Regulation of HIF-1α stability by lysine methylation

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The level and activity of critical regulatory proteins in cells are tightly controlled by several tiers of post-translational modifications. HIF-1α is maintained at low levels under normoxia conditions 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-1α 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-1α, which triggers degradation of the protein by the ubiquitin-proteasome system, whereas LSD1 removes the methyl group, leading to stabilization of HIF-1α under hypoxia conditions. In cells from knock-in mice with a mutation preventing HIF-1α methylation (Hif1αKKK), HIF-1α levels were increased in both normoxic and hypoxic conditions. Hif1αKKK knock-in mice displayed increased hematological parameters, such as red blood cell count and hemoglobin concentration. They also displayed pathological phenotypes; retinal and tumor-associated angiogenesis as well as tumor growth were increased in Hif1αKKK knock-in mice. Certain human cancer cells exhibit mutations that cause defects in HIF-1α stability. In summary, this newly identified methylation-based regulation of HIF-1α stability constitutes another layer of regulation that is independent of previously identified mechanisms. [BMB Reports 2016; 49(5): 245-246]

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

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Hypoxia-inducible factor-1α (HIF-1α) 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-1α is tightly regulated, primarily by ubiquitin-mediated degradation. In the presence of physiological concentrations of oxygen (normoxia), HIF-1α 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-1α, which leads to its translocation to the nucleus, where it heterodimerizes with HIF-1β and induces the expression of target genes. In addition to controlling HIF-1α transcriptional activity by regulating HIF-1α stability, other means of regulation include SUMOylation, acetylation, and phosphorylation.

Recently, we identified another control mechanism of HIF-1α stability (Fig. 1). We will discuss the following three aspects of this new discovery: molecular mechanisms, physiological significance as revealed by a Hif1αKKK knock-in mouse model, and clinical relevance to human cancers. In general, the cellular level of HIF-1α is very low in normoxic conditions. In the presence of MG132, a proteasome inhibitor, we were able to identify methylation of HIF-1α on the 32nd lysine residue, and found that this methylation is mediated by SET7/9 methyltransferase in the nucleus. Interestingly, the level of HIF-1α methylation in the presence of MG132, which was measured by a HIF-1α 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-1α without proteasome inhibitor treatment. HIF-1α methylation could be a signal for poly-ubiquitination by an unidentified E3 ligase that results in the degradation of HIF-1α, which is independent of the cytosolic destabilization mechanism of HIF-1α. We sought to identify the underlying mechanism controlling the stabilization of HIF-1α from early to later periods of hypoxia. On the basis of decreased methylation of HIF-1α, we found that a demethylating enzyme, LSD1, removes a methyl group from HIF-1α to stabilize it. The HIF-1α protein level under hypoxia was higher in Lsd1-deficient mouse embryonic fibroblasts (MEFs) compared to control MEFs. Therefore, SET7/9 and LSD1 are newly identified regulators of hypoxia that control...
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Fig. 1. Schematic model of regulation of HIF-1α protein stability. Under normoxic conditions, HIF-1α protein stability is regulated by PHD-dependent hydroxylation in the cytoplasm. Hydroxylated HIF-1α is degraded by 26S proteasomes to maintain low HIF-1α protein levels. In contrast, SET7/9-dependent methylation and LSD1-dependent demethylation of HIF-1α regulate protein stability primarily in the nucleus in a hydroxylation-independent manner during normoxia. Upon hypoxia, HIF-1α is stabilized by LSD1-dependent demethylation in the nucleus.

HIF-1α stability.

The physiological relevance of this new mechanism was evaluated with a Hif1α<sup>KA/KA</sup> knock-in mouse model, in which HIF-1α resists methylation. These mice are largely indistinguishable from their wild-type counterparts in growth, fertility, and life-span. HIF-1α levels are slightly higher in several tissues from Hif1α<sup>KA/KA</sup> mice compared to those from wild-type mice; the difference becomes more prominent after treating the mice with a prolyl hydroxylase inhibitor, dimethyloxalylglycine (DMOG), which protects HIF-1α from cytosolic degradation. This phenomenon can be explained as follows: HIF-1α that resists degradation in the cytosol is normally degraded in the nucleus by SET7/9-mediated methylation followed by ubiquitination and proteasomal degradation. However, methylation-defective HIF-1α accumulates in the nucleus after resisting degradation in the cytosol. Along with higher levels of HIF-1α protein, Hif1α<sup>KA/KA</sup> mice display increased hematocrit, red blood cell count, and hemoglobin levels. One well-known function of HIF-1α is to promote angiogenesis by activating the transcription of angiogenic factors. Two types of angiogenesis, retinal and tumor-associated, were both significantly elevated in Hif1α<sup>KA/KA</sup> mice along with increased expression of VEGF in both cases. Although the phenotype was not prominent under normal physiological conditions, Hif1α<sup>KA/KA</sup> mice showed clear alterations in the methylation-derived clearance system in pathological situations.

Another intriguing characteristic of Hif1α<sup>KA/KA</sup> cells and mice is an increased tendency for tumorigenesis. MEFs derived from Hif1α<sup>KA/KA</sup> mice showed enhanced cell migration as well as colony formation. MDA-MB231 breast cancer cells that stably express methylation-resistant HIF-1α K32A protein form more and larger tumors in athymic nude mice compared to cells expressing wild-type HIF-1α. In order to determine if this newly found mechanism has any relevance to human cancer, we searched databases for HIF-1α 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-1α proteins are resistant to methylation-dependent degradation and cells expressing the mutant HIF-1α exhibit increased migration. Although this hypothesis requires systemic validation, it is highly possible that these HIF-1α mutations contribute to the development and/or progression of human cancers.

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