

Praca poglądowa/Review paper

Regulation of HIF-1 α Stability by Prolyl Hydroxylases in HNSCC

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Abstract

Hypoxia is a common feature of the tumor microenvironment and plays an important role in the progression and treatment resistance of head and neck squamous cell carcinoma (HNSCC). Reduced oxygen availability triggers molecular adaptation to hypoxic stress, with hypoxia-inducible factor 1 α (HIF-1 α) acting as a central regulator of this response. Under normoxic conditions, HIF-1 α is hydroxylated by prolyl hydroxylase domain enzymes (PHD1, PHD2, and PHD3), recognized by the von Hippel-Lindau protein, and subsequently degraded by the proteasome. In hypoxia, reduced PHD activity leads to HIF-1 α stabilization, and activation of genes involved in angiogenesis, glucose metabolism, cell survival, and tumor progression. Increasing evidence indicates that dysregulation of PHD expression may contribute to altered HIF-1 α signaling in cancer, including HNSCC. In addition, epigenetic mechanisms such as DNA methylation may influence PHD expression and consequently modulate the cellular response to hypoxia. This review summarizes the role of hypoxia and the PHD–HIF-1 α axis in HNSCC, with particular emphasis on the regulation of HIF-1 α stability and the potential contribution of epigenetic mechanisms to this pathway.

Keywords: HNSCC, HIF-1 α , hypoxia, PHDs, prolyl hydroxylases, epigenetics

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Hypoxia, HIF-1 α , and prolyl hydroxylase regulation in HNSCC

Head and neck cancers (HNC) are the sixth most common cancers worldwide and are associated with a high mortality rate [1]. Approximately 90% of HNC cases are head and neck squamous cell carcinomas (HNSCC), with a 5-year survival rate of 40–50% [2]. HNSCC represents a heterogeneous group of malignancies arising from different anatomical sites of the upper respiratory and gastrointestinal tracts, including the oral cavity, larynx, and pharynx. One of the major factors contributing to the high mortality of HNSCC is the frequent diagnosis at an advanced stage, partly due to the lack of reliable biomarkers for early detection [3]. The major risk factors for HNSCC include tobacco smoking and chewing, as well as alcohol consumption [4]. Infection with human papillomavirus (HPV), particularly HPV-16 and HPV-18, represents another important risk factor and is primarily associated with oropharyngeal cancers [5]. Treatment of locally advanced HNSCC generally involves surgical resection combined with radiotherapy and/or chemotherapy, while immune checkpoint inhibitors such as nivolumab and pembrolizumab are also used in selected patients [6].

Despite advances in diagnosis and treatment, the clinical outcome of HNSCC remains poor. An important biological feature contributing to tumor progression and treatment resistance is the presence of hypoxia within the tumor microenvironment (TME). Hypoxia is a characteristic feature of solid tumors, including HNSCC, and results primarily from an imbalance between oxygen supply and consumption. Although rapid tumor growth can stimulate neoangiogenesis, the newly formed blood vessels are frequently structurally and functionally abnormal, resulting in inefficient oxygen delivery [7]. Consequently, hypoxia develops within poorly vascularized regions of the tumor and represents an important selective pressure promoting tumor adaptation.

Hypoxia contributes to an aggressive tumor phenotype by activating adaptive mechanisms that promote neovascularization, cellular migration and invasion, metastatic potential, and resistance to anticancer treatment, including chemotherapy, radiotherapy, and immunotherapy [8]. Tumor cells adapt to low oxygen availability through extensive metabolic and transcriptional reprogramming. One important adaptation is a shift toward glycolytic metabolism, which allows ATP production to be maintained despite limited oxygen availability and contributes to lactate accumulation and acidification of the tumor microenvironment [9]. Together with increased invasiveness, metastatic potential, and genomic instability, these changes promote tumor survival and progression. A major molecular mechanism underlying this adaptive response is mediated by hypoxia-inducible factors (HIFs), which function as central transcriptional regulators of cellular responses to reduced oxygen availability [10].

HIF-1 α as a central mediator of the hypoxic response

HIFs are heterodimeric transcription factors composed of an oxygen-sensitive α subunit and a constitutively expressed β subunit, also known as aryl hydrocarbon receptor nuclear translocator (ARNT) [11]. Among the HIF family members, HIF-1 α is one of the major mediators of the cellular response to hypoxia and plays an important role in tumor adaptation. Under normoxic conditions, HIF-1 α is highly unstable, with a half-life of approximately 5 minutes [12]. Its stability is primarily controlled by oxygen-dependent post-translational hydroxylation, which provides a direct molecular link between oxygen availability and transcriptional regulation.

Under normoxic conditions, HIF-1 α is hydroxylated at specific proline residues, including Pro402 and Pro564, by prolyl hydroxylase domain enzymes (PHDs) [13]. Hydroxylation enables recognition of HIF-1 α by the von Hippel–Lindau tumor suppressor protein (pVHL), increasing its affinity for HIF-1 α by more than 1,000-fold [14]. pVHL forms part of an E3 ubiquitin ligase complex that promotes ubiquitination and subsequent proteasomal degradation of HIF-1 α [15]. In addition, factor inhibiting HIF (FIH) hydroxylates an asparagine residue at position 803 within the C-terminal transactivation domain, thereby inhibiting the recruitment of transcriptional coactivators [16]. Thus, under normoxia, coordinated hydroxylation by PHDs and FIH keeps HIF-1 α transcriptional activity low.

In contrast, hypoxia directly inhibits these oxygen-dependent hydroxylation reactions because molecular oxygen is required as a substrate for PHD and FIH activity [17]. As a result, HIF-1 α escapes hydroxylation and pVHL-mediated degradation, leading to its stabilization and accumulation in the cytoplasm. Stabilized HIF-1 α subsequently translocates into the nucleus, where it dimerizes with the constitutively expressed HIF- β subunit [18]. The resulting transcriptional complex binds hypoxia-responsive elements (HREs) within target genes and recruits

transcriptional cofactors, leading to activation of genes involved in angiogenesis, metabolism, erythropoiesis, and cell survival. Important HIF-1 α target genes include vascular endothelial growth factor (VEGF), erythropoietin (EPO), and glucose transporters such as GLUT1 and GLUT3 [17]. The mechanism of HIF-1 α is demonstrated on Figure 1.

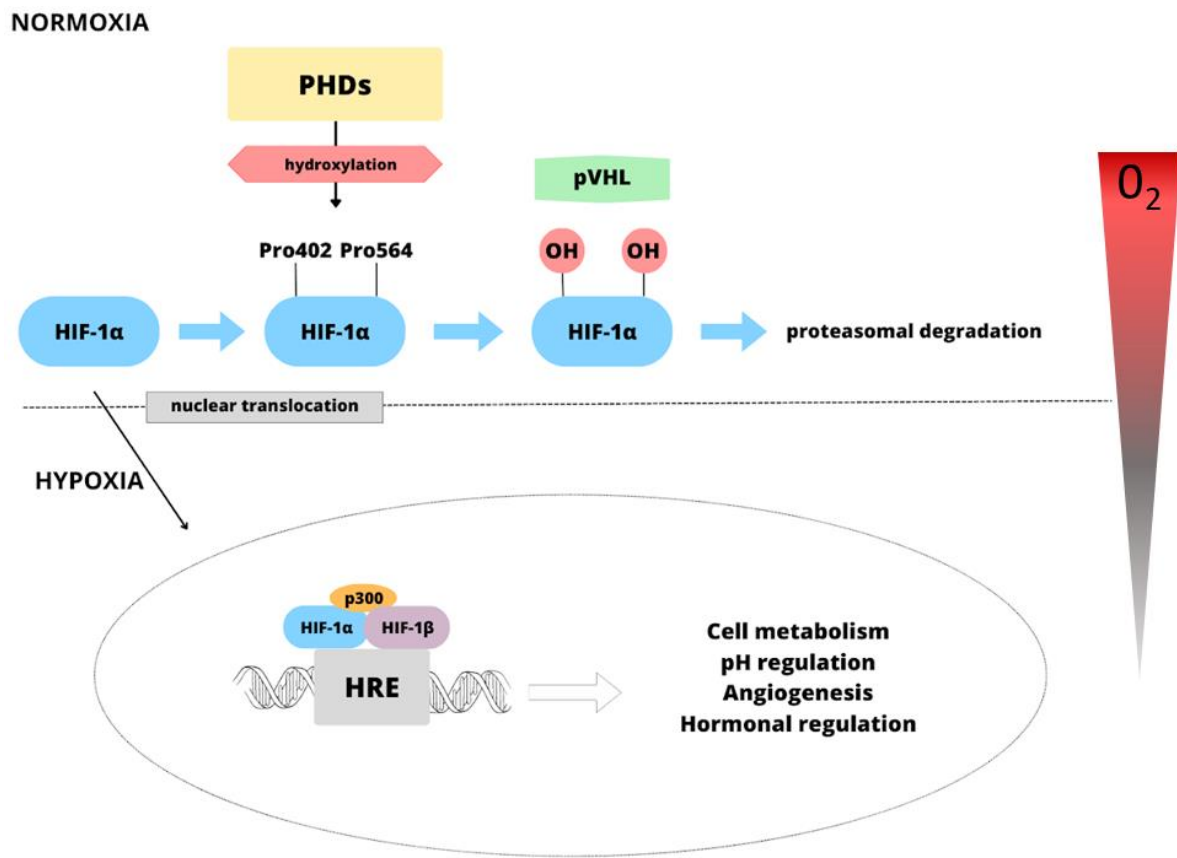


Figure 1. Mechanism of HIF activity depending on oxygen availability. Under hypoxic conditions, HIF-1 α is activated by translocating to the nucleus and binding to HIF-1 β . The active form of HIF-1 α recognizes the HRE (hypoxia response element) sequence and regulates the expression of various genes. Contrarily, in a normoxic environment HIF-1 α undergoes hydroxylation on proline residues (402 and 564 positions) by PHDs and is subsequently polyubiquitinated by the von-Hippel-Lindau protein (pVHL), which causes proteasomal degradation of the complex.

Therefore, the PHD–HIF-1 α axis represents a key oxygen-sensing mechanism that determines whether HIF-1 α is rapidly degraded under normoxia or stabilized and transcriptionally active under hypoxia. Dysregulation of this regulatory system may therefore have important consequences for tumor progression.

Prolyl hydroxylases as regulators of HIF-1 α stability

Hydroxylation of HIF-1 α is catalyzed by three prolyl hydroxylases: PHD1, PHD2, and PHD3, also known as EGLN2, EGLN1, and EGLN3, respectively. PHDs belong to the family of non-heme iron- and 2-oxoglutarate-dependent dioxygenases and require oxygen, Fe²⁺, and α -ketoglutarate for enzymatic activity [19]. Although PHD1, PHD2, and PHD3 share structural features, they differ in their tissue distribution, subcellular localization, and biological functions. PHD2 (EGLN1) is expressed in most tissues and appears to play the predominant role in regulating HIF-1 α stability under normoxic conditions [20].

The importance of PHD2 in HIF regulation has been demonstrated *in vivo*. Mice lacking *phd2* exhibit severe placental abnormalities that result in embryonic lethality [21]. In contrast, deletion of *phd1* or *phd3* is not lethal and can increase tolerance to hypoxia by promoting a metabolic shift from aerobic toward anaerobic glucose

metabolism [22]. Moreover, *phd2* deletion in adult mice results in excessive angiogenesis, vasodilation, and severe erythrocytosis associated with activation of the renal erythropoietin pathway, effects that are not observed following deletion of *phd1* or *phd3* [23]. These observations highlight the distinct and non-redundant functions of individual PHD isoforms in the regulation of the hypoxic response.

Importantly, PHD activity is not determined solely by oxygen availability. Changes in PHD expression and activity may also occur during tumor development and can influence HIF signaling. PHD1, PHD2, and PHD3 have been reported to be differentially expressed at the mRNA and protein levels in various tumor types compared with corresponding non-malignant tissues [24]. In colorectal cancer, reduced expression of PHD1, PHD2, and PHD3 transcripts was observed in tumor tissue compared with non-malignant tissue [25]. Similarly, reduced PHD1 and PHD2 expression in lung cancer was associated with poorer patient prognosis and larger tumor size [26].

In HNSCC, alterations in PHD expression may also contribute to tumour biology and treatment response. Higher PHD2 expression has been associated with poorer tumour differentiation and increased proliferation of HNSCC cells, whereas PHD2 expression was reported to be lower in normal tissues and well-differentiated tumours. Moreover, low PHD2 expression, particularly low nuclear PHD2 expression, has been associated with a more favourable response to radiotherapy [27, 28]. In contrast, PHD3 expression was elevated in tumour tissues compared with anatomically matched non-cancerous tissues from patients with HNSCC. Inhibition of PHD3 expression reduced cell survival under hypoxic conditions, suggesting that PHD3 upregulation may contribute to cell survival during hypoxia, potentially through regulation of cell-cycle progression [29]. Interestingly, HPV-positive HNSCC cells exhibited higher levels of both HIF-1 α and PHD2 protein under normoxic and hypoxic conditions, indicating that HPV status may influence the regulation of the HIF/PHD axis in HNSCC [30].

These observations suggest that PHD enzymes may have context-dependent functions in cancer that extend beyond their canonical role as negative regulators of HIF signaling. However, the mechanisms responsible for altered PHD expression in tumors remain incompletely understood.

Epigenetic regulation of PHD expression under hypoxia

One potential mechanism regulating PHD expression is epigenetic modification. Epigenetic mechanisms, including DNA methylation, histone modifications, and non-coding RNAs, regulate gene expression without altering the underlying DNA sequence [31]. Among these mechanisms, DNA methylation involves the addition of a methyl group to the C5 position of cytosine, generating 5-methylcytosine (5-mC) [32]. Methylation of promoter-associated CpG islands can repress gene transcription by interfering with transcription factor binding or promoting the recruitment of transcriptional repressors [32].

Aberrant DNA methylation is frequently observed in HNSCC and may contribute to tumor progression and treatment resistance. Increased DNA methylation has been reported in radioresistant HNSCC cells compared with radiosensitive cells [33]. Moreover, tumor-specific DNA methylation patterns may have potential as biomarkers for early cancer detection because of their relative biological stability [34].

Importantly, hypoxia itself can influence epigenetic regulation. Reduced oxygen availability has been associated with decreased activity of TET proteins involved in DNA demethylation, potentially resulting in increased methylation of promoter regions and altered gene expression [35]. Epigenetic mechanisms may also directly affect the expression of PHD enzymes. For example, reduced PHD3 expression in prostate, melanoma, breast, and renal cancer cell lines has been associated with hypermethylation of CpG islands within the promoter region of *PHD3* [24]. Similarly, in colorectal cancer, reduced *PHD3* expression in tumor tissue was accompanied by methylation of CpG islands within its regulatory promoter region [25].

Conclusions

Collectively, these observations suggest that the regulation of PHD expression may represent an additional layer of control over HIF-1 α activity. Thus, the hypoxic response in HNSCC is determined not only by oxygen availability but also by the molecular mechanisms controlling the expression and activity of HIF-1 α regulators. Further understanding of the PHD–HIF-1 α axis may therefore provide important insights into how hypoxia contributes to HNSCC progression and treatment resistance.

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