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Regulation of HIF-1 α stability by lysine methylationSung Hee Baek^{1,*} and Keun Il Kim^{2,*}

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Abbreviations: HIF-1 α , hypoxia-inducible factor-1 α ; PHD, proline hydroxylase domain; VHL, von Hippel-Lindau; CUL2, cullin 2; LSD1, lysine-specific demethylase 1; DMOG, dimethyloxalylglycine; MEF, mouse embryonic fibroblast

Perspective to: Yunho Kim et al (2016), Methylation-dependent regulation of HIF-1 α stability restricts retinal and tumor angiogenesis, *Nature Communications*, 7:10347, doi: 10.1038/ncomms10347

Abstract

The protein level and activity of critical regulatory proteins in cells are tightly controlled by several tiers of post-translational modifications. HIF-1 α is maintained at low levels under normoxia by the collaboration between PHD proteins and the VHL-containing E3 ubiquitin ligase complex. We recently identified a new physiologically relevant mechanism that regulates HIF-1 α stability in the nucleus in response to cellular oxygen levels. This mechanism is based on the collaboration between the SET7/9 methyltransferase and the LSD1 demethylase. SET7/9 adds a methyl group to HIF-1 α , which triggers degradation of the protein by the ubiquitin-proteasome system, whereas LSD1 removes the methyl group, leading to stabilization of HIF-1 α under hypoxia. In cells from a knock-in mouse model that harbor a mutation preventing HIF-1 α methylation (*Hif1 α ^{KA/KA}*), HIF-1 α levels increased in both normoxic and hypoxic conditions. With increased HIF-1 α , *Hif1 α ^{KA/KA}* knock-in mice displayed increased hematological parameters, such as red blood cell count and hemoglobin concentrations. In pathological aspects, retinal and tumor-associated angiogenesis as well as tumor growth were increased in *Hif1 α ^{KA/KA}* knock-in mice. Certain human cancer cells exhibit mutations that cause defects in HIF-1 α methylation. In summary, this newly identified methylation-based regulation of HIF-1 α stability constitutes another layer of regulation independent of previously identified mechanisms.

Hypoxia-inducible factor-1 α (HIF-1 α) is a key transcriptional regulator responsible for the adaptation of cells and tissues to a state of low oxygen (hypoxia). Since uncontrolled expression of hypoxia-inducible genes is harmful to normal physiology, the cellular level of HIF-1 α is tightly regulated, primarily by ubiquitin-mediated degradation. In the presence of physiological concentrations of oxygen (normoxia), HIF-1 α is hydroxylated on proline residues by proline hydroxylase domain (PHD) proteins (PHD1/PHD2/PHD3). Hydroxylated proline residues serve as a marker for recognition by the VHL-containing CUL2 E3 ubiquitin ligase complex. On the other hand, the lack of oxygen in hypoxia triggers reduced hydroxylation and increased stability of HIF-1 α which leads to its translocation to the nucleus, where HIF-1 α heterodimerizes with HIF-1 β and together induce the expression of target genes. In addition to controlling HIF-1 α transcriptional activity by regulating HIF-1 α stability, other means of regulation include SUMOylation, acetylation, and phosphorylation.

Recently, we identified another mechanism of control for the stability of HIF-1 α (Figure 1). We will discuss the following three aspects of this new discovery: molecular mechanisms, physiological significance revealed by *Hif1 α ^{KA/KA}* knock-in mouse model, and clinical relevance to human cancers. In general, the cellular level of HIF-1 α is very low in normoxic conditions. In the presence of MG132, a proteasome inhibitor, we were able to identify methylation of HIF-1 α on the 32nd lysine residue, and found that this methylation is mediated by SET7/9 methyltransferase in the nucleus. Interestingly, the level of HIF-1 α methylation in the presence of MG132, which was measured by a HIF-1 α methylation-specific antibody, is high in normoxia, decreases upon initiation of hypoxia, and increases again after longer exposure to hypoxia. This inversely correlates with the protein level of HIF-1 α without proteasome inhibitor treatment. HIF-1 α methylation could be a signal for poly-ubiquitination by an unidentified E3 ligase resulting in the degradation of HIF-1 α , which is independent of cytosolic destabilization mechanism of HIF-1 α . We sought to

identify the underlying mechanism controlling the stabilization of HIF-1 α from early to later periods of hypoxia. On the basis of decreased methylation of HIF-1 α , we found that a demethylating enzyme, LSD1, removes a methyl group from HIF-1 α to stabilize it. The HIF-1 α protein level under hypoxia was higher in the *Lsd1*-deficient mouse embryonic fibroblasts (MEFs) compared to control MEFs. Therefore, SET7/9 and LSD1 are newly identified regulators of hypoxia that control HIF-1 α stability.

The physiological relevance of this new mechanism was evaluated with a *Hif1 α ^{KA/KA}* knock-in mouse model, in which HIF-1 α resists methylation. These mice are largely indistinguishable from their wild-type counterpart in growth, fertility, and life-span. HIF-1 α levels are slightly higher in several tissues from *Hif1 α ^{KA/KA}* mice compared to those from wild-type; however, the difference became more prominent after treating the mice with a prolyl hydroxylase inhibitor, dimethyloxalylglycine (DMOG), which protects HIF-1 α from cytosolic degradation. This phenomenon can be explained as follows: HIF-1 α that resisted degradation in the cytosol is normally degraded in the nucleus by SET7/9-mediated methylation followed by ubiquitination and proteasomal degradation. However, the methylation-defective HIF-1 α accumulates in the nucleus after resisting degradation in the cytosol. With higher levels of HIF-1 α protein, *Hif1 α ^{KA/KA}* mice display increased hematocrit, red blood cell count, and amount of hemoglobin. One well-known function of HIF-1 α is to promote angiogenesis by activating the transcription of angiogenic factors. Two types of angiogenesis, retinal and tumor-associated, were both significantly elevated in *Hif1 α ^{KA/KA}* mice with increased expression of VEGF. Although the phenotype was not prominent under normal physiological conditions, *Hif1 α ^{KA/KA}* mice showed clear significance of methylation-derived clearance system in pathological situations.

Another intriguing characteristic of the cells and mice with *Hif1 α ^{KA/KA}* genotype is increased tendency to tumorigenesis. MEFs derived from *Hif1 α ^{KA/KA}* mice showed enhanced

cell migration as well as colony formation. MDA-MB231 breast cancer cells that stably express methylation-resistant HIF-1 α K32A protein formed more and larger tumors in athymic nude mice compared to cells expressing wild-type HIF-1 α . In order to determine if this newly found mechanism has any relevance to human cancer, we searched databases for HIF-1 α mutations in human cancers and identified two frequently occurring mutations, S28Y and R30Q. Both are situated near the methylation site at K32; however, a mutation in K32 itself was not detected. Both S28Y and R30Q mutant HIF-1 α proteins are resistant to methylation-dependent degradation and the cells expressing the mutant HIF-1 α exhibit increased migration. Although it needs to be validated more systematically, it is highly possible that these HIF-1 α mutations might contribute to the development and/or progression of human cancers.

Main text: 730 words

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FIGURE LEGEND**Figure 1. Schematic model for regulation of HIF-1 α protein stability**

Under normoxic condition, HIF-1 α protein stability is regulated by PHD-dependent hydroxylation in the cytoplasm. The hydroxylated HIF-1 α is degraded by 26S proteasomes to maintain low HIF-1 α protein levels. In contrast, SET7/9-dependent methylation and LSD1-dependent demethylation of HIF-1 α regulate protein stability primarily in the nucleus in a hydroxylation-independent manner during normoxia. Upon hypoxia, HIF-1 α is stabilized by LSD1-dependent demethylation in the nucleus.

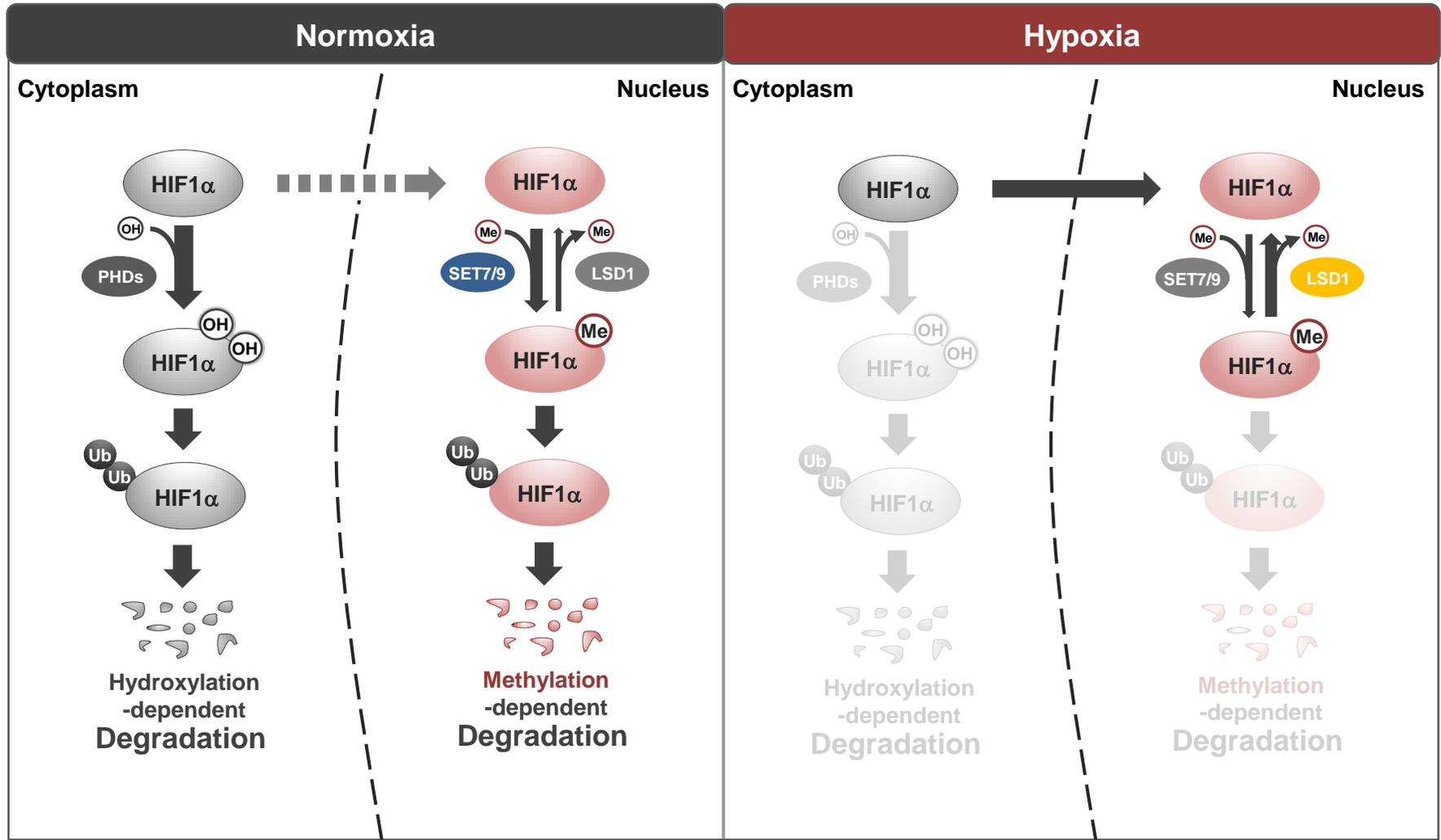


Figure 1 (Baek and Kim)