Prolyl 4 Hydroxylase: A Critical Target in the Pathophysiology of Diseases

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Prolyl 4 hydroxylases (P4H) are iron- and 2-oxoglutarate-dependent dioxygenase enzymes and hypoxia-inducible transcription factor (HIF)-P4Hs play a critical role in the regulating oxygen homeostasis in the local tissues as well as in the systemic circulation. Over a period of time, a number of prolyl hydroxylase inhibitors and activators have been developed. By employing the pharmacological tools and transgenic knock out animals, the critical role of these enzymes has been established in the pathophysiology of number of diseases including myocardial infarction, congestive heart failure, stroke, neurodegeneration, inflammatory disease, respiratory diseases, retinopathy and others. The present review discusses the different aspects of these enzymes including their pathophysiological role in disease development.

Key Words: Hypoxia inducible factor, Inflammation, Ischemia, Prolyl hydroxylase

INTRODUCTION

Prolyl hydroxylases belong to the family of iron- and 2-oxoglutarate-dependent dioxygenase enzyme and its several distinct isofoms have been identified. The hypoxia-inducible factor (HIF) prolyl hydroxylase enzymes, termed as prolyl hydroxylase domain (PHD), play an important role in oxygen regulation in the physiological network. Its three isoforms including PHD1, PHD2 and PHD3 have been identified in different tissues and organs [1].

Cells recognize and respond to hypoxia by accumulating the transcription factor hypoxia-inducible factor 1 (HIF-1), composed of an oxygen-sensitive inducible HIF-1α and a constitutive HIF-1β subunits. PHD enzyme are involved in the degradation of HIF-1α sub-unit by regulating its hydroxylation of 402/504 proline residues. Under hypoxic conditions, the lack of oxygen leads to stabilization of HIF-1α to form HIF heterodimer which is subsequently translocated to the nucleus. The binding of the HIF-heterodimer to specific DNA sequences within the nucleus, named hypoxia-responsive elements, triggers the trans-activation of target genes. The nature of target gene and type of expressed proteins may vary depending upon the type of tissues and disease conditions. The present review describes the different aspects of these PHD enzymes including the therapeutic implications of its modulators in different disease states.

TYPES OF PROLYL 4-HYDROXYLASES

Collagen Prolyl 4-Hydroxylases

Collagen prolyl 4-hydroxylases (C-P4Hs) are located within the lumen of the endoplasmic reticulum and catalyze the hydroxylation of prolines in -X-Pro-Gly- sequences in collagens and more than 15 other proteins that have collagen-like domains [2]. These C-P4Hs have a central role in the biosynthesis of collagens as 4-hydroxyproline residues are essential for the formation of the collagen triple helix.

1. Nematode collagen P4Hs

The nematode C. elegans has a large gene family of more than 150 members that encodes cuticle collagens [3]. The PHY-1/PHY-2/PDI2 mixed tetramer is the main P4H form in wild-type C. elegans, along with a small amount of...
PHY-1/ PDI dimers, while PHY-2/PDI dimers have not been detected. The PHY-1, PHY-2, and PDI subunits of nema-

todes C-P4H are complementary to \( \alpha \) (I), \( \alpha \) (II), and \( \beta \) sub-

units of vertebrates C-P4H. \( \alpha \) subunit from \( D. \ melanogaster \) has been cloned and characterized and con-

sists of 516 amino acid residues and shows 34% sequence identity to the vertebrate \( \alpha \) subunits and the C. elegans PHY-1, respectively [5]. C-P4Hs have also been cloned and characterized from the parasite filarial nematodes \( O. volvulus \) and \( B. malayi \) [6,7].

2. Plant and viral P4Hs

Although plants have no collagens, yet 4-hydroxyproline is found in many plant glycoproteins. Unlike the animal P4Hs, partially purified P4Hs from unicellular and multi-
cellular green algae have been shown to be monomERIC in nature [8]. In addition, higher plant P4Hs has also been noted to exist as monomers [9]. Partly purified plant P4Hs

have been shown to effectively hydroxylate poly (L-proline) [10]. However, plant P4Hs have been unable to hydroxylate free prolines suggesting that a poly (L-proline) type II helix conformation is required in the substrate for hydroxylation [10]. None of the animal P4Hs use poly (L-proline) as a substrate, but it has been noted to be an effective com-

petitive inhibitor of the former [11,12]. \( A. thaliana \) genome has been noted to contain six genes encoding \( \alpha \)-subunit like short polypeptides of 280~332 residues that show 21~37% amino acid sequence identity to the cata-
ytic C-terminal regions of the human P4H \( \alpha \) subunits [13].

4-Hydroxyproline has been reported to be absent in viral and bacterial proteins, but viral and bacterial genomes are also known to encode polypeptides with proline-rich repeats and even short collagen-like sequences [14-16]. A viral P4H has been cloned from an algal virus, \( P. bursaria \) Chlorella virus-1 (PBCV-1). PBCV-1 P4H was found to hydroxylate prolines in both positions in the -Pro-Ala-Pro-Lys-

sequences but those preceding the alanines are hydroxylated more efficiently [15].

3. Vertebrate collagen P4Hs

Collagen P4Hs, from all vertebrate sources so far has been studied, are composed of \( \alpha \), \( \beta \) tetramers in which the \( \beta \) subunit is identical to protein disulphide isomerase (PDI) [11,12]. C-P4H has long been assumed to be of one type only, with no isoenzymes, however, now several iso-

forms of catalytic \( \alpha \) subunit have been identified in hu-

mans, mice, \( C. elegans \) and \( D. melanogaster \) [5,17,18]. Both the \( \alpha \) (I) and \( \alpha \) (II) subunits asso-

ciate with \( \beta \) subunit to form \( \alpha \) (I) \( \beta \) or \( \alpha \) (II) \( \beta \) tet-

ramers, called the type I and type II enzymes, respectively [17,19]. Insect cell co-expression data strongly argue against the existence of mixed vertebrate \( \alpha \) (I) \( \alpha \) (II) \( \beta \) \( \beta \) tetramers [17].

The human \( \alpha \) (I) subunit consists of 517 amino acids and a signal peptide of 17 additional residues, whereas the \( \alpha \) (II) subunit consists of 514 amino acids and a signal peptide of 21 residues. The overall amino acid sequence identity between the human \( \alpha \) (I) and \( \alpha \) (II) subunits is 64%, and highest degree of identity (80%), is observed within the cata-
ytic C-terminal regions [17,20]. Type I C-P4H is the main form in the most cell types and tissues, while the type II enzyme has been shown to represent approximately 30% of the total P4H activity in cultured human WI-38 and HT-1080 cells and approximately 5~15% in various chick embryo tissues [17]. However, type II P4H represents at least 70% and 80% of the total P4H activity in cultured mouse chondrocytes and cartilage, respectively [21], and is thus likely to have a major role in the development of carti-
lage, cartilagenous bone and capillaries in vertebrates.

### HYPOXIA INDUCIBLE FACTOR-PROLYL 4-HYDROXYLASE (HIF-P4H) SYSTEM

**Discovery, types and distribution of HIF-1**

Semenza and Wang discovered the HIF-1, a protein with DNA binding activity, by identifying the presence of hypo-

xia response element (HRE; 5'-RCGTG-3') in the erythropoietin gene [22]. The two isoforms or subunits of HIF-1 viz., HIF-1 \( \alpha \) (inducible) and HIF-1 \( \beta \) (constitutive) have been identified that form a heterodimeric complex to regu-
late target gene in response to hypoxia [23]. HIF-1 \( \beta \), also known as the aryl hydrocarbon nuclear translocator (ARNT), was originally identified as a binding partner of the aryl hydrocarbon receptor [24] and was known much earlier as compared to its binding partner HIF-1 \( \alpha \) [25]. The subsequent studies revealed the ubiquitous presence of HIF-1 \( \alpha \) in the human and the mouse tissues and described its general role in multiple physiological responses to hypo-

xia [26]. HIF-1 \( \alpha \) and HIF-1 \( \beta \) proteins belong to the basic helix-loop-helix–Per-ARNT-Sim (bHLH-PAS) protein family [23] and bHLH and PAS motifs are essential for dimeri-

zation of these subunits and subsequent DNA binding [27]. HIF-2 \( \alpha \) (also termed as HIF-like factor and HIF-related factor) was identified and cloned in the lung, endothelium, and carotid body [28-30]. HIF-3 \( \alpha \), mainly expressed in the Purkinje cells of the cerebellum and corneal epithelium, was subsequently discovered. A splice variant of HIF-3 \( \alpha \) does not possess endogenous transactivation activity and mainly functions as inhibitory PAS (IPAS) to prevent the binding of HIF-1 \( \alpha \) to its target DNA binding site [31].

Structurally, HIF-1 \( \alpha \) protein possesses N-terminal (N-

TAD) and C-terminal (C-TAD) as two transactivation do-

mains in its C-terminal half part that are involved in activat-
ing the transcriptional process [32]. The C-TAD particu-

larly interacts with CBP/p300 (acting as co-activators) to activate gene transcription [33]. The hydroxylation of an asparagine residue in the C-TAD inhibits the association of HIF-1 \( \alpha \) with co-activators CBP/p300 and thus inhibits its transcriptional activity [34,35]. HIF-1 \( \alpha \) also possess an oxygen-dependent degradation domain (ODDD) that mediates oxygen-regulated stability [35].

**HIF-P4H enzymes**

HIF-P4Hs, a novel and distinct family of cytoplasmic prolyl 4-hydroxylases, plays a critical role in the regulation of the hypoxia-inducible transcription factor (HIF-1 \( \alpha \) [36,37]. No overall amino acid sequence homology has been detected between the collagen-P4Hs and HIF-P4Hs, with the exception of critical residues in catalytic domain. It has been reported that human type I and type II collagen-P4Hs do not hydroxylate 19-residue synthetic peptide correspond-
ing to the sequence around Pro\(^ \alpha \) in HIF-1 \( \alpha \) [38]. HIF-
P4Hs have been identified in humans, \( C. elegans \) and \( D. melanogaster \). Three human cytoplasmic HIF-4 P4H isoen-

zymes that hydroxylate HIF-1 \( \alpha \) have been identified. The three different human HIF-P4Hs shows a 42~59% se-
function identity to one another but no distinct sequence similarity to the collagen P4Hs [37,39,40]. Like collagen-P4Hs these novel enzymes require Fe\(^{2+}\), 2-oxoglutarate, O\(_2\), and ascorbate as co-factor for catalytic activity. Although all three enzymes are widely expressed in many tissues, they exhibit tissue-specific overexpression. P4H2 are abundant in adipose tissue [41], P4H3 in the heart and placenta [41,42], and P4H1 in the testis [42]. The differences of the enzyme activity of P4Hs, sub-cellular localization and tissue distribution enables a graded or tissue-specific response to hypoxia.

Three isoforms of HIF-P4Hs have nearly identical Km value for O\(_2\), indicating that changes in the O\(_2\) are likely to have similar effects on the catalytic activities of all three isoenzymes. These Km values have been found to be slightly higher than the concentration of dissolved O\(_2\) in air and much higher than the Km for O\(_2\) of the type I collagen-P4H. The difference in Km values may correspond to different functions of the two classes of P4H. HIF-P4Hs have been found to act as effective oxygen sensors, their Km values for O\(_2\) are close to atmospheric oxygen concentrations and even small decreases in O\(_2\) have been noted to influence their activities. However, type I collagen-P4H has been known to act in situations with very low O\(_2\) concentrations, which is in wounds and tissues of low vascularity. Therefore, the Km values for O\(_2\) of type I collagen-P4H is much lower.

**Gene regulatory function and mechanism of HIF-1**

The hypoxia-inducible transcription factors (HIFs) has been noted to play a central role in the regulation of cellular and systemic O\(_2\) homeostasis [22]. The functional role of HIF-1\(\alpha\) in regulating target gene expression in response to hypoxia is mainly under the control of oxygen sensing. HIF-1\(\alpha\) dioxygenases also termed as prolyl-4-hydroxylase-domain (P4HD) containing enzymes [23]. These enzymes are also termed as HIF-prolyl hydroxylase (PHH) and Egg-laying Nine (EGLN) and its three isoforms have been identified that include PHD1/PHF3/EGLN2, PHD2/PHF2/ EGLN1, and PHD3/PHF1/EGLN3 [37]. These belong to the superfamily of non-heme iron (Fe\(^{2+}\))-containing 2-oxoglutarate (2OG)-dependent oxygenases and these enzymes sense the cellular oxygen and use it as a co-substrate in hydroxylation reaction [43]. During normoxia (normal oxygen concentration), P4H enzymes rapidly hydroxylate the proline residues i.e., proline 402 (Pro402) and 564 (Pro564) located within ODDDD on the de novo synthesized cytoplasmic HIF-1\(\alpha\) [44-46]. Once the two proline residues are converted to hydroxyproline, the hydroxylated HIF-1\(\alpha\) fits accurately on the surface pocket of von Hippel–Lindau (pVHL) in a highly specific manner [47,48]. Before binding to HIF-1\(\alpha\), the pVHL associates with other proteins elongin C, elongin B, cullin-2, and Rbx1 to form the VCB-Cul2 E3 ligase complex [49]. The subsequent binding of HIF-1\(\alpha\) to this multiprotein E3 complex causes polyubiquitination of HIF-1\(\alpha\), ultimately leading to its degradation by 26S proteasome [25].

However, hydroxylation of proline residues of HIF-1\(\alpha\) does not occur during hypoxia due to inhibition of P4HD enzymes which in turn prevents its degradation. The persisting HIF-1\(\alpha\) forms a stable hetero-dimer with HIF-\(\beta\) using bHLH and PAS motifs [38,49]. HIF-1\(\alpha\)-HIF-\(\beta\) hetero-dimer translocates into the nucleus and binds to the HIF-responsive elements in a number of hypoxia-inducible genes, such as those for erythropoietin, vascular endothelial growth factor and glycolytic enzymes etc (Fig. 1) [38,49]. Interestingly, the gene for the \(\alpha\) (I) subunit of human type I collagen-P4H has been shown to be one of the hypoxia-inducible target genes of HIF-1\(\alpha\) [50]. HIF-1\(\alpha\) has been termed as ‘master regulator of gene expression’ during hypoxia and the consequences of HIF-1\(\alpha\) activation are manifold [51]. HIF-1\(\alpha\) has been known for its ability to stimulate glycolysis, angiogenesis and erythropoiesis at cellular level. HIF-1\(\alpha\) has been reported to modulate the expression of many genes involved in cell survival including insulin-like growth factor-2 (IGF-2), transforming growth factor-\(\alpha\) (TGF-\(\alpha\)) and nitric oxide synthase-2 (NOS-2) [52-54]. HIF-1\(\alpha\) also has the property to increase the glucose uptake and anaerobic metabolism through activation of glucose transporters GLUT-1 and GLUT-3, and glycolytic enzymes such as phosphoglycerate kinase-1 (PGK-1) and aldolase A (ALDA) [55]. These changes increase the tolerance of cells to hypoxic conditions. HIF-1\(\alpha\) has also been shown to modulate activities at the tissue level by up-regulating genes involved in angiogenesis and blood flow, including genes for vascular endothelial growth factor (VEGF) and heme oxygenase-1 (HO-1) [56]. Finally, HIF-1\(\alpha\) has also been noted to exert effects at the level of the whole organism, by inducing the erythropoietin (EPO) gene responsible for the generation of hemoglobin and consequently the oxygen-carrying capacity of blood.

**Stabilization of HIF-1\(\alpha\) due to decreased functioning of P4H enzymes**

The stability regulation and subsequent trans-activational functions of HIF-1\(\alpha\) are mainly controlled by its post-translational modifications, such as hydroxylation, ubiquitination, acetylation, and phosphorylation [57]. It has been reported that mutation of both proline residues disrupts the interaction of HIF-1\(\alpha\) with pVHL and increases its stability in the presence of normal oxygen levels, whereas mutation of either proline alone only partially stabilizes HIF-1\(\alpha\) [45]. The half-life of HIF-1\(\alpha\) may also be increased by inactivating the P4HDs by 2OG analogs [38,40]. Fe\(^{2+}\) at the active site of the P4HDs is loosely bound by two histi-
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ferring RNAs (siRNAs) leads to cardioprotection against myocardial ischaemia.

Recently, it has been reported that the orally absorbed PHD inhibitor enzyme, GSK360A, modulates HIF-1α signaling to protect the failing heart following myocardial infarction [1]. The cardiomyocyte-specific knock out of PHD2 has been associated with protection from acute myocardial ischemic injury [80]. The earlier studies also demonstrated that the hearts of HIF-P4H-2 hypomorphic mice are more resistant to acute ischemia-reperfusion injury [81]. The con-jugated linoleic acid-induced blockade of PHD1 and induction of HIF-2α in myocardium is associated with up-regulation of pyruvate dehydrogenase lipoamide kinase isozyme 4 (PDK4) by activation of PPARα. It is described to reprogram the basal metabolism and protect against oxidative damage in myocardium in mice [82].

**Congestive heart failure (CHF)**

Left ventricular (LV) remodeling after myocardial infarction (MI) involves disruption of supporting structures, myocyte hypertrophy, and collagen deposition both at the site of infarction and at areas remote from the infarct. This process is adaptive initially but may progress to LV dilatation and dysfunction [83]. The final common pathway for collagen formation involves the activation of HIF-P4H, and accordingly, the inhibition of this enzyme after MI has been proposed to prevent interstitial fibrosis. Nwogu et al. [84] substantiated this proposal by showing the decreased fibrosis and collagen deposition in the presence of FG041, an orally available P4H inhibitor. In another study, P4H inhibitor was shown to improve the LV dysfunction and reduce the imbalance of matrix turnover and hypertrophy associated gene expression [85]. The conditional inactivation of PHD2 in mice sufficient to activate a subset of HIF target genes has been associated with premature mortality characterized by marked venous congestion and dilated cardiomyopathy [86].

**Cancer**

The activation of the HIF-1α system has been observed in numerous cancers due to induction of a hypoxic environment in the wake of rapidly dividing cancerous cells [87]. The different molecular mechanisms have been proposed for the up-regulation of the HIF-1α system in tumorogenesis [88] and it is apparent that its down-regulation may be an attractive target for cancer therapy. Bordoli and co-workers demonstrated that down-regulation of PHD2 leads to increased tumor growth in a hormone-dependent mammary carcinoma and the clinical samples of human breast cancer showed significantly shorter survival times of patients with low-level PHD2 over a period of 10 years. In the absence of PHD2, an increase in amphiregulin was reported and its levels were normalized after PHD2 reconstitution. Amphiregulin is an angiogenesis-related antibody and is regulated on the transcriptional level specifically by HIF-2, but not HIF-1. Accordingly, it has been proposed that PHD2/HIF-2/amphiregulin signaling is critical in regulating breast tumor progression and PHD2 is a potential tumor suppressor in breast cancer [89]. The studies have also indicated that PHDs function as tumor suppressors in human colorectal cancer (CRC). A recent clinical study has described that the low expression of PHD2 in CRC predicts poor survival (in early stage tumors) independent of HIF-1α [90]. Heindryckx and co-workers described the increased hepato-carcinogenesis and development of cholangiocarcinoma in PHD2 deficient mice in response to diethylnitrosamine, a carcinogenic agent. The growth of these tumors is limited as they rapidly outgrow their vascular supply and become hypoxic. Therefore, it is proposed that deficiency of PHD2-induced stabilization of HIF-1α promotes angiogenesis to accelerate the growth of these tumors in mutant mice suggesting PHD2 as good target for potential therapeutic intervention [91]. On the contrary, Klotzsche-von Ameln and co-workers demonstrated the anti-tumor effects due to inhibition of oxygen sensor PHD2 in tumor cells through matrix metalloproteinase-induced TGF-β activation pathway [92].

**Respiratory diseases**

Bronchopulmonary dysplasia (BPD), a disorder of pre-term newborns, is characterized by impairment in lung microvasculature development and distal airway formation. It has been reported that pharmacological inhibition of HIF-P4H with FG-4045 is associated with an increased angiogenesis in lung due to augmented growth factors like vascular endothelial growth factor (VEGF) and platelet-endothelial cell adhesion molecule 1 (PECAM-1) [93]. The augmentation of lung angiogenesis with elevated HIF-1α in preterm model of BPD, suggests the potential usefulness of HIF-P4H inhibitors in the management of BPD in pre-term newborns. The involvement of prolyl hydroxylases in the process of hypoxic pulmonary vascular remodeling in chronic obstructive pulmonary disease (COPD) via regulation of HIF-1α gene expression has been demonstrated. The levels of HIF-1α mRNA and protein levels in COPD group are shown to significantly higher as compared to normal subjects [94].

**Peripheral vascular diseases**

Peripheral vascular disease is one of ischemic disease and its treatment is still unsatisfactory. Using the rat sponge model for angiogenesis, Warnecke et al. [95] provided the evidences of increased vascularization with local injection of HIF prolyl hydroxylase inhibitor and projected it as novel target for the treatment of peripheral ischemic diseases.
Within hours after a single application of HIF prolyl hydroxylase inhibitors such as L-Mim and S956711, the induction of cytotoxic genes including HO-1 was demonstrated suggesting their capability to mediate acute protection against hypoxic damage. Accordingly, it has been proposed that the topical application of HIF-P4H inhibitors could be clinically useful to augment vascularization in peripheral artery disease or to preserve organ transplant [95]. A recent study has shown that although the HIF1α levels in vein wall are not affected during thrombosis, yet its up-regulation in local vein wall promotes angiogenesis to recanalize the veins and resolve the thrombus [96].

**Stroke**

Stroke is an ischemic disease of the brain and its treatment like other ischemic diseases is still unsatisfactory. There have been studies suggesting that the HIF-1α prolyl hydroxylases are inhibited during ischemic preconditioning and pharmacological inhibitors of these enzymes may be viable targets for stroke therapy (Bergeron et al., 2000). Siddiq et al. [97] demonstrated the up-regulation of HIF dependent target genes like enolase, VEGF, P21\textsuperscript{\textit{mitogen}} and erythropoietin in the embryonic cortical neurons \textit{in vitro} and even in adult rat brain \textit{in vivo} as a consequence of HIF-P4H inhibition. The expression of these genes due to HIF-P4H inhibition has been shown to prevent oxidative stress-induced death \textit{in vitro} and ischemic injury \textit{in vivo}. Recently, the inhibition of prolyl hydroxylase and subsequent stabilization of HIF activity with oral administration of TM6008 has been shown to protect the neurons in the forebrain from focal ischemia by inhibiting apoptosis [98].

**Neurodegenerative diseases**

The HIF prolyl hydroxylase inhibitors are shown to prevent mitochondrial toxins-induced neuronal death, thus, implicating their therapeutic potential for Huntington's disease and Alzheimer's disease [99]. The pharmacological inhibition of prolyl hydroxylases by 3,4-dihydroxybenzoate (DHB) administration has been shown to produce protection against MPTD-induced neurotoxicity, animal model of Parkinson's disease [100]. The \textit{in vitro} studies have demonstrated that DHB attenuates LPS-mediated induction of nitric oxide synthase and pro-inflammatory cytokines in murine BV2 microglial cells. Furthermore, it was also shown to reduce ROS production and activation of NF-κB and MAPK pathways possibly due to up-regulation of HO-1 levels. The \textit{in vivo} treatment with DHB also suppressed MPTD-induced microglial activation suggesting that its beneficial neuroprotective properties may be due to inhibition of microglial activation via HO-1 induction [101]. The earlier studies have shown HIF prolyl hydroxylase inhibition increases cell viability and potentiates dopamine release in dopaminergic cells and hence, prolyl hydroxylases may represent novel targets for therapeutic intervention in disorders characterized by dopamine homeostasis dysregulation like Parkinson's disease [102].

**Kidney diseases**

The intrinsic HIF activation is sub-maximal in acute kidney injury and the augmentation of HIF has been shown to ameliorate the acute disease manifestations of the kidney [103]. The activation of HIF has been shown to protect the kidney from acute ischemic cell death, while it promotes fibrosis in experimental models of chronic kidney diseases. Kapitsinou and co-workers demonstrated that the pharmacologic inhibition of HIF prolyl hydroxylase before acute kidney injury ameliorates fibrosis and prevents the development of anemia. Accordingly, the pre-ischemic targeting of the PHD/HIF pathway has been suggested as an effective therapeutic strategy for the prevention of chronic kidney disease resulting from acute injury [104].

**Inflammation and related diseases**

Using a pharmacologic approach leading to HIF-1α stabilization, and genetic manipulation of HIF-1α homologs in zebrafish, Elks and co-workers demonstrated the key role of HIF-1α in neutrophil inflammation. Both approaches suggested that the activated HIF-1α delays resolution of inflammation and its activation leads to reduced neutrophil apoptosis and increased retention of neutrophils at the site of tissue injury, thereby delaying the resolution phase [105]. Using neutrophils from mice deficient in PHD3, the unique role for PHD3 in prolonging neutrophil survival during hypoxia (distinct from other hypoxia-associated changes in neutrophil function and metabolic activity) has been demonstrated. The reduced neutrophil survival due to PHD3 deficiency was associated with up-regulation of the proapoptotic mediator Siva1 and loss of its binding target Bel-xL. An increased neutrophil apoptosis and clearance in PHD3-deficient mice has also been reported \textit{in vivo} models of inflammation (acute lung injury model and acute mouse model of colitis) [106].

The studies have shown that HIF prolyl hydroxylase inhibitors are protective in mouse models of inflammatory bowel disease (IBD). The PHD1(-/-), but not PHD2(+/-) or PHD3(-/-), mice are shown to lose susceptible to the development of dextran sulphate sodium-induced colitis in terms of reduction in weight loss, disease activity, colon histology, neutrophil infiltration, and cytokine expression. Furthermore, the reduced susceptibility of PHD1(-/-) mice to colitis was associated with increased density of colonic epithelial cells due to decreased apoptosis and enhanced epithelial barrier function [107].

**Oxygen-induced retinopathy**

The major side effect of oxygen therapy for preterm infants is retinopathy which in turn leads to blindness in children. Duan and co-workers demonstrated that the exposure of 75% oxygen leads to degradation of retinal HIF-α proteins in the neonatal Smice expressing normal amounts of PHD2 and it was accompanied by massive loss of the retinal microvessels. PHD2 deficiency significantly stabilized HIF-1α (HIF-2α to some extent), in neonatal retinal tissues, to protect retinal microvessels from oxygen-induced obliteration. Accordingly, it has been proposed that there is close association between PHD2-dependent HIF-α degradation and oxygen-induced retinal microvascular obliteration, and PHD2 may serve as promising therapeutic target to prevent oxygen-induced retinopathy [108].

**Preconditioning**

Three HIF-P4Hs (HIF-P4H1, HIF-P4H2, and HIF-P4H3) affect the proteosome-mediated degradation of HIF by cata-
lyzing the hydroxylation of key proline residues in the HIF-1α subunit under normoxic conditions. When oxygen tension is reduced, HIF-P4H-mediated hydroxylation does not occur and HIF-1α accumulates in the nucleus leading to enhanced HIF-mediated gene transcription [109]. The involvement of HIF-P4Hs in preconditioning in the various tissues has been recently shown by many workers. Bernhardt et al. [110] has reported that hypoxic preconditioning or HIF-P4Hs inhibitors confer renal protection in ischemic acute renal failure [110]. Ran et al. [111] reported that hypoxic preconditioning shows neuroprotection against sustained ischemic insult and HIF-P4Hs inhibitors mimic this hypoxic preconditioning reflecting the involvement of HIF-P4Hs in hypoxic preconditioning. Prolyl 4-hydroxylation inhibition-induced preconditioning is also involved in myocardial protection. From our own laboratory, it was demonstrated that pharmacological preconditioning with EDBH (HIF-P4Hs inhibitor) mimicked the cardioprotective effects of remote renal preconditioning. Administration of α-KG (HIF-P4Hs activator) and diethylidithiocarbamic acid (NFκB inhibitor) were shown to abolish the cardioprotective effects of remote renal preconditioning and EDBH. Therefore, it was proposed that inhibition of HIF-P4H has a key role in remote renal preconditioning-induced cardioprotection and HIF-P4H inhibition triggers a transduction pathway involving NFκB activation [112].

In the model of cultured hippocampal slices, the application of anoxia preconditioning before oxygen-glucose deprivation is shown to prevent the neuronal damage and suppression of HIF-1α and HIF-3α mRNA expression. Furthermore, the effects of HIF prolyl-hydroxylase inhibition with 2,4-pyridinedicarboxylic acid diethyl ester pre-treatment were similar to anoxia preconditioning. It suggests that anoxia preconditioning increases anti-ischemic neuronal resistance which correlates with the changes of HIF-1α and HIF-3α expression [113]. It has been described that ischemic preconditioning produces reinforcing effect on HIF-1 accumulation during the subsequent hypoxic injury and HIF-1 induction during hypoxic preconditioning produces reinforcing effect to accumulate HIF-1 and develops a tolerance against a subsequent hypoxic neuronal injury [114].

Others

The loss of the oxygen sensor PHD1 is suggested to make the skeletal muscles and liver more resistant to ischemia reperfusion injury, thereby projecting it as critical target in ischemia reperfusion-induced injury in these organs [115]. Muz and co-workers described that PHD-2 is the major isoform that regulates HIF-α levels in RA fibroblast-like synoviocytes (RA FLS) suggesting the major importance of this enzyme in hypoxia- and angiogenesis-dependent inflammatory diseases such as RA [116].

CONCLUSION

The preclinical studies have projected prolyl 4-hydroxylase as critical target in the pathophysiology of number of diseases. Therefore, its pharmacological modulators may also be clinically effective in management of diseases.

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