (1997). Defective placental vasculogenesis causes embryonic lethality in VHL-deficient mice. *Proc. Natl. Acad. Sci. USA* **94**, 9102-9107. doi:10.1073/pnas.94.17.9102

Goda, N., Ryan, H. E., Khadivi, B., McNulty, W., Rickert, R. C. and Johnson, R. S. (2003). Hypoxia-inducible factor 1alpha is essential for cell cycle arrest during hypoxia. *Mol. Cell. Biol.* **23**, 359-369. doi:10.1128/MCB.23.1.359-369.2003

Guo, J., Chakraborty, A. A., Liu, P., Gan, W., Zheng, X., Inuzuka, H., Wang, B., Zhang, J., Zhang, L., Yuan, M. et al. (2016). pVHL suppresses kinase activity of Akt in a proline-hydroxylation-dependent manner. *Science* **353**, 929-932. doi:10.1126/science.aad5755

Gustafsson, M. V., Zheng, X., Pereira, T., Gradin, K., Jin, S., Lundkvist, J., Ruas, J. L., Poellinger, L., Lendahl, U. and Bondesson, M. (2005). Hypoxia requires notch signaling to maintain the undifferentiated cell state. *Dev. Cell* **9**, 617-628. doi:10.1016/j.devcel.2005.09.010

Haase, V. H. (2017). HIF-prolyl hydroxylases as therapeutic targets in erythropoiesis and iron metabolism. *Hemodial. Int.* **21** Suppl. 1, S110-S124. doi:10.1111/hdi.12567

Haase, V. H., Glickman, J. N., Socolovsky, M. and Jaenisch, R. (2001). Vascular tumors in livers with targeted inactivation of the von Hippel-Lindau tumor suppressor. *Proc. Natl. Acad. Sci. USA* **98**, 1583-1588. doi:10.1073/pnas.98.4.1583

Hayes, J. D., Dinkova-Kostova, A. T. and Tew, K. D. (2020). Oxidative Stress in Cancer. *Cancer Cell* **38**, 167-197. doi:10.1016/j.ccell.2020.06.001

Hewitson, K. S., Lienard, B. M., McDonough, M. A., Clifton, I. J., Butler, D., Soares, A. S., Oldham, N. J., McNeill, L. A. and Schofield, C. J. (2007). Structural and mechanistic studies on the inhibition of the hypoxia-inducible transcription factor hydroxylases by tricarboxylic acid cycle intermediates. *J. Biol. Chem.* **282**, 3293-3301. doi:10.1074/jbc.M608337200

Hirose, K., Morita, M., Ema, M., Mimura, J., Hamada, H., Fujii, H., Saijo, Y., Gotoh, O., Sogawa, K. and Fujii-Kuriyama, Y. (1996). cDNA cloning and tissue-specific expression of a novel basic helix-loop-helix/PAS factor (Arnt2) with close sequence similarity to the aryl hydrocarbon receptor nuclear translocator (Arnt). *Mol. Cell. Biol.* **16**, 1706-1713. doi:10.1128/MCB.16.4.1706

Hirsila, M., Koivunen, P., Gunzler, V., Kivirikko, K. I. and Myllyharju, J. (2003). Characterization of the human prolyl 4-hydroxylases that modify the hypoxia-inducible factor. *J. Biol. Chem.* **278**, 30772-30780. doi:10.1074/jbc.M304982200

Holscher, M., Silter, M., Krull, S., von Ahlen, M., Hesse, A., Schwartz, P., Wielockx, B., Breier, G., Katschinski, D. M. and Zieseniss, A. (2011). Cardiomyocyte-specific prolyl-4-hydroxylase domain 2 knock out protects from acute myocardial ischemic injury. *J. Biol. Chem.* **286**, 11185-11194. doi:10.1074/jbc.M110.186809

Hu, C. J., Wang, L. Y., Chodosh, L. A., Keith, B. and Simon, M. C. (2003). Differential roles of hypoxia-inducible factor 1alpha (HIF-1alpha) and HIF-2alpha in hypoxic gene regulation. *Mol. Cell. Biol.* **23**, 9361-9374. doi:10.1128/MCB.23.24.9361-9374.2003

Hu, C. J., Iyer, S., Sataur, A., Covello, K. L., Chodosh, L. A. and Simon, M. C. (2006). Differential regulation of the transcriptional activities of hypoxia-inducible factor 1 alpha (HIF-1alpha) and HIF-2alpha in stem cells. *Mol. Cell. Biol.* **26**, 3514-3526. doi:10.1128/MCB.26.9.3514-3526.2006

Huang, M., Nguyen, P., Jia, F., Hu, S., Gong, Y., de Almeida, P. E., Wang, L., Nag, D., Kay, M. A., Giaccia, A. J. et al. (2011). Double knockdown of prolyl hydroxylase and factor-inhibiting hypoxia-inducible factor with nonviral minicircle gene therapy enhances stem cell mobilization and angiogenesis after myocardial infarction. *Circulation* **124**, S46-S54.

Huang, Y., Kapere Ochieng, J., Kempen, M. B., Munck, A. B., Swagemakers, S., van Ijcken, W., Grosveld, F., Tibboel, D. and Rottier, R. J. (2013). Hypoxia inducible factor 3alpha plays a critical role in alveolarization and distal epithelial cell differentiation during mouse lung development. *PLoS ONE* **8**, e57695. doi:10.1371/journal.pone.0057695

Hyvarinen, J., Hassinen, I. E., Sormunen, R., Maki, J. M., Kivirikko, K. I., Koivunen, P. and Myllyharju, J. (2010a). Hearts of hypoxia-inducible factor prolyl 4-hydroxylase-2 hypomorphic mice show protection against acute ischemia-reperfusion injury. *J. Biol. Chem.* **285**, 13646-13657. doi:10.1074/jbc.M109.084855

Hyvarinen, J., Parikka, M., Sormunen, R., Ramet, M., Tryggvason, K., Kivirikko, K. I., Myllyharju, J. and Koivunen, P. (2010b). Deficiency of a transmembrane prolyl 4-hydroxylase in the zebrafish leads to basement membrane defects and compromised kidney function. *J. Biol. Chem.* **285**, 42023-42032. doi:10.1074/jbc.M110.145904

Ibuki, M., Shoda, C., Miwa, Y., Ishida, A., Tsubota, K. and Kurihara, T. (2020). Lactoferrin has a therapeutic effect via HIF inhibition in a murine model of choroidal neovascularization. *Front. Pharmacol* **11**, 174. doi:10.3389/fphar.2020.00174

Ivan, M., Haberberger, T., Gervasi, D. C., Michelson, K. S., Gunzler, V., Kondo, K., Yang, H., Sorokina, I., Conaway, R. C., Conaway, J. W. et al. (2002). Biochemical purification and pharmacological inhibition of a mammalian prolyl hydroxylase acting on hypoxia-inducible factor. *Proc. Natl. Acad. Sci. USA* **99**, 13459-13464. doi:10.1073/pnas.192342099

Iyer, N. V., Kotch, L. E., Agani, F., Leung, S. W., Laughner, E., Wenger, R. H., Gassmann, M., Gearhart, J. D., Lawler, A. M., Yu, A. Y. et al. (1998). Cellular and developmental control of O2 homeostasis by hypoxia-inducible factor 1 alpha. *Genes Dev.* **12**, 149-162. doi:10.1101/gad.12.2.149

Jain, S., Maltepe, E., Lu, M. M., Simon, C. and Bradfield, C. A. (1998). Expression of ARNT, ARNT2, HIF1 alpha, HIF2 alpha and Ah receptor mRNAs in the developing mouse. *Mech. Dev.* **73**, 117-123. doi:10.1016/S0925-4773(98)00038-0

Jeong, J. W., Bae, M. K., Ahn, M. Y., Kim, S. H., Sohn, T. K., Bae, M. H., Yoo, M. A., Song, E. J., Lee, K. J. and Kim, K. W. (2002). Regulation and destabilization of HIF-1alpha by ARD1-mediated acetylation. *Cell* **111**, 709-720. doi:10.1016/S0092-8674(02)01085-1

Jiang, X., Tian, W., Tu, A. B., Pasupneti, S., Shuffle, E., Dahms, P., Zhang, P., Cai, H., Dinh, T. T., Liu, B. et al. (2019). Endothelial hypoxia-inducible factor-2alpha is required for the maintenance of airway microvasculature. *Circulation* **139**, 502-517. doi:10.1161/CIRCULATIONAHA.118.036157

Kaelin, W. G. (2007). Von Hippel-Lindau disease. *Annu. Rev. Pathol.* **2**, 145-173. doi:10.1146/annurev.pathol.2.010506.092049

Kapitsinou, P. P., Liu, Q., Unger, T. L., Rha, J., Davidoff, O., Keith, B., Epstein, J. A., Moores, S. L., Erickson-Miller, C. L. and Haase, V. H. (2010). Hepatic HIF-2 regulates erythropoietic responses to hypoxia in renal anemia. *Blood* **116**, 3039-3048. doi:10.1182/blood-2010-02-270322

Kapitsinou, P. P., Sano, H., Michael, M., Kobayashi, H., Davidoff, O., Bian, A., Yao, B., Zhang, M. Z., Harris, R. C., Duffy, K. J. et al. (2014). Endothelial HIF-2 mediates protection and recovery from ischemic kidney injury. *J. Clin. Invest.* **124**, 2396-2409. doi:10.1172/JCI69073

Kappel, A., Ronicke, V., Damert, A., Flamme, I., Risau, W. and Breier, G. (1999). Identification of vascular endothelial growth factor (VEGF) receptor-2 (Flk-1) promoter/enhancer sequences sufficient for angioblast and endothelial cell-specific transcription in transgenic mice. *Blood* **93**, 4284-4292. doi:10.1182/blood.V93.12.4284

Kasper, L. H., Boussouar, F., Boyd, K., Xu, W., Biesen, M., Rehg, J., Baudino, T. A., Cleveland, J. L. and Brindle, P. K. (2005). Two transactivation mechanisms cooperate for the bulk of HIF-1-responsive gene expression. *EMBO J.* **24**, 3846-3858. doi:10.1038/sj.emboj.7600846

Keith, B., Adelman, D. M. and Simon, M. C. (2001). Targeted mutation of the murine arylhydrocarbon receptor nuclear translocator 2 (Arnt2) gene reveals partial redundancy with Arnt. *Proc. Natl. Acad. Sci. USA* **98**, 6692-6697. doi:10.1073/pnas.121494298

Kim, Y., Nam, H. J., Lee, J., Park, D. Y., Kim, C., Yu, Y. S., Kim, D., Park, S. W., Bhin, J., Hwang, D. et al. (2016). Methylation-dependent regulation of HIF-1alpha stability restricts retinal and tumour angiogenesis. *Nat. Commun.* **7**, 10347. doi:10.1038/ncomms10347

Kleszka, K., Leu, T., Quinting, T., Jastrow, H., Pechlivanis, S., Fandrey, J. and Schreiber, T. (2020). Hypoxia-inducible factor-2alpha is crucial for proper brain development. *Sci. Rep.* **10**, 19146. doi:10.1038/s41598-020-75838-4

Kobayashi, H., Liu, J., Urrutia, A. A., Burmakin, M., Ishii, K., Rajan, M., Davidoff, O., Saifudeen, Z. and Haase, V. H. (2017). Hypoxia-inducible factor prolyl-4-hydroxylation in FOXD1 lineage cells is essential for normal kidney development. *Kidney Int.* **92**, 1370-1383. doi:10.1016/j.kint.2017.06.015

Kocabas, F., Xie, L., Xie, J., Yu, Z., DeBerardinis, R. J., Kimura, W., Thet, S., Elshamy, A. F., Abouellail, H., Muralidhar, S. et al. (2015). Hypoxic metabolism in human hematopoietic stem cells. *Cell Biosci* **5**, 39. doi:10.1186/s13578-015-0020-3

Koeppen, M., Lee, J. W., Seo, S. W., Brodsky, K. S., Kreth, S., Yang, I. V., Buttrick, P. M., Eckle, T. and Eltzschig, H. K. (2018). Hypoxia-inducible factor 2-alpha-dependent induction of amphiregulin dampens myocardial ischemia-reperfusion injury. *Nat. Commun.* **9**, 816. doi:10.1038/s41467-018-03105-2

Koivunen, P., Hirsila, M., Gunzler, V., Kivirikko, K. I. and Myllyharju, J. (2004). Catalytic properties of the asparaginyl hydroxylase (FIH) in the oxygen sensing pathway are distinct from those of its prolyl 4-hydroxylases. *J. Biol. Chem.* **279**, 9899-9904. doi:10.1074/jbc.M312254200

Koivunen, P., Tiainen, P., Hyvarinen, J., Williams, K. E., Sormunen, R., Klaus, S. J., Kivirikko, K. I. and Myllyharju, J. (2007). An endoplasmic reticulum transmembrane prolyl 4-hydroxylase is induced by hypoxia and acts on hypoxia-inducible factor alpha. *J. Biol. Chem.* **282**, 30544-30552. doi:10.1074/jbc.M704988200

Kok, F. O., Shin, M., Ni, C. W., Gupta, A., Grosse, A. S., van Impel, A., Kirchmaier, B. C., Peterson-Maduro, J., Kourkoulis, G., Male, I. et al. (2015). Reverse genetic screening reveals poor correlation between morpholino-induced and mutant phenotypes in zebrafish. *Dev. Cell* **32**, 97-108. doi:10.1016/j.devcel.2014.11.018

Krishnan, J., Ahuja, P., Bodenmann, S., Knapik, D., Perriard, E., Krek, W. and Perriard, J. C. (2008). Essential role of developmentally activated hypoxia-

inducible factor 1alpha for cardiac morphogenesis and function. *Circ. Res.* **103**, 1139-1146. doi:10.1161/01.RES.0000338613.89841.c1

Lando, D., Peet, D. J., Whelan, D. A., Gorman, J. J. and Whitelaw, M. L. (2002). Asparagine hydroxylation of the HIF transactivation domain a hypoxic switch. *Science* **295**, 858-861. doi:10.1126/science.1068592

Lange, C., Turrero Garcia, M., Decimo, I., Bifari, F., Eelen, G., Quaegebeur, A., Boon, R., Zhao, H., Boeckx, B., Chang, J. et al. (2016). Relief of hypoxia by angiogenesis promotes neural stem cell differentiation by targeting glycolysis. *EMBO J.* **35**, 924-941. doi:10.15252/embj.201592372

Lee, M. Y., Luciano, A. K., Ackah, E., Rodriguez-Vita, J., Bancroft, T. A., Eichmann, A., Simons, M., Kyriakides, T. R., Morales-Ruiz, M. and Sessa, W. C. (2014). Endothelial Akt1 mediates angiogenesis by phosphorylating multiple angiogenic substrates. *Proc. Natl. Acad. Sci. USA* **111**, 12865-12870. doi:10.1073/pnas.1408472111

Li, H., Ko, H. P. and Whitlock, J. P. (1996). Induction of phosphoglycerate kinase 1 gene expression by hypoxia. Roles of Arnt and HIF1alpha. *J. Biol. Chem.* **271**, 21262-21267. doi:10.1074/jbc.271.35.21262

Li, J., Tao, T., Xu, J., Liu, Z., Zou, Z. and Jin, M. (2020). HIF1alpha attenuates neuronal apoptosis by upregulating EPO expression following cerebral ischemiareperfusion injury in a rat MCAO model. *Int. J. Mol. Med.* **45**, 1027-1036.

Liu, Y. V., Baek, J. H., Zhang, H., Diez, R., Cole, R. N. and Semenza, G. L. (2007). RACK1 competes with HSP90 for binding to HIF-1alpha and is required for O(2)-independent and HSP90 inhibitor-induced degradation of HIF-1alpha. *Mol. Cell* **25**, 207-217. doi:10.1016/j.molcel.2007.01.001

Liu, X., Chen, Z., Xu, C., Leng, X., Cao, H., Ouyang, G. and Xiao, W. (2015). Repression of hypoxia-inducible factor alpha signaling by Set7-mediated methylation. *Nucleic Acids Res.* **43**, 5081-5098. doi:10.1093/nar/gkv379

Loinard, C., Ginouves, A., Vilar, J., Cochain, C., Zouggari, Y., Recalde, A., Duriez, M., Levy, B. I., Pouyssegur, J., Berra, E. et al. (2009). Inhibition of prolyl hydroxylase domain proteins promotes therapeutic revascularization. *Circulation* **120**, 50-59. doi:10.1161/CIRCULATIONAHA.108.813303

Lu, C. W., Lin, S. C., Chen, K. F., Lai, Y. Y. and Tsai, S. J. (2008). Induction of pyruvate dehydrogenase kinase-3 by hypoxia-inducible factor-1 promotes metabolic switch and drug resistance. *J. Biol. Chem.* **283**, 28106-28114. doi:10.1074/jbc.M803508200

Luo, W., Lin, B., Wang, Y., Zhong, J., O'Meally, R., Cole, R. N., Pandey, A., Levchenko, A. and Semenza, G. L. (2014). PHD3-mediated prolyl hydroxylation of nonmuscle actin impairs polymerization and cell motility. *Mol. Biol. Cell* **25**, 2788-2796. doi:10.1091/mbc.e14-02-0775

Lv, B., Li, F., Han, J., Fang, J., Xu, L., Sun, C., Hua, T., Zhang, Z., Feng, Z. and Jiang, X. (2017). Hif-1alpha Overexpression improves transplanted bone mesenchymal stem cells survival in rat MCAO stroke model. *Front Mol Neurosci* **10**, 80.

Madan, E., Parker, T. M., Pelham, C. J., Palma, A. M., Peixoto, M. L., Nagane, M., Chandaria, A., Tomas, A. R., Canas-Marques, R., Henriques, V. et al. (2019). HIF-transcribed p53 chaperones HIF-1alpha. *Nucleic Acids Res.* **47**, 10212-10234. doi:10.1093/nar/gkz766

Mahon, P. C., Hirota, K. and Semenza, G. L. (2001). FIH-1: a novel protein that interacts with HIF-1alpha and VHL to mediate repression of HIF-1 transcriptional activity. *Genes Dev.* **15**, 2675-2686. doi:10.1101/gad.924501

Makino, Y., Cao, R., Svensson, K., Bertilsson, G., Asman, M., Tanaka, H., Cao, Y., Berkenstam, A. and Poellinger, L. (2001). Inhibitory PAS domain protein is a negative regulator of hypoxia-inducible gene expression. *Nature* **414**, 550-554. doi:10.1038/35107085

Maltepe, E., Schmidt, J. V., Baunoch, D., Bradfield, C. A. and Simon, M. C. (1997). Abnormal angiogenesis and responses to glucose and oxygen deprivation in mice lacking the protein ARNT. *Nature* **386**, 403-407. doi:10.1038/386403a0

Mandl, M., Lieberum, M. K. and Depping, R. (2016). A HIF-1alpha-driven feed-forward loop augments HIF signalling in Hep3B cells by upregulation of ARNT. *Cell Death Dis* **7**, e2284. doi:10.1038/cddis.2016.187

Mathieu, J., Zhou, W., Xing, Y., Sperber, H., Ferreccio, A., Agoston, Z., Kuppusamy, K. T., Moon, R. T. and Ruohola-Baker, H. (2014). Hypoxia-inducible factors have distinct and stage-specific roles during reprogramming of human cells to pluripotency. *Cell Stem Cell* **14**, 592-605. doi:10.1016/j.stem.2014.02.012

Maxwell, P. H., Wiesener, M. S., Chang, G. W., Clifford, S. C., Vaux, E. C., Cockman, M. E., Wykoff, C. C., Pugh, C. W., Maher, E. R. and Ratcliffe, P. J. (1999). The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. *Nature* **399**, 271-275. doi:10.1038/20459

Metzen, E., Berchner-Pfannschmidt, U., Stengel, P., Marxsen, J. H., Stolze, I., Klinger, M., Huang, W. Q., Wotzlaw, C., Hellwig-Burgel, T., Jelkmann, W. et al. (2003). Intracellular localisation of human HIF-1 alpha hydroxylases: implications for oxygen sensing. *J. Cell Sci.* **116**, 1319-1326. doi:10.1242/jcs.00318

Michaud, J. L., DeRossi, C., May, N. R., Holdener, B. C. and Fan, C. M. (2000). ARNT2 acts as the dimerization partner of SIM1 for the development of the hypothalamus. *Mech. Dev.* **90**, 253-261. doi:10.1016/S0925-4773(99)00328-7

Min, J. H., Yang, H., Ivan, M., Gertler, F., Kaelin, W. G., Jr. and Pavletich, N. P. (2002). Structure of an HIF-1alpha -pVHL complex: hydroxyproline recognition in signaling. *Science* **296**, 1886-1889. doi:10.1126/science.1073440

Minamishima, Y. A., Moslehi, J., Bardeesy, N., Cullen, D., Bronson, R. T. and Kaelin, W. G.Jr. (2008). Somatic inactivation of the PHD2 prolyl hydroxylase causes polycythemia and congestive heart failure. *Blood* **111**, 3236-3244. doi:10.1182/blood-2007-10-117812

Morita, M., Ohneda, O., Yamashita, T., Takahashi, S., Suzuki, N., Nakajima, O., Kawauchi, S., Ema, M., Shibahara, S., Udono, T. et al. (2003). HLF/HIF-2alpha is a key factor in retinopathy of prematurity in association with erythropoietin. *EMBO J.* **22**, 1134-1146. doi:10.1093/emboj/cdg117

Moser, S. C., Bensaddek, D., Ortmann, B., Maure, J. F., Mudie, S., Blow, J. J., Lamond, A. I., Swedlow, J. R. and Rocha, S. (2013). PHD1 links cell-cycle progression to oxygen sensing through hydroxylation of the centrosomal protein Cep192. *Dev. Cell* **26**, 381-392. doi:10.1016/j.devcel.2013.06.014

Motta, S., Minici, C., Corrada, D., Bonati, L. and Pandini, A. (2018). Ligand-induced perturbation of the HIF-2alpha:ARNT dimer dynamics. *PLoS Comput. Biol.* **14**, e1006021. doi:10.1371/journal.pcbi.1006021

Mowat, F. M., Luhmann, U. F., Smith, A. J., Lange, C., Duran, Y., Harten, S., Shukla, D., Maxwell, P. H., Ali, R. R. and Bainbridge, J. W. (2010). HIF-1alpha and HIF-2alpha are differentially activated in distinct cell populations in retinal ischaemia. *PLoS One* **5**, e11103. doi:10.1371/journal.pone.0011103

Myllykoski, M., Sutinen, A., Koski, M. K., Kallio, J. P., Raasakka, A., Myllyharju, J., Wierenga, R. K. and Koivunen, P. (2021). Structure of transmembrane prolyl 4-hydroxylase reveals unique organization of EF and dioxygenase domains. *J. Biol. Chem.* **296**, 100197. doi:10.1074/jbc.RA120.016542

Mylonis, I., Chachami, G., Samiotaki, M., Panayotou, G., Paraskeva, E., Kalousi, A., Georgatsou, E., Bonanou, S. and Simos, G. (2006). Identification of MAPK phosphorylation sites and their role in the localization and activity of hypoxia-inducible factor-1alpha. *J. Biol. Chem.* **281**, 33095-33106. doi:10.1074/jbc.M605058200

Nagao, K., Taniyama, Y., Kietzmann, T., Doi, T., Komuro, I. and Morishita, R. (2008). HIF-1alpha signaling upstream of NKX2.5 is required for cardiac development in Xenopus. *J. Biol. Chem.* **283**, 11841-11849. doi:10.1074/jbc.M702563200

Nakayama, K., Frew, I. J., Hagensen, M., Skals, M., Habelhah, H., Bhoumik, A., Kadoya, T., Erdjument-Bromage, H., Tempst, P., Frappell, P. B. et al. (2004). Siah2 regulates stability of prolyl-hydroxylases, controls HIF1alpha abundance, and modulates physiological responses to hypoxia. *Cell* **117**, 941-952. doi:10.1016/j.cell.2004.06.001

Nasyrov, E., Nolan, K. A., Wenger, R. H., Marti, H. H. and Kunze, R. (2019). The neuronal oxygen-sensing pathway controls postnatal vascularization of the murine brain. *FASEB J.* **33**, 12812-12824. doi:10.1096/fj.201901385RR

Niklasson, C. U., Fredlund, E., Monni, E., Lindvall, J. M., Kokaia, Z., Hammarlund, E. U., Bronner, M. E. and Mohlin, S. (2021). Hypoxia inducible factor-2alpha importance for migration, proliferation, and self-renewal of trunk neural crest cells. *Dev. Dyn.* **250**, 191-236. doi:10.1002/dvdy.253

NobelPrize.Org (2019). The Nobel Prize in Physiology or Medicine 2019.

Novais, E. J., Choi, H., Madhu, V., Suyama, K., Anjo, S. I., Manadas, B., Shapiro, I. M., Salgado, A. J. and Risbud, M. V. (2021). Hypoxia and hypoxia-inducible factor-1alpha regulate endoplasmic reticulum stress in nucleus pulposus cells: implications of endoplasmic reticulum stress for extracellular matrix secretion. *Am. J. Pathol.* **191**, 487-502. doi:10.1016/j.ajpath.2020.11.012

Obach, M., Navarro-Sabate, A., Caro, J., Kong, X., Duran, J., Gomez, M., Perales, J. C., Ventura, F., Rosa, J. L. and Bartrons, R. (2004). 6-Phosphofructo-2-kinase (pfkfb3) gene promoter contains hypoxia-inducible factor-1 binding sites necessary for transactivation in response to hypoxia. *J. Biol. Chem.* **279**, 53562-53570. doi:10.1074/jbc.M406096200

Oktay, Y., Dioum, E., Matsuzaki, S., Ding, K., Yan, L. J., Haller, R. G., Szweda, L. I. and Garcia, J. A. (2007). Hypoxia-inducible factor 2alpha regulates expression of the mitochondrial aconitase chaperone protein frataxin. *J. Biol. Chem.* **282**, 11750-11756. doi:10.1074/jbc.M611133200

Olenchock, B. A., Moslehi, J., Baik, A. H., Davidson, S. M., Williams, J., Gibson, W. J., Chakraborty, A. A., Pierce, K. A., Miller, C. M., Hanse, E. A. et al. (2016). EGLN1 Inhibition and Rerouting of alpha-Ketoglutarate Suffice for Remote Ischemic Protection. *Cell* **164**, 884-895. doi:10.1016/j.cell.2016.02.006

Olson, E., Mahar, K. M., Morgan, L., Fillmore, C., Holland, C. and Lavery, L. (2019). Randomized phase I trial to evaluate the safety, tolerability, pharmacokinetics, and pharmacodynamics of topical daprodustat in healthy volunteers and in patients with diabetic foot ulcers. *Clin Pharmacol Drug Dev* **8**, 765-778. doi:10.1002/cpdd.704

Paliege, A., Rosenberger, C., Bondke, A., Sciesielski, L., Shina, A., Heyman, S. N., Flippin, L. A., Arend, M., Klaus, S. J. and Bachmann, S. (2010). Hypoxia-inducible factor-2alpha-expressing interstitial fibroblasts are the only renal cells that express erythropoietin under hypoxia-inducible factor stabilization. *Kidney Int.* **77**, 312-318. doi:10.1038/ki.2009.460

Palomaki, S., Pietila, M., Laitinen, S., Pesala, J., Sormunen, R., Lehenkari, P. and Koivunen, P. (2013). HIF-1alpha is upregulated in human mesenchymal stem cells. *Stem Cells* **31**, 1902-1909. doi:10.1002/stem.1435

Peng, J., Zhang, L., Drysdale, L. and Fong, G. H. (2000). The transcription factor EPAS-1/hypoxia-inducible factor 2alpha plays an important role in vascular

remodeling. *Proc. Natl. Acad. Sci. USA* **97**, 8386-8391. doi:10.1073/pnas.140087397

Percy, M. J., Zhao, Q., Flores, A., Harrison, C., Lappin, T. R., Maxwell, P. H., McMullin, M. F. and Lee, F. S. (2006). A family with erythrocytosis establishes a role for prolyl hydroxylase domain protein 2 in oxygen homeostasis. *Proc. Natl. Acad. Sci. USA* **103**, 654-659. doi:10.1073/pnas.0508423103

Percy, M. J., Chung, Y. J., Harrison, C., Mercieca, J., Hoffbrand, A. V., Dinardo, C. L., Santos, P. C., Fonseca, G. H., Gualandro, S. F., Pereira, A. C. et al. (2012). Two new mutations in the HIF2A gene associated with erythrocytosis. *Am. J. Hematol.* **87**, 439-442. doi:10.1002/ajh.23123

Perelli, R. M., O'Sullivan, M. L., Zarnick, S. and Kay, J. N. (2021). Environmental oxygen regulates astrocyte proliferation to guide angiogenesis during retinal development. *Development* **148**, dev199418. doi:10.1242/dev.199418

Quaegebeur, A., Segura, I., Schmieder, R., Verdegem, D., Decimo, I., Bifari, F., Dresselaers, T., Eelen, G., Ghosh, D., Davidson, S. M. et al. (2016). Deletion or inhibition of the oxygen sensor PHD1 protects against ischemic stroke via reprogramming of neuronal metabolism. *Cell Metab.* **23**, 280-291. doi:10.1016/j.cmet.2015.12.007

Rajagopalan, S., Olin, J., Deitcher, S., Pieczek, A., Laird, J., Grossman, P. M., Goldman, C. K., McEllin, K., Kelly, R. and Chronos, N. (2007). Use of a constitutively active hypoxia-inducible factor-1alpha transgene as a therapeutic strategy in no-option critical limb ischemia patients: phase I dose-escalation experience. *Circulation* **115**, 1234-1243. doi:10.1161/CIRCULATIONAHA.106.607994

Rankin, E. B., Biju, M. P., Liu, Q., Unger, T. L., Rha, J., Johnson, R. S., Simon, M. C., Keith, B. and Haase, V. H. (2007). Hypoxia-inducible factor-2 (HIF-2) regulates hepatic erythropoietin in vivo. *J. Clin. Invest.* **117**, 1068-1077. doi:10.1172/JCI30117

Ravi, R., Mookerjee, B., Bhujwalla, Z. M., Sutter, C. H., Artemov, D., Zeng, Q., Dillehay, L. E., Madan, A., Semenza, G. L. and Bedi, A. (2000). Regulation of tumor angiogenesis by p53-induced degradation of hypoxia-inducible factor 1alpha. *Genes Dev.* **14**, 34-44.

Reisz-Porszasz, S., Probst, M. R., Fukunaga, B. N. and Hankinson, O. (1994). Identification of functional domains of the aryl hydrocarbon receptor nuclear translocator protein (ARNT). *Mol. Cell. Biol.* **14**, 6075-6086.

Rieger, A., Menzel, M., Rainov, N. G., Sanchin, L., Rot, S., Furka, I., Gorombey, Z. and Burkert, W. (1997). Continuous monitoring of the partial pressure of oxygen in cerebral venous blood. *Neurosurgery* **41**, 462-467; discussion 467-468. doi:10.1097/00006123-199708000-00027

Roberts, A. M., Watson, I. R., Evans, A. J., Foster, D. A., Irwin, M. S. and Ohh, M. (2009). Suppression of hypoxia-inducible factor 2alpha restores p53 activity via Hdm2 and reverses chemoresistance of renal carcinoma cells. *Cancer Res.* **69**, 9056-9064. doi:10.1158/0008-5472.CAN-09-1770

Rodesch, F., Simon, P., Donner, C. and Jauniaux, E. (1992). Oxygen measurements in endometrial and trophoblastic tissues during early pregnancy. *Obstet. Gynecol.* **80**, 283-285.

Rodriguez, J., Herrero, A., Li, S., Rauch, N., Quintanilla, A., Wynne, K., Krstic, A., Acosta, J. C., Taylor, C., Schlisio, S. et al. (2018). PHD3 regulates p53 protein stability by hydroxylating proline 359. *Cell Rep* **24**, 1316-1329. doi:10.1016/j.celrep.2018.06.108

Rouault-Pierre, K., Lopez-Onieva, L., Foster, K., Anjos-Afonso, F., Lamrissi-Garcia, I., Serrano-Sanchez, M., Mitter, R., Ivanovic, Z., de Verneuil, H., Gribben, J. et al. (2013). HIF-2alpha protects human hematopoietic stem/progenitors and acute myeloid leukemic cells from apoptosis induced by endoplasmic reticulum stress. *Cell Stem Cell* **13**, 549-563. doi:10.1016/j.stem.2013.08.011

Ryan, H. E., Lo, J. and Johnson, R. S. (1998). HIF-1 alpha is required for solid tumor formation and embryonic vascularization. *EMBO J.* **17**, 3005-3015. doi:10.1093/emboj/17.11.3005

Saini, Y., Harkema, J. R. and LaPres, J. J. (2008). HIF1alpha is essential for normal intrauterine differentiation of alveolar epithelium and surfactant production in the newborn lung of mice. *J. Biol. Chem.* **283**, 33650-33657. doi:10.1074/jbc.M805927200

Saini, Y., Proper, S. P., Dornbos, P., Greenwood, K. K., Kopec, A. K., Lynn, S. G., Grier, E., Burgoon, L. D., Zacharewski, T. R., Thomas, R. S. et al. (2015). Loss of Hif-2alpha rescues the hif-1alpha deletion phenotype of neonatal respiratory distress in mice. *PLoS ONE* **10**, e0139270.

Saitou, M., Furuse, M., Sasaki, H., Schulzke, J. D., Fromm, M., Takano, H., Noda, T. and Tsukita, S. (2000). Complex phenotype of mice lacking occludin, a component of tight junction strands. *Mol. Biol. Cell* **11**, 4131-4142. doi:10.1091/mbc.11.12.4131

Sano, M., Minamino, T., Toko, H., Miyauchi, H., Orimo, M., Qin, Y., Akazawa, H., Tateno, K., Kayama, Y., Harada, M. et al. (2007). p53-induced inhibition of Hif-1 causes cardiac dysfunction during pressure overload. *Nature* **446**, 444-448. doi:10.1038/nature05602

Sarkar, K., Cai, Z., Gupta, R., Parajuli, N., Fox-Talbot, K., Darshan, M. S., Gonzalez, F. J. and Semenza, G. L. (2012). Hypoxia-inducible factor 1 transcriptional activity in endothelial cells is required for acute phase cardioprotection induced by ischemic preconditioning. *Proc. Natl. Acad. Sci. USA* **109**, 10504-10509. doi:10.1073/pnas.1208314109

Schipani, E., Ryan, H. E., Didrickson, S., Kobayashi, T., Knight, M. and Johnson, R. S. (2001). Hypoxia in cartilage: HIF-1alpha is essential for chondrocyte growth arrest and survival. *Genes Dev.* **15**, 2865-2876.

Scortegagna, M., Ding, K., Oktay, Y., Gaur, A., Thurmond, F., Yan, L. J., Marck, B. T., Matsumoto, A. M., Shelton, J. M., Richardson, J. A. et al. (2003). Multiple organ pathology, metabolic abnormalities and impaired homeostasis of reactive oxygen species in Epas1-/- mice. *Nat. Genet.* **35**, 331-340. doi:10.1038/ng1266

Semenza, G. L. (2011). Regulation of metabolism by hypoxia-inducible factor 1. *Cold Spring Harb. Symp. Quant. Biol.* **76**, 347-353. doi:10.1101/sqb.2011.76.010678

Semenza, G. L. (2019). Pharmacologic Targeting of Hypoxia-Inducible Factors. *Annu. Rev. Pharmacol. Toxicol.* **59**, 379-403. doi:10.1146/annurev-pharmtox-010818-021637

Settelmeier, S., Schreiber, T., Maki, J., Byts, N., Koivunen, P., Myllyharju, J., Fandrey, J. and Winning, S. (2020). Prolyl hydroxylase domain 2 reduction enhances skeletal muscle tissue regeneration after soft tissue trauma in mice. *PLoS One* **15**, e0233261. doi:10.1371/journal.pone.0233261

Shao, Z., Zhang, Y. and Powell-Coffman, J. A. (2009). Two distinct roles for EGL-9 in the regulation of HIF-1-mediated gene expression in Caenorhabditis elegans. *Genetics* **183**, 821-829. doi:10.1534/genetics.109.107284

Siggaard-Andersen, O. and Huch, R. (1995). The oxygen status of fetal blood. *Acta Anaesthesiol. Scand. Suppl.* **107**, 129-135. doi:10.1111/j.1399-6576.1995.tb04347.x

Skuli, N., Majmundar, A. J., Krock, B. L., Mesquita, R. C., Mathew, L. K., Quinn, Z. L., Runge, A., Liu, L., Kim, M. N., Liang, J. et al. (2012). Endothelial HIF-2alpha regulates murine pathological angiogenesis and revascularization processes. *J. Clin. Invest.* **122**, 1427-1443. doi:10.1172/JCI57322

So, J. H., Kim, J. D., Yoo, K. W., Kim, H. T., Jung, S. H., Choi, J. H., Lee, M. S., Jin, S. W. and Kim, C. H. (2014). FIH-1, a novel interactor of mindbomb, functions as an essential anti-angiogenic factor during zebrafish vascular development. *PLoS ONE* **9**, e109517. doi:10.1371/journal.pone.0109517

Song, D., Li, L. S., Heaton-Johnson, K. J., Arsenault, P. R., Master, S. R. and Lee, F. S. (2013). Prolyl hydroxylase domain protein 2 (PHD2) binds a Pro-Xaa-Leu-Glu motif, linking it to the heat shock protein 90 pathway. *J. Biol. Chem.* **288**, 9662-9674. doi:10.1074/jbc.M112.440552

Steinhoff, A., Pientka, F. K., Mockel, S., Kettelhake, A., Hartmann, E., Kohler, M. and Depping, R. (2009). Cellular oxygen sensing: Importins and exportins are mediators of intracellular localisation of prolyl-4-hydroxylases PHD1 and PHD2. *Biochem. Biophys. Res. Commun.* **387**, 705-711. doi:10.1016/j.bbrc.2009.07.090

Stothers, C. L., Luan, L., Fensterheim, B. A. and Bohannon, J. K. (2018). Hypoxia-inducible factor-1alpha regulation of myeloid cells. *J. Mol. Med. (Berl.)* **96**, 1293-1306. doi:10.1007/s00109-018-1710-1

Takeda, K., Ho, V. C., Takeda, H., Duan, L. J., Nagy, A. and Fong, G. H. (2006). Placental but not heart defects are associated with elevated hypoxia-inducible factor alpha levels in mice lacking prolyl hydroxylase domain protein 2. *Mol. Cell. Biol.* **26**, 8336-8346. doi:10.1128/MCB.00425-06

Takeda, K., Cowan, A. and Fong, G. H. (2007). Essential role for prolyl hydroxylase domain protein 2 in oxygen homeostasis of the adult vascular system. *Circulation* **116**, 774-781. doi:10.1161/CIRCULATIONAHA.107.701516

Takeda, K., Aguila, H. L., Parikh, N. S., Li, X., Lamothe, K., Duan, L. J., Takeda, H., Lee, F. S. and Fong, G. H. (2008). Regulation of adult erythropoiesis by prolyl hydroxylase domain proteins. *Blood* **111**, 3229-3235. doi:10.1182/blood-2007-09-114561

Takeda, K., Duan, L. J., Takeda, H. and Fong, G. H. (2014). Improved vascular survival and growth in the mouse model of hindlimb ischemia by a remote signaling mechanism. *Am. J. Pathol.* **184**, 686-696. doi:10.1016/j.ajpath.2013.11.032

Takeda, Y., Costa, S., Delamarre, E., Roncal, C., Leite de Oliveira, R., Squadrito, M. L., Finisguerra, V., Deschoemaeker, S., Bruyere, F., Wenes, M. et al. (2011). Macrophage skewing by Phd2 haplodeficiency prevents ischaemia by inducing arteriogenesis. *Nature* **479**, 122-126. doi:10.1038/nature10507

Tian, H., McKnight, S. L. and Russell, D. W. (1997). Endothelial PAS domain protein 1 (EPAS1), a transcription factor selectively expressed in endothelial cells. *Genes Dev.* **11**, 72-82. doi:10.1101/gad.11.1.72

Tian, H., Hammer, R. E., Matsumoto, A. M., Russell, D. W. and McKnight, S. L. (1998). The hypoxia-responsive transcription factor EPAS1 is essential for catecholamine homeostasis and protection against heart failure during embryonic development. *Genes Dev.* **12**, 3320-3324. doi:10.1101/gad.12.21.3320

Tomita, S., Ueno, M., Sakamoto, M., Kitahama, Y., Ueki, M., Maekawa, N., Sakamoto, H., Gassmann, M., Kageyama, R., Ueda, N. et al. (2003). Defective brain development in mice lacking the Hif-1alpha gene in neural cells. *Mol. Cell. Biol.* **23**, 6739-6749. doi:10.1128/MCB.23.19.6739-6749.2003

Uniacke, J., Holterman, C. E., Lachance, G., Franovic, A., Jacob, M. D., Fabian, M. R., Payette, J., Holcik, M., Pause, A. and Lee, S. (2012). An oxygen-regulated switch in the protein synthesis machinery. *Nature* **486**, 126-129. doi:10.1038/nature11055

Wang, G. L. and Semenza, G. L. (1995). Purification and characterization of hypoxia-inducible factor 1. *J. Biol. Chem.* **270**, 1230-1237. doi:10.1074/jbc.270.3.1230

Wang, G. L., Jiang, B. H., Rue, E. A. and Semenza, G. L. (1995). Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O2 tension. *Proc. Natl. Acad. Sci. USA* **92**, 5510-5514. doi:10.1073/pnas.92.12.5510

Wang, Q., He, Z., Huang, M., Liu, T., Wang, Y., Xu, H., Duan, H., Ma, P., Zhang, L., Zamvil, S. S. et al. (2018). Vascular niche IL-6 induces alternative macrophage activation in glioblastoma through HIF-2alpha. *Nat. Commun.* **9**, 559. doi:10.1038/s41467-018-03050-0

Weidemann, A., Krohne, T. U., Aguilar, E., Kurihara, T., Takeda, N., Dorrell, M. I., Simon, M. C., Haase, V. H., Friedlander, M. and Johnson, R. S. (2010). Astrocyte hypoxic response is essential for pathological but not developmental angiogenesis of the retina. *Glia* **58**, 1177-1185. doi:10.1002/glia.20997

Wu, D., Potluri, N., Lu, J., Kim, Y. and Rastinejad, F. (2015). Structural integration in hypoxia-inducible factors. *Nature* **524**, 303-308. doi:10.1038/nature14883

Xue, W., Cai, L., Tan, Y., Thistlethwaite, P., Kang, Y. J., Li, X. and Feng, W. (2010). Cardiac-specific overexpression of HIF-1{alpha} prevents deterioration of glycolytic pathway and cardiac remodeling in streptozotocin-induced diabetic mice. *Am. J. Pathol.* **177**, 97-105. doi:10.2353/ajpath.2010.091091

Yamashita, T., Ohneda, O., Nagano, M., Iemitsu, M., Makino, Y., Tanaka, H., Miyauchi, T., Goto, K., Ohneda, K., Fujii-Kuriyama, Y. et al. (2008). Abnormal heart development and lung remodeling in mice lacking the hypoxia-inducible factor-related basic helix-loop-helix PAS protein NEPAS. *Mol. Cell. Biol.* **28**, 1285-1297. doi:10.1128/MCB.01332-07

Yang, X., Yang, S., Wang, C. and Kuang, S. (2017). The hypoxia-inducible factors HIF1alpha and HIF2alpha are dispensable for embryonic muscle development but essential for postnatal muscle regeneration. *J. Biol. Chem.* **292**, 5981-5991. doi:10.1074/jbc.M116.756312

Yoshizawa, T., Hinoi, E., Jung, D. Y., Kajimura, D., Ferron, M., Seo, J., Graff, J. M., Kim, J. K. and Karsenty, G. (2009). The transcription factor ATF4 regulates glucose metabolism in mice through its expression in osteoblasts. *J. Clin. Invest.* **119**, 2807-2817. doi:10.1172/JCI39366

Zhang, N., Fu, Z., Linke, S., Chicher, J., Gorman, J. J., Visk, D., Haddad, G. G., Poellinger, L., Peet, D. J., Powell, F. et al. (2010). The asparaginyl hydroxylase factor inhibiting HIF-1alpha is an essential regulator of metabolism. *Cell Metab.* **11**, 364-378. doi:10.1016/j.cmet.2010.03.001

Zheng, X., Zhai, B., Koivunen, P., Shin, S. J., Lu, G., Liu, J., Geisen, C., Chakraborty, A. A., Moslehi, J. J., Smalley, D. M. et al. (2014). Prolyl hydroxylation by EglN2 destabilizes FOXO3a by blocking its interaction with the USP9x deubiquitinase. *Genes Dev.* **28**, 1429-1444. doi:10.1101/gad.242131.114

Zhou, C. H., Zhang, X. P., Liu, F. and Wang, W. (2015). Modeling the interplay between the HIF-1 and p53 pathways in hypoxia. *Sci. Rep.* **5**, 13834. doi:10.1038/srep13834

Zhu, C., Yu, J., Pan, Q., Yang, J., Hao, G., Wang, Y., Li, L. and Cao, H. (2016). Hypoxia-inducible factor-2 alpha promotes the proliferation of human placenta-derived mesenchymal stem cells through the MAPK/ERK signaling pathway. *Sci. Rep.* **6**, 35489. doi:10.1038/srep35489

Zimmermann, A. S., Morrison, S. D., Hu, M. S., Li, S., Nauta, A., Sorkin, M., Meyer, N. P., Walmsley, G. G., Maan, Z. N., Chan, D. A. et al. (2014). Epidermal or dermal specific knockout of PHD-2 enhances wound healing and minimizes ischemic injury. *PLoS ONE* **9**, e93373. doi:10.1371/journal.pone.0093373