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Review

New Insights into Protein Hydroxylation and Its Important Role in Human Diseases

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ABSTRACT

Protein hydroxylation is a post-translational modification catalyzed by 2-oxoglutarate-dependent dioxygenases. The hydroxylation modification can take place on various amino acids, including but not limited to proline, lysine, asparagine, aspartate and histidine. A classical example of this modification is hypoxia inducible factor alpha (HIF-α) prolyl hydroxylation, which affects HIF-α protein stability via the Von-Hippel Lindau (VHL) tumor suppressor pathway, a Cullin 2-based E3 ligase adaptor protein frequently mutated in kidney cancer. In addition to protein stability regulation, protein hydroxylation may influence other post-translational modifications or the kinase activity of the modified protein (such as Akt and DYRK1A/B). In other cases, protein hydroxylation may alter protein-protein interaction and its downstream signaling events *in vivo* (such as OTUB1, MAPK6 and eEF2K). In this review, we highlight the recently identified protein hydroxylation targets and their pathophysiological roles, especially in cancer settings. Better understanding of protein hydroxylation will help identify novel therapeutic targets and their regulation mechanisms to foster development of more effective treatment strategies for various human cancers.

Contents

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1. Introduction

1.1. 2-oxoglutarate-dependent dioxygenases

2-oxoglutarate (2-OG)-dependent dioxygenases are iron-containing enzymes that pair substrate oxidation to the conversion of 2-OG to succinate and carbon dioxide [1]. The 2-OG-dependent oxygenase catalyzes the hydroxylation of proline and lysine residues, which was first identified in collagen biosynthesis [2]. Subsequently, it has been demonstrated that this type of post-translational modification also affects intracellular proteins. Specifically, the hypoxia-inducible factors (HIFs) have been found to be hydroxylated on both proline and asparagine residues by the prolyl hydroxylase domain proteins PHD1, PHD2, PHD3 (also called Egl nine homolog EglN2, EglN1, and EglN3) and factor inhibiting HIF (FIH), respectively [3]. Up to date, about seventy 2-OG dependent dioxygenases have been identified in the human genome [4]. According to their biological functions, it is possible to distinguish them into three major subclasses: histone demethylases, DNA/RNA demethylases/hydroxylases and protein hydroxylases [5].

Histone methylation is performed by the family of lysine and arginine methyltransferases, which can transfer up to three methyl groups to histone lysines and asparagines, subsequently triggering different activities according to the recruitment of specific effector proteins by the readers [6]. Importantly, the JumonjiC (JMJC) domain-containing histone demethylases, which can be further divided into seven subfamilies (KDM2–8), remove methyl groups from all three methyl lysine states, with concomitant production of succinate, carbon dioxide, and subsequent release of formaldehyde [7,8].

Eukaryotic DNA and RNA methylation is primarily catalyzed by DNA and RNA methyltransferases, respectively, which epigenetically modulate cell fate without altering the nucleic acid sequence. DNA/RNA can be also methylated by endogenous and/or exogenous alkylating agents in a process referred to as methylation damage [9]. In humans, both regulatory and aberrant methylation can be oxidatively reversed by a non-heme iron-dependent dioxygenase superfamily composed of nine members (alkylation repair homologs ALKBH1–8, and fat-mass and obesity-associated FTO) [9]. Recently, the ten-eleven translocation (TET)/J-binding protein (JBP) family proteins (TET1, TET2, and TET3; JBP1 and JBP2), also belonging to the family of iron- and 2-OG-dependent dioxygenases, have been found to oxidize 5-methylcytosine [10].

In this review, we will mainly focus on protein hydroxylases. The primary residue to be hydroxylated in proteins is proline, and accordingly the hydroxylases that catalyze this reaction are called prolyl hydroxylases. In addition to proline, asparagine, aspartate, lysine and histidine have also been found to be hydroxylated in cells [11–14].

1.2. Prolyl Hydroxylases

The HIFs are transcription factors that coordinate cellular responses to low oxygen levels, aiming at increased oxygen delivery and reduced oxygen consumption [3]. Transcriptionally active HIFs are heterodimers composed of an alpha and ARNT (aryl hydrocarbon receptor nuclear translocator) subunits. In humans, the alpha subunit has three isoforms, namely HIF-1α, HIF-2α and HIF-3α. Different from the ARNT subunit that is constitutively expressed, the protein stabilization of the alpha subunit is oxygen-sensitive [3].

In normoxic conditions, HIFs have been found to be hydroxylated on one or two proline residues by the prolyl hydroxylase domain proteins PHD1/EglN2, PHD2/EglN1, and PHD3/EglN3 [15]. Specifically in regards to HIF-1α, its hydroxylation on proline 402 (Pro402) and 564 (Pro564) by PHDs triggers its recognition by a multimeric E3 ubiquitin ligase complex formed by the von Hippel-Lindau tumor suppressor protein (pVHL), elongin B and C, Cullin 2 (CUL2) and RING-box 1 (RBX1) proteins. Homologous to the Skp, Cullin, F-box containing (SCF) family of E3 ubiquitin ligase complexes, this complex targets HIF-1α for ubiquitin-mediated proteasomal degradation (Fig. 1A) [16]. It is important to point out that even though all three enzymes contribute to HIF regulation, PHD2/EglN1 is the major prolyl hydroxylase mediating HIF-1α hydroxylation *in vivo* [17–20].

Under hypoxic conditions, HIFs are not hydroxylated and, thus, can bind to the hypoxia-response elements (HRE) in the promoter of more than one hundred target genes involved in cell survival in low oxygen conditions [21]. These targeted genes include metabolic genes such as the glucose transporter 1 (GLUT1), the vascular endothelial growth factor (VEGF) that promotes angiogenesis, and erythropoietin (EPO) that controls erythropoiesis. Transforming growth factor alpha (TGFα), implicated in cell proliferation and survival, and C-X-C chemokine receptor type 4 (CXCR4), important for cell migration and invasion, are also characterized HIF targets (Fig. 1B) [22]. As all of these pathways promote the growth and progression of many tumors, HIFs are generally considered as attractive therapeutic targets in cancer.

Since the identification of HIF hydroxylation, much effort has been devoted to better understand whether and how prolyl hydroxylation regulates signaling pathways beyond the HIF-pathway. Over the course of the last decades, a handful of substrates have been identified as *bona fide* PHD substrates. Among them, the centrosomal protein of 192 kDa CEP192 and the inhibitor of nuclear factor kappa-B kinase subunit beta IKBKB are PHD1 substrates [23,24], while the isoform 2 of pyruvate kinase PKM2 and the β-adrenergic receptor II are PHD3 substrates. Since these targets have been reviewed recently [5,25], this review will largely focus on other most recently identified substrates.

1.3. Regulation of Prolyl Hydroxylases

PHD/EglN protein expression is tissue- and context-dependent. PHD2/EglN1 is ubiquitously expressed, whereas PHD1/EglN2 and PHD3/EglN3 are mainly observed in testis and heart, respectively [26]. PHDs are transcriptionally regulated by their downstream target HIF pathway [27,28]. Of note, HIF-induced PHD3/EglN3 expression provides a negative feedback regulating HIFs in hypoxic conditions [29]. Furthermore, PHD2/EglN1 and PHD1/EglN2 can be transcriptionally regulated by estrogen pathways in breast cancer cells [30–34]. At the protein level, the abundance of PHD3/EglN3, and to a lesser extent of PHD1/EglN2, is regulated by the E3 ubiquitin ligases SIAH1/2 in a proteasomal-dependent manner [35]. However, the manipulation of PHD/EglN catalytic activities has been a mystery for a long time. Previous research has reviewed extensively on how PHDs/EglNs serve as important nutrient sensors since their activity is regulated by the