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Cellular Signal Transduction of the Hypoxia Response

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Cells induce the hypoxia responses to adapt to the environment when organisms are exposed to a low oxygen environment. The hypoxia response leads to the activation of multiple cellular signalling pathways involved in regulation of respiration, metabolism, cell survival and so forth. Hypoxia-Inducible-Factor (HIF) pathway plays a central role during the hypoxia response as its expression and activity are regulated in an oxygen-dependent manner and it also regulates the expression of multiple hypoxia responsive genes. The expression of HIF is regulated by proline hydroxylation, which is mediated by HIF prolyl-hydroxylase named PHD. The hydroxylated HIF-α subunit is degraded via the ubiquitin–proteasome pathway. The PHD activity needs to be strictly regulated to ensure the stabilization of HIF under hypoxic conditions, because PHD leads to HIF degradation. This review describes the regulatory mechanism of HIF stability and activity under normoxia and hypoxic conditions. Furthermore, the role of the HIF-independent pathways during the hypoxia response, which is as important as the HIF pathway, will also be described.

Key words: HIF, PHD, prolyl-hydroxylation, Siah2, ubiquitination.

Abbreviations: HIF, Hypoxia-Inducible Factor; PHD, prolyl-hydroxlase domain containing; TCA, tricarboxylic acid; ATP, adenosine triphosphate; EMSA, electrophoretic mobility shift assay; PAS, Per-Amt-Sim; HRE, hypoxia response element; PGK, phosphoglycerate kinase; ARNT, aryl hydrocarbon receptor nuclear translocator; EPO, erythropoietin; VEGF, vascular-endothelial growth factor; ODD, oxygen-dependent degradation; SCF, Skpl-Cull-F box protein; MEF, mouse embryonic fibroblast; FKBP, FK506-binding protein; SPRY, Sprouty; mTOR, mammalian target of rapamycin; AMPK, AMP-activated protein kinase; PML, promyelocytic leukemia; NF-κB, nuclear factor-kappa B; HDAC, histone deacetylase.

Organisms are constantly exposed to oxygen that allows them to produce energy efficiently. The oxygen concentration in the atmosphere is 20.9%; however, at high altitudes air pressure becomes lower along with a decrease in the partial oxygen pressure, a hypoxia condition. Hypoxia is a detrimental condition for organisms due to limited supply of oxygen that is necessary for the efficient energy production.

In order to survive such conditions, organisms need to alter their physiological functions to adapt to the environment. The oxygen concentration in the human body is not even and there is a gradient of oxygen concentration. The cells located nearby the blood vessels obtain oxygen more efficiently than the cells apart from these vessels. Therefore, hypoxic regions occur throughout the body. Most of tissues or organs in the human body are hypoxic in comparison to the oxygen concentration in the air and it is normally within the range of 2–9% (*1*). This tissue hypoxic condition is referred to 'physiological normoxia', where the tissues do not necessarily activate the hypoxia response.

When cells encounter hypoxic conditions, they change their physiology into a 'hypoxic mode'. One of the major changes in the cells is their metabolic state. Cells utilize the tricarboxylic acid (TCA) cycle and oxidative-phosphorylation in the mitochondria as well as glycolysis for energy production under aerobic conditions. Cells under hypoxia, however, rely on the glycolysis pathway for energy production since the oxidative phosphorylation process requires oxygen (*2*). Whereas glycolysis produces only 2 adenosine triphospate (ATP) molecules from one glucose molecule, oxidative-phosphorylation generates 36–38 ATP molecules per a glucose molecule. Under a hypoxia condition, cells upregulate glucose uptake, induce the expression of glycolytic enzymes, and inhibit the enzymes leading to the TCA cycle, such as pyruvate dehydrogenase to shift to an anaerobic mode of energy production (*3*,*4*). At the same time, cells decrease the metabolic rate, such as translation rate, to limit cellular activity in an oxygen-limited environment. Efficient uptake of oxygen is also promoted by an increase in the respiration rate, which is mediated by an 'oxygen-sensing organ', the carotid body in mammals (*5*). Furthermore, an increase in the number of erythrocytes, which carry oxygen in blood, occurs to further provide oxygen to the peripheral tissues (*6*). All these responses are connected to the efficient uptake of oxygen and reducing the expenditure of energy in order to overcome the hypoxic conditions.

HYPOXIA-INDUCIBLE FACTOR PATHWAY

Hypoxia-Inducible Factor (HIF) was first identified as an activity in the nuclear protein which is upregulated under hypoxic conditions (*7*). The 'activity' binds to the

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3′-flanking region of the EPO gene in a hypoxia treated sample, which was detected by the electrophoretic mobility shift assay (EMSA) experiment. Later, the protein responsible for the activity was identified in a large-scale purification of HeLa nuclear extract as 120-kDa (α subunit) and 94-kDa (β subunit) proteins (*8*).

HIF is a heterodimeric transcription factor consisting of an α subunit and a β subunit. Expression of the α subunit is dependent on the oxygen level, and it is expressed under hypoxic conditions. Meanwhile, the β subunit is not regulated by oxygen and it is constitutively expressed under both normoxia and hypoxia conditions. HIF-α protein contains a basic helix–loop–helix-Per-Arnt-Sim (PAS) region and it dimerizes with the β subunit of HIF (*9*). Upon formation of the HIF heterodimer (α and β subunit interaction), the protein becomes an active transcription factor, and transactivates numbers of target genes. HIF binds to the hypoxia response element (HRE; typical HRE sequence is ACGTG) on the promoter region of the target genes. HIF family protein has three α subunits (1α, 2α and 3α) and two β subunits (ARNT, ARNT2) (*10*). HIF-1α and -2α are the two isoforms extensively studied. These proteins are both potent activators of hypoxia-inducible genes. Although HIF-1α and -2α share many common target genes, the tissue distribution of the proteins is different. While HIF-1α expression is ubiquitously expressed throughout the tissues, HIF-2α expression is limited to certain cell types such as in glomerular endothelial cells in kidney, hepatocytes in liver, and endothelial cells in the hippocampal region (*11*). Furthermore, there are target genes that are specifically induced by either HIF-1α or -2α. For example, HIF-1α induces PGK-1, which is involved in energy metabolism, while HIF-2α specifically induces Oct4 that is involved in stem cell maintenance (*12*). Interestingly, while HIF-1α inhibits the c-Myc-dependent transactivation (*13*), HIF-2α cooperates with c-Myc to promote cell proliferation (*14*). Alternatively, HIF-3α functions as a negative regulator of transcription. By alternative splicing, HIF-3α forms inhibitory PAS domain protein under hypoxic conditions, which binds to HIF-1α and prevents the activity of functional HIF complex (*15*). However, it is not clear whether HIF-3α functions as a transcription factor in a manner similar to that of the other two. ARNT, the β subunit of HIF, is an essential component of HIF transcription factor, and it is commonly used by HIF-1α and -2α. There are two ARNT isoforms, and the expression of ARNT2 is high in the kidney and brain. The role of ARNT is not limited to the hypoxia response. It also forms a heterodimer with AhR, a dioxin receptor, and functions as a transcription factor involved in toxic and biological effects caused by dioxin (*16*).

HIF transactivates a wide variety of genes involved in the hypoxia response such as erythropoietin (EPO) which induces red blood cell production (*17*), vascular-endothelial growth factor (VEGF) which promotes angiogenesis (*18*), and *GLUT*1 which increases the efficiency of the glucose uptake (*19*). These genes could be grouped into several groups based on their functions. The groups include cell death/survival, glucose metabolism, angiogenesis, erythropoiesis and pH regulation (*20*). By inducing sets of genes, HIF maintains the homeostasis during the hypoxia. Importantly, many of these target genes are connected to the growth and/or evasion of tumours as well. Therefore, HIF is also considered to be a good target for anti-cancer drugs.

Inactivation of HIF-1α or HIF-2α in mice both causes embryonic lethality. HIF-1α knockout (KO) mice becomes lethal at E8.5, whereas HIF-2α at E9.5 (*21–24*). Embryos die of different causes: HIF-1α$^{-/-}$ embryos die due to cardiac and vascular defects, while HIF-2α$^{-/-}$ embryos die from different causes such as bradycardia, vascular defects and incomplete lung maturation, depending on the genetic background. The difference in the KO phenotypes highlights the non-overlapping roles of the HIF-1α and HIF-2α regulated physiological responses.

PROLYL-HYDROXYLASES REGULATE HIF-α EXPRESSION

HIF-α proteins are highly unstable in cells under normoxia conditions, whereas they are stabilized under hypoxic conditions (*25*). The increased expression of HIF-1α under hypoxic conditions is mainly achieved by the regulation at the protein level. This is further demonstrated by the identification of oxygen-dependent degradation domain (ODD), which is required for HIF downregulation under normoxia conditions (*26*). HIF is actively degraded by the ubiquitin–proteasome system in normoxia and ODD serves as a degradation domain. Proteasome inhibitor increases HIF-1α under normoxia conditions to the level comparable to hypoxic treated cells, indicating the active degradation of HIF-1α by proteasome in normoxia (*26*). Therefore, HIF-1α is synthesized on one hand, but degraded on the other hand under normoxia conditions. The ubiquitin ligase regulating the HIF-α expression under normoxia is the pVHL complex (Fig. 1). The pVHL complex consists of von-Hippel Lindau protein, elongin B, elongin C, cullin2 and Rbx1 (*27*,*28*). The complex forms the SCF type ubiquitin ligase, and pVHL is the key protein which recognizes HIF-1α as a substrate, in a manner similar to that of the F box protein of the SCF complex (*29*). VHL is a gene mutated in the von-Hippel Lindau disease patient (*30*). Von-Hippel Lindau disease causes clear cell carcinoma in the kidney and pheochromocytoma or hemangioblastoma in the brain. The patients have higher HIF expression levels, due to the lack of the degradation machinery. Constant expression and activation of HIF is characteristic of cells prone to tumour formation. Since pVHL regulates the HIF-α expression constantly under normoxia, it therefore becomes important to elucidate how such degradation becomes inhibited under hypoxic conditions. A post-translational modification of HIF-α protein is considered to be the answer to this question.

HIF-α is modified post-translationally by phosphorylation, SUMOylation, ubiquitination and hydroxylation (*31–35*). Among these modifications, proline hydroxylation is the key modification to regulate the ubiquitination-dependent degradation of HIF-α (Fig. 1). In mammals, there are three hydroxylases, named PHD1, PHD2 and PHD3 (PHD stands for the prolyl hydroxylase domain containing) (*36*). These enzymes are able to hyrdoxylate the proline residues of HIF-α protein.

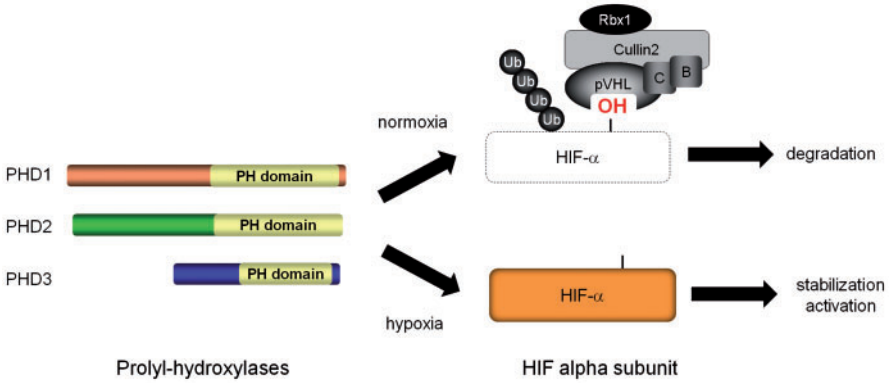

Fig. 1. **Hydroxylation and ubiquitination-dependent degradation of HIF-α subunit.** PHDs hydroxylate the proline residues of the HIF-α subunits. When the α subunit is hydroxylated, it is efficiently captured by pVHL. pVHL constitutes an ubiquitin ligase complex together with cullin2, elongin B, elongin C, Rbx1 and ubiquitinates HIF-α subunit. The ubiquitinated α subunit is degraded by proteasome. Since PHDs' activity is attenuated under hypoxic condition, HIF-α is mostly not subjected to hydroxylation. HIF-α without hydroxylation is stable and becomes active as a transcription factor under hypoxia condition. PH domain: prolyl-hydroxylase domain; B: elongin B; C: elongin C; Ub: ubiquitin.

Hydroxylated HIF-α is efficiently captured by the pVHL protein, and targeted for ubiquitination (Fig. 1). Meanwhile, HIF-α cannot interact with pVHL without proline hydroxylation, therefore HIF-α will not undergo ubiquitination and thus become stabilized. In *Caenorhabditis elegans*, mutation of *Egln*9 gene causes an increased HIF-α expression in normoxia (*36*). *Egln*9 encodes a protein which is a worm counterpart of PHDs that belongs to the 4-prolyl hydroxylase family.

Several co-factors are required for PHDs to hydroxylate HIF. The co-factors are 2-oxoglutarate, Fe^{2+}, ascorbic acid, and oxygen. Since PHDs require oxygen, it is active under normoxic condition. Conversely, under hypoxic conditions where the supply of oxygen is limited, the activity of PHDs becomes lower (the enzymatic activity decreases to 50% at 1% O_2 for PHD3 and 2% O_2 for PHD2) (*37*). Therefore, this constitutes one mechanism to inhibit the HIF-α hydroxylation under hypoxic conditions which thus leads to its stabilization.

The KOs of three PHDs have been generated in mice. The PHD2 KO mouse displays an embryonic lethal phenotype due to an aberrant formation of the placenta (*38*). Since PHDs are negative regulators of HIF-α expression, the increased expression of HIF-1α is found in the placenta and heart of PHD2 KO animals. A conditional KO of PHD2 shows polycythemia, which is consistent with the human disease caused by a mutation of *PHD2* gene also causing familial erythrocytosis (*39*,*40*). PHD1 KO mice induce hypoxia tolerance in skeletal muscles, which protects the cells from lethal ischemic injury. The hypoxia tolerance is primarily dependent on the HIF-2α pathway, which is upregulated in the $PHD1^{-/-}$ muscle (*41*). PHD3 has a pro-apoptotic function in neuronal cells which is independent of the HIF pathway (*42*). KO of PHD3 is associated with a decreased rate of neuronal death during development and increases the size of superior cervical ganglion. This causes a discordance of the sympathoadrenal system (*43*). The phenotype is linked to the HIF-independent role of PHD3.

UBIQUITIN LIGASE SIAH UNDER HYPOXIC CONDITIONS

Siah is a human (or mammalian) homologue of the *Drosophila Sina* gene. Sina was originally identified as a mutant with abnormal eye formation (*44*,*45*). Sina targets the transcriptional repressor Tramtrack for degradation, which is involved in eye formation in flies. Siah contains an N-terminal RING finger domain as well as a zinc-finger domain, which is a catalytic center for ubiquitin ligase activity. There are two isoforms of Siah in mammals, Siah1 and 2. Although these two proteins are encoded by different genes, they are highly homologous other than at the N-terminal which is highly divergent. Both Siah1 and Siah2 are potent E3 ligases, and by degrading multiple substrates, Siahs regulate various cellular responses. Those substrates include, N-CoR, c-myb involved in transcriptional regulation, 2-oxoglutarate dehydrogenase in metabolism, TRAF2, β-catenin, and DCC in signal transduction (*46*). Siah1 KO mice display a smaller body size and male infertility (*47*). Meanwhile, Siah2 KO mice display an expansion of myeloid progenitor cells in the bone marrow, and Siah2 mutant bone marrow produces more osteoclasts *in vitro* than wild-type bone marrow (*48*).

PHD3 is one of the enzymes regulating the expression of HIF-1α through hydroxylation. Co-immunoprecipitation experiments show that Siah2 and PHD3 interact *in vivo* and PHD3 is actively degraded (Fig. 2) (*49*). Siah2 KO mouse embryonic fibroblasts (MEFs) show upregulation of PHD3. Accordingly expression of HIF-1α, a target of PHD3, is downregulated in Siah2 KO MEF. Furthermore, a Siah1/Siah2 double knockout (DKO) MEFs displays almost no expression of HIF-1α and VEGF, which is a target gene of HIF-1α. Importantly, the silencing of PHD3 by RNAi in DKO MEFs rescues the expression of HIF-1α in hypoxia, indicating that regulation of PHD3 by Siah2 is the key mechanism for HIF-1α expression in these cell types. Therefore, Siah plays an important

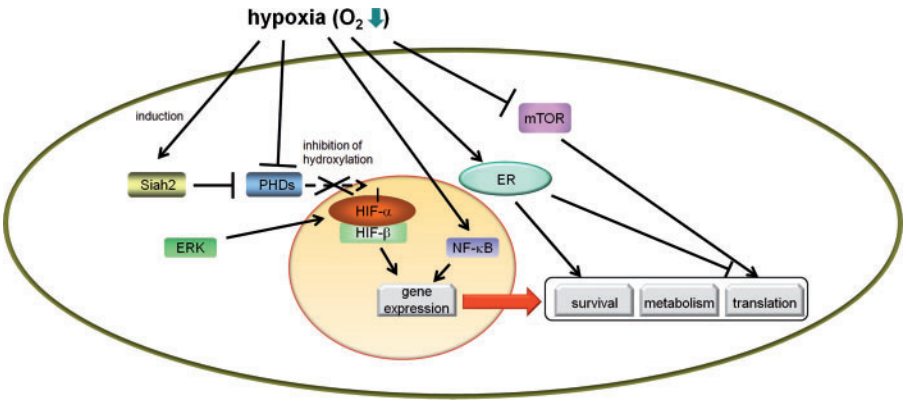

Fig. 2. **Hypoxia-activated signalling pathways.** When cells are exposed to hypoxic condition, multiple cellular signalling pathways are altered. *Siah*2 expression is induced and actively targets PHD3. PHDs require oxygen for the activity, and the ability of PHDs to hydroxylate HIF-α is decreased. Inhibition of PHDs is a key mechanism to stabilize HIF-α protein under hypoxia. Stabilized HIF-α forms an active transcription factor with HIF-β and upregulates numbers of hypoxia-inducible genes expression. ERK phosphorylates HIF-α and positively regulates its transcriptional activity. NF-κB pathway, which is activated under hypoxia condition, also plays an important role in the hypoxic gene expression. Although the mechanism is not clear yet, hypoxia conditions cause ER stress and activate the unfolded protein response pathway. It is mainly involved in the regulation of cell viability and translational inhibition. mTOR activity is inhibited under hypoxia condition. Hypoxia causes inhibition of mRNA translation, and suppression of mTOR activity is the key mechanism responsible for it. ER: endoplasmic reticulum; mTOR: mammalian target of rapamycin; ERK: extracellular signal-regulated kinase.

role in the stabilization of HIF-1α under hypoxic conditions (Fig. 2). Recently, FKBP38 was identified as a molecule which promotes the degradation of PHD2 (*50*).

Although Siah1 and Siah2 share a number of functions, their expressional regulation is different. The *Siah1* gene is upregulated by genotoxic stress, such as UV irradiation (*51*). On the other hand, *Siah2* mRNA is found to be upregulated under hypoxic conditions as early as 2 h and reaches a maximum level at around 5 h. The activity of Siah2 to target PHD3 is enhanced under hypoxic conditions. Therefore, mRNA induction is one of the mechanisms that Siah2 gains activity under hypoxic conditions. The mechanism underlying increased *Siah2* mRNA under hypoxia is unknown. In addition, p53 is upregulated under hypoxic conditions (*52*); therefore, it is possible that *Siah1* is induced under hypoxic conditions as well.

Mice alter their respiration and metabolic rate, produce cytokines such as EPO or VEGF to adapt to the hypoxic conditions. When Siah2 KO mice are maintained under mild hypoxic conditions (7%), the ability to properly adapt to hypoxic conditions is impaired (*49*). First, the increase in the haemoglobin concentration, which represents the number and/or capability of red blood cells in mice, is significantly lower in comparison to that observed in wild type mice. Second, the ventilatory response is affected. While wild-type mice respond to hypoxic conditions by increasing the respiration rate, and decreasing the metabolic rate, Siah2 KO mice further decrease the metabolic rate but cannot increase the respiration rate. The respiration defect observed in the Siah2 KO mice resembles the phenotype of HIF-1α heterozygous mice (*53*), which suggests that the KO mice phenotype of Siah2 is connected to decreased expression of HIF-1α.

TUMOUR FORMATION AND HYPOXIA

A hypoxic environment is often found in growing tumours. Growing cancer cells form a tumour with a hypoxic region in the inner region of the tumour (intratumoural hypoxia) (*54*). The intratumoural hypoxia could even cause death of many cancer cells, but the cancer cells under the sustained hypoxic conditions are often found to be resistant to radiation or chemotherapeutic agents. Under such conditions, HIF-α becomes stabilized. The activity of HIF, which allows normal cells to adapt to hypoxic conditions, is utilized by tumour cells. An overexpression of HIF-α protein is found in numerous tumours, such as the stomach, pancreas, lung, liver and so forth (*55*). HIF-α expression supports cancer cells to grow, survive, invade, target and metastasize. Therefore, hypoxia condition contributes to multiple activities in cancer.

Siah2 has been implicated in cancer. The inhibition of Siah2 can suppress the growth of pancreatic cancer, lung cancer and melanoma which are mediated by Ras pathway (*56–58*). Siah2 KO mice lack the ability to properly respond to hypoxia. How would the inhibition of Siah2 activity affect tumour formation in mice? Two ways to inhibit the Siah2 activity have been examined; (i) introducing a catalytically inactive mutant which functions as dominant negative (RING mutant form), and (ii) introducing a partial sequence of Siah2 binding protein (PHYL) which would compete with the substrates. Both methods were employed in a mouse melanoma model (*58*), showing that both suppressed tumuorigenesis, but in a different manner. The RING mutant mainly suppressed the growth of the tumour, and therefore the rate of metastasis was decreased as well. Meanwhile, PHYL suppressed metastasis, but not the size of primary tumour. One of the main pathways

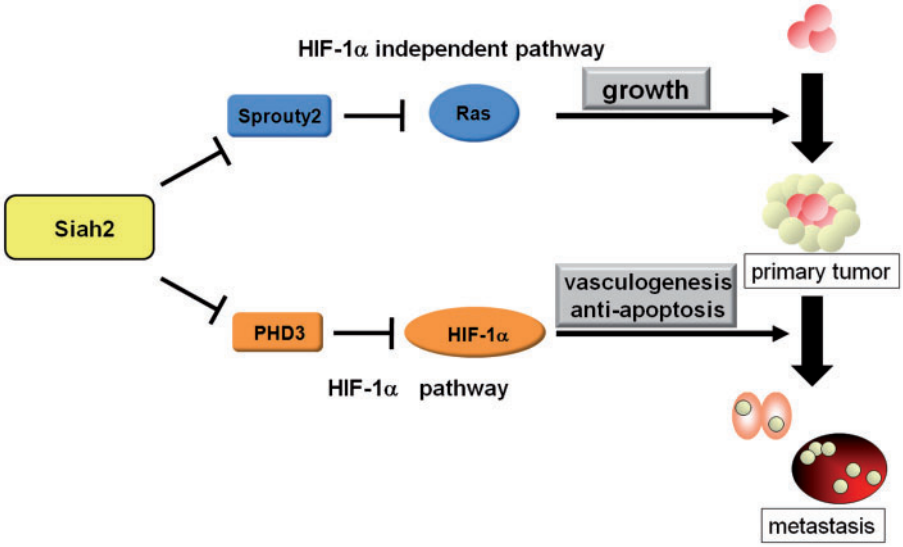

Fig. 3. **Regulation of HIF 1α-dependent and -independent pathway by Siah2.** Siah2 has a major role in tumorigenesis by regulating two of its substrates in mouse melanoma. First, Siah2 activates the Ras pathway by down-regulation of its negative regulator, Sprouty2. Ras pathway is involved in the growth of melanoma. Second, Siah2 stabilizes and activates HIF-1α by down-regulating PHD3 expression. HIF-1α is required for the tumour metastasis by regulating the vasculogenesis and cell viability in the melanoma. Inhibition of Siah2 activity inactivates both of these pathways and suppresses the tumour formation and metastasis to liver and lung.

affected by PHYL is the HIF-1α pathway via Siah2 and PHD3 (Fig. 3). Apart from PHYL, Siah2 RING mutant mainly inhibited the Sprouty (SPRY) 2 pathway, which is involved in the inhibition of Ras-ERK activation (Fig. 3). As Siah2 targets SPRY2 for degradation, inhibition of Siah2 activity increased the SPRY2 expression and inhibited the Ras-ERK kinase pathway. A similar effect of PHYL was also seen in a mouse mammary tumour model. The introduction of a PHYL fragment in the mammary tumour reduced the growth and angiogenesis of tumour which is caused by the decreased level of HIF-1α expression (*59*).

COMPLEX FORMATION AND REGULATION OF PHD3 ACTIVITY

PHD3 is unique because it has a shorter amino acid sequence in comparison to PHD1 or PHD2. PHD3 interacts with itself, and certain portions of PHD3 exist as a dimer or oligomer under normoxia. Interestingly, when this structure is exposed to hypoxic conditions, it forms a large complex (*60*). This complex is oxygen sensitive and when it is exposed to normoxia again (re-oxygenation), it will become a smaller structure. This fact suggests that some oxygen-sensing system may therefore exist in this process.

PHD3 in the large complex has a reduced ability to hydroxylate HIF-1α *in vitro*. Therefore, the formation of a complex is another mechanism to inhibit the prolyl-hydroxylation of HIF-α by PHD3 under hypoxia. The precise mechanism of how the complex formation affects the PHD3 activity remains unclear; however, since the effect was seen in an *in vitro* experiment, it is possible that the complex makes PHD3 inaccessible to the HIF-α protein or some of the co-factors, which are required for PHD activity. Various processes could trigger the change in the protein–protein interaction that is induced or altered by hypoxia, leading to the formation of a large complex. These could include post-translational modification, the induction of some adaptor proteins, and the structural change of the protein.

Based on our study to identify the complex-containing protein, we identified molecules involved in energy metabolism, translation and cell structure organization (K.Nakayama, unpublished data). Some of the proteins increased the interaction with PHD3 under hypoxia, which would serve as a force to form a large complex. Another PHD3 complex has been identified by different approach. This complex is mainly found under normoxia conditions and contains proteins such as the 26S proteasome component, chaperones and ubiquitin (*61*). Therefore, these two complexes are probably two different structures. However, it is interesting to consider that PHD3 may also have a function apart from HIF-α hydroxylation, to alter the activity of proteins by serving as a scaffold and forming large complexes, both in normoxia and hypoxia.

HIF-INDEPENDENT PATHWAY

The role and regulation of the HIF pathway has been described. However, the HIF-independent pathway is equally important during the hypoxia response. Some of the major signalling pathways which show altered activity under hypoxic conditions are outlined below (Fig. 2).

Mammalian target of rapamycin pathway—Protein translation is generally inhibited to save the energy under severe hypoxic conditions. The mammalian target of rapamycin (mTOR) complex I, which phosphorylates S6 kinase and 4E-BP, is involved in the hypoxic

inhibition of protein translation by inhibiting ribosomal biogenesis and cap-dependent translation, respectively. The mTOR activity is inhibited under hypoxic conditions (*62*). The hypoxic inhibition of mTOR activity is mediated by the hypoxia-inducible gene, REDD1 through TSC1/TSC2, an inhibitor of mTOR (*63*). However, it does not require AMPK activity, which plays a role in mTOR inhibition upon energy depletion. In addition, PML also regulates the activity of mTOR independent of this pathway. PML interacts with mTOR and sequesters it into nuclear bodies (*64*). The regulation of mTOR activity under hypoxic conditions is critical and, the constitutive activation of S6 kinase, which is a downstream effector of mTOR pathway, promotes cell death under hypoxic conditions (*65*).

Endoplasmic reticulum stress—When cells are exposed to endoplasmic reticulum (ER) stress and unfolded proteins accumulate in the ER, cells activate unfolded protein response (UPR) to maintain the protein quality or induce to cell death. UPR is activated under hypoxic conditions which is mediated by three key players; namely, PERK, IRE1 and ATF6. PERK is immediately activated upon hypoxic exposure, and then phosphorylates eIF2 to inhibit the translation (*66*). Activated IRE1 promotes the splicing of X-box binding protein (XBP1) pre-mRNA. The inhibition of XBP1 by siRNA resulted in an increased apoptosis under hypoxic conditions (*67*). As a part of UPR, many chaperones such as GRP78 (Bip), ORP150 and GRP94 are also induced under hypoxic conditions.

Nuclear factor-kappa B pathway—The nuclear factor-kappa B (NF-κB) pathway, one of the key transcription factors in the immune response, also plays an important role during hypoxia response. The NF-κB pathway is activated under hypoxic conditions, which induces HIF-1α mRNA. Conversely, the induction of the *NF-κB* gene by HIF-1α is also observed in neutrophils, and is critical for their survival. The NF-κB pathway activated in the tumour plays an important role in tumour angiogenesis (*68*). PHD2 is downregulated in different human cancer cells, and this is linked to the increase in tumour growth. Downregulation of PHD2 elevates the NF-κB activity and upregulates the expression of *IL*-8 and *angiogenin* gene expression, which causes angiogenesis in tumours. PHD1 interacts with and regulates the activity of IKK-β (*69*). Although the hydroxylation of IKK-β by PHD1 is not proven, it is possible that hypoxic activation of NF-κB is regulated by the PHD1-IKK-β axis. Analysis of IKK-$\beta^{-/-}$ animals indicates that NF-κB activity is essential for HIF-1α and its target gene expression (*70*). While the hypoxia-activated NF-κB pathway positively regulates the gene expression, it has also been demonstrated that NF-κB negatively regulates the *MCP-1* gene expression by interacting with histone deacetylase, HDAC2 (*71*).

CLOSING REMARK

The hypoxia response alters numerous physiological activities. Although the HIF pathway has been the central focus for hypoxia studies, the hypoxia response involves multiple pathways besides the HIF pathway. An important question remains, namely whether any cross talk exists between these pathways which may thus play a role in the adaptation to different modes of hypoxia, such as the acute phase or chronic phase.

Siah2 regulates both the HIF pathway and the HIF-independent pathway through different targets, i.e. PHD3 and SPRY2. The inhibition of Siah2 inhibits both HIF-dependent and -independent pathways, which could suppress the two important tumorigenesis processes; namely, growth and metastasis (Fig. 3). Using drugs targeting the HIF-independent pathway in combination with the HIF-inhibitors would regulate the hypoxia response more effective, since they are expected to inhibit the hypoxia response broadly.

What is an oxygen sensor that initiate the hypoxia response? PHD enzymes are proposed to be an oxygen sensor because they require oxygen molecules to regulate the HIF expression. However, it remains unknown whether or not PHDs regulate other pathways (hydroxylates other molecules besides HIF-α), and if those pathways contributes to the regulatory mechanism of the hypoxia response. Probably, PHD system is not the sole oxygen sensor, and there would be some other molecules (systems) that are responsible for modulating the hypoxic signalling cascades. Finally, if various oxygen sensors do exist in cells, then the question remains as to whether or not a mechanism would exist to integrate all the sensors (activity, sensitivity, expression, intracellular localization, etc.)? Answers to this question might provide valuable insight into the mechanism of how such cells sense oxygen, which could thus potentially serve as a drug target to modulate the broader hypoxia response.

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CONFLICT OF INTEREST

None declared.

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