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Review article

Prolyl hydroxylase domain enzymes and their role in cell signaling and cancer metabolism

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ABSTRACT

The prolyl hydroxylase domain (PHD) enzymes regulate the stability of the hypoxia-inducible factor (HIF) in response to oxygen availability. During oxygen limitation, the inhibition of PHD permits the stabilization of HIF, allowing the cellular adaptation to hypoxia. This adaptation is especially important for solid tumors, which are often exposed to a hypoxic environment. However, and despite their original role as the oxygen sensors of the cell, PHD are currently known to display HIF-independent and hydroxylase-independent functions in the control of different cellular pathways, including mTOR pathway, NF-kB pathway, apoptosis and cellular metabolism. In this review, we summarize the recent advances in the regulation and functions of PHD in cancer signaling and cell metabolism.

Contents

Abbreviations: αKG, 2-oxoglutarate; AMPK, AMP-activated protein kinase; ATF4, activating transcription factor 4; DDR, DNA damage response; DMOG, dimethyloxaloylglycine; eEF2, eukaryotic elongation factor 2; EGFR, epidermal growth factor receptor; HCLK2, Caenorhabditis elegans biological clock protein CLK-2; HIF, hypoxia inducible factor; HRE, hypoxia-response element; Hsp90, heat shock protein 90; KIF1Bβ, kinesin-like protein; mTOR, mammalian target of rapamycin; NF-kB, nuclear factor-kappa B; ODD, oxygen dependent degradation; PCBP, poly-r(C)-binding protein 1; PDH, pyruvate dehydrogenase; PKM2, pyruvate kinase isozymes M2; PHD, prolyl hydroxylase; pVHL, von Hippel-Lindau tumor suppressor; ROS, reactive oxygen species; TCA, tricarboxylic acid cycle.

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1. Introduction

Among the different hallmarks of cancer (Hanahan and Weinberg, 2011), metabolic transformation plays a key role in the adaptation of cancer cells to a changing environment. Due to the rapid proliferation of cancer cells, solid tumors are often exposed to low oxygen and nutrient availabilities. The stabilization of the hypoxia-inducible factor (HIF) upon oxygen restriction in the cancer cell coordinates the transcriptional response to low oxygen levels. Known as the oxygen sensors of the cell in metazoans, the prolyl hydroxylase domain (PHD) protein family plays a central role in the regulation of HIF stability. PHD enzymes were first described about 10 years after HIF discovery. This family belongs to a family of 2-oxoglutarate(αKG)-dependent, non-haem iron-binding dioxygenases. PHD were discovered in *Caenorhabditis elegans* (EGL-9), and since then they have been described in different organisms, such as mammals (EGLN1-4), rat (SM-20), *Drosophila melanogaster* (CG1114), *Dictyostelium*, the fission yeast (*Schizosaccharomyces pombe*), or even in photosynthetic organisms such as *Chlamydomonas reinhardtii* (Boulahbel et al., 2009). In mammalian cells, there are three different genes encoding three isoforms of PHD, called EGLN1 (encoding PHD2), EGLN2 (encoding PHD1), and EGLN3 (encoding PHD3). An endoplasmic reticulum transmembrane prolyl hydroxylase (TM-HIF-P4H) has also been identified with an activity similar to HIF prolyl hydroxylase but the C-terminal catalytic region is closely related to collagen prolyl hydroxylase (Koivunen et al., 2007b; Oehme et al., 2002). PHD1 and PHD2 are two longer isoforms with respectively 407 and 426 amino acids in humans. They share a highly conserved hydroxylase domain at their C-terminal domain, but a divergent and poorly characterized N-terminal domain. The shorter isoform PHD3, with only 239 amino acids, has the hydroxylase domain and also a divergent N-terminal sequence (Bruick and Mcknight, 2001; Epstein et al., 2001; Ivan et al., 2002).

All three isoforms are expressed in all tissues but at different levels. PHD2 is found in most tissues, whereas PHD1 is more expressed in testes, brain, kidney, heart, and liver, and PHD3 is present mostly in the heart (Cioffi et al., 2003). Although three main isoforms are reported and studied, different alternatively spliced isoforms have been also described (Hirsilä et al., 2003). For example, gain or loss of function of different splicing forms of the PHD3 gene are reported to regulate the hypoxia response pathway (Cervera et al., 2006). Besides, two different isoforms of PHD1, produced by alternative translational initiation, have very similar activity on the HIF system, raising a question of the regulation of other non-HIF targets (Tian et al., 2006). So far, alternatively spliced PHD2 transcripts encode catalytically inactive polypeptides (Hirsilä et al., 2003). The reason of the presence of different splicing isoforms of PHD family is still unclear.

The hydroxylation activity of PHD is oxygen-dependent. As mentioned above, the main target is the transcription factor HIFα, (three main subunits described, HIF-1α, HIF-2α and HIF-3α) that regulates cell response to hypoxic conditions. In the presence of oxygen, PHD (mostly PHD2, at least *in vivo*) hydroxylate HIF-1α in its oxygen-dependent degradation (ODD) domain at two proline residues (P402 and P564). Both proline residues are found in a conserved motif with a sequence like −Leu-X-X-Leu-Ala-Pro, and the substitution of flanking leucine or alanine residues has little effect on prolyl hydroxylation (Epstein et al., 2001; Huang et al., 2002). The hydroxylation leads to the binding of HIFα to the von Hippel–Lindau (pVHL) tumor suppressor protein and induces its ubiquitination and subsequent proteolytic degradation by the E3 ubiquitin ligase complex (Berra et al., 2006). Under hypoxia, PHD are inactivated and HIFα is stabilized, thus interacting with HIFβ, allowing the expression of target genes (Kaelin and Ratcliffe, 2008).

Despite the important role of PHD in oxygen sensing and HIF regulation, there is now strong evidences that PHD have additional functions in different pathways. Various publications have reported non-HIF substrates and also hydroxylase-independent functions of PHD. In this review, we will summarize both the HIF-dependent and HIF-independent functions and regulation of PHD inside the eukaryotic cell.

2. Upstream of PHD: metabolic components controlling PHD activity

PHD activity depends on different upstream inputs, such as oxygen and αKG as co-substrates, or iron and ascorbate as co-factors (Fig. 1).

2.1. Oxygen

A member of the dioxygenase family, PHD are able to incorporate both atoms of dioxygen into their products, and they are, as a consequent, sensitive to oxygen level. The isotopic study using ^{18}O showed that one oxygen atom from dioxygen is used in the oxidative decarboxylation of αKG to generate succinate and CO_2, and the other oxygen atom is used for the hydroxylation of a proline residue of the targeted HIFα molecule (McNeill et al., 2002). The presence of oxygen is crucial for PHD activity and it cannot be substituted by an H_2O molecule. Different studies have measured the affinity of PHD by oxygen using HIFα peptide substrates. The apparent K_M for oxygen (the concentration of oxygen that supports a half-maximal initial catalytic rate) is closer to 100 μM (Ehrismann et al., 2007; Koivunen et al., 2006). Compared with others dioxygenases, this K_M is particularly high, and certainly higher than the intracellular oxygen level (10–30 μM). It means that PHD activity relies on oxygen levels when all other substrates and co-factors are available. This is the biochemical basis of the function of PHD as oxygen sensors. PHD are also regulated by O_2 availability through E3 ubiquitin ligases Siah1a and Siah2 activity. Under hypoxia, Siah2 transcription is stimulated, leading to PHD1 and PHD3 proteasomal degradation (Nakayama et al., 2004). This study present an additional layer of complexity in the regulation of PHD in response to oxygen level.

Low oxygen levels lead to the production of Reactive Oxygen Species (ROS) generated by complex III of the mitochondrial electron transport chain (Chandel et al., 1998). Several works confirmed the role of ROS in the control of HIFα stability by genetic or pharmacological inhibition of mitochondria activity (Brunelle et al., 2005; Chandel et al., 2000; Guzy et al., 2005; Mansfield et al., 2005; Pan et al., 2006). According to this, ROS production inhibits PHD enzymes by regulating the level of Fe(II), ascorbate or Krebs cycle intermediates which have an impact on PHD activity (Gerald et al., 2004; Hagen, 2012; Li et al., 2014). Nonetheless, different works denied the role of ROS in HIF accumulation. In mitochondria-deficient HeLa cells, ROS production is very low and HIF can still be stabilized under hypoxia (Enomoto et al., 2002). Another result showed that mitochondria regulate HIF-1α protein stabilization and accumulation by regulating the intracellular oxygen availability not by producing ROS from complex III (Chua et al., 2010).