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REVIEW

# Epigenetic regulators: multifunctional proteins modulating hypoxia-inducible factor-α protein stability and activity

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**Abstract** The hypoxia-inducible factor (HIF) is a heterodimeric transcription factor governing a transcriptional program in response to reduced $O_2$ availability in metazoans. It contributes to physiology and pathogenesis of many human diseases through its downstream target genes. Emerging studies have shown that the transcriptional activity of HIF is highly regulated at multiple levels and the epigenetic regulators are essential for HIF-mediated transactivation. In this review, we will discuss the comprehensive regulation of HIF transcriptional activity by different types of epigenetic regulators.

**Keywords** Hypoxia-inducible factor · Epigenetic writer · Epigenetic eraser · Epigenetic reader · ATP-dependent chromatin remodeler · Chromatin reprogramming · Gene regulation

## Introduction

The hypoxia-inducible factor (HIF) is a master transcriptional factor consisting of an inducible α subunit and a constitutively expressed β subunit in response to low oxygen in metazoans [1]. Three α subunits (HIF-1α, HIF-2α, and HIF-3α) and two β subunits (HIF-1β, also known as ARNT, and ARNT2) have been cloned thus far [2–5]. Three HIF-1α mRNA transcripts are encoded by human *HIF1A* gene, whereas only one HIF-2α mRNA transcript is transcribed from human *EPAS1* gene (Fig. 1). HIF-1α and HIF-2α share about 48% overall amino acid sequence identity and contain the same functional domains: basic helix–loop–helix (bHLH) domain, Per-Arnt-Sim (PAS) domain, oxygen-dependent degradation (ODD) domain, N-terminal transactivation domain (N-TAD), inhibitory domain (ID), and C-terminal transactivation domain (C-TAD) (Fig. 1). A total of 19 distinct HIF-3α transcripts exist in the human genome database due to alternative mRNA splicing but only 8 variants may encode HIF-3α proteins. Human HIF-3α isoforms 1 and 9 contain a N-TAD and a unique leucine zipper (LZIP) domain and are shown to activate gene transcription in human cells (Fig. 1) [6]. However, HIF-3α isoform 4 lacks the transactivation domain and LZIP domain, and functions as a transcriptional inhibitor for HIF-1 and HIF-2 [7]. HIF-1β is ubiquitously expressed, but ARNT2 expression is restricted to the brain and kidney in rat and mouse [8, 9]. Hundreds of genes have been discovered to be regulated by HIF in various cell types and their protein products regulate erythropoiesis, angiogenesis, metabolism, pH homeostasis, stem cell maintenance, autophagy, immune evasion, and cell migration/invasion [10]. Therefore, HIF-1 and HIF-2 play an important role in development, physiology, and diseases, such as cancer, heart disease, sleep apnea, and trauma [10]. Recent studies showed that HIF-3α overexpression causes aberrant late branching morphogenesis, alveolar formation, and epithelial differentiation during lung development in transgenic mice [11]. HIF-3α knockout impairs pulmonary endothelial cell proliferation and angiogenic potential

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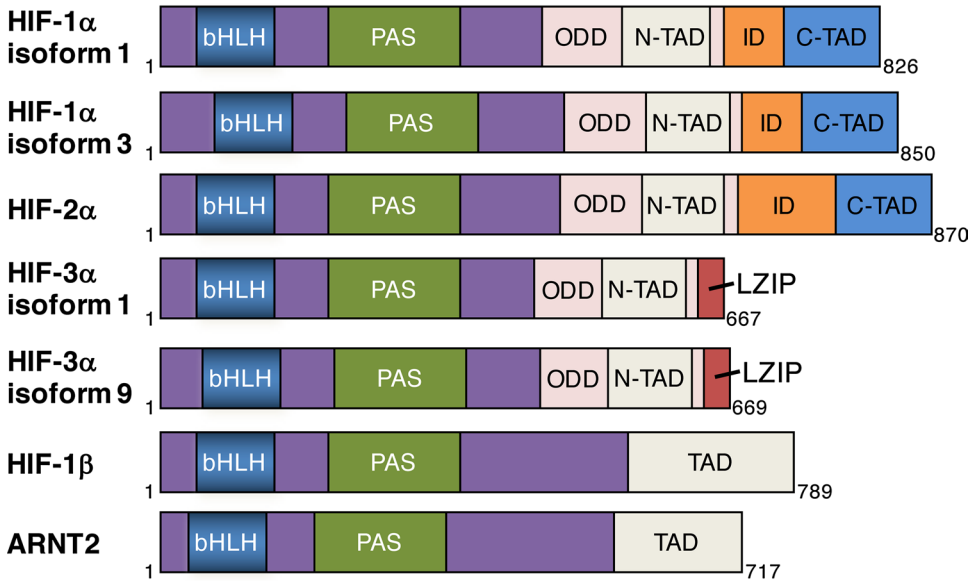


**Fig. 1** The structure scheme of active human HIF-α isoforms and human HIF-β. The functional domains of each isoform are indicated. *bHLH* basic helix-loop-helix, *PAS* Per-Arnt-Sim, *ODD* oxygen-dependent degradation, *N-TAD* N-terminal transactivation domain, *ID* inhibitory domain, *C-TAD* C-terminal transactivation domain, *LZIP* leucine zipper

[12], suggesting a critical role of HIF-3α in physiology and pathology.

The transcriptional activity of HIF, particularly HIF-1 and HIF-2, is regulated at both mRNA and protein levels. Under normoxic conditions, HIF-α is hydroxylated on proline (Pro) residues (Pro 402 and Pro 564 for human HIF-1α; Pro 405 and Pro 531 for human HIF-2α; Pro 406 and Pro 492 for human HIF-3α isoform 9) by a family of prolyl hydroxylases (PHDs) in the presence of the cofactors (iron, α-ketoglutarate, and ascorbate) and the substrate $O_2$ [13]. Prolyl hydroxylated HIF-α is recognized by von Hippel-Lindau (VHL), which recruits the Cullin-2/Elongin-B/C ubiquitin E3 ligase complex to induce HIF-α protein degradation in the 26*S* proteasome (Fig. 2) [14]. Under hypoxic conditions, prolyl hydroxylation of HIF-α is impaired, leading to stabilization of HIF-α protein (Fig. 2). HIF-α is then translocated into the nucleus and dimerized with HIF-1β. The heterodimer binds to the hypoxia response element

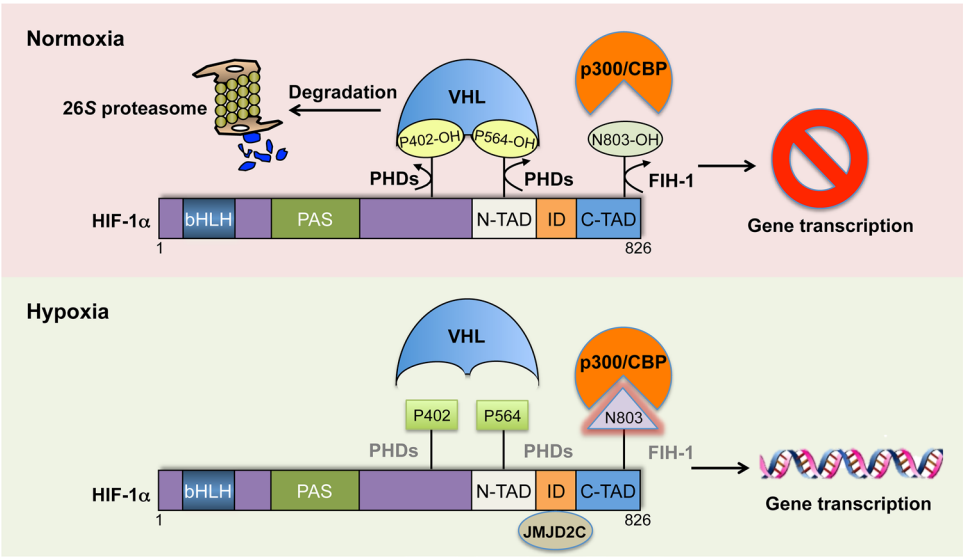


**Fig. 2** $O_2$-dependent regulation of HIF-1 activity. Under normoxia, human HIF-1α is hydroxylated on proline (P) residues 402 and 564 by a family of prolyl hydroxylases (PHDs). Prolyl hydroxylated HIF-1α is recognized by von Hippel-Lindau (VHL), which recruits the Cullin-2/Elongin-B/C ubiquitin E3 ligase complex to induce HIF-1α protein degradation in the 26*S* proteasome. On the other hand, human HIF-1α is also hydroxylated on asparagine (N) 803 residue by another dioxygenase factor inhibiting HIF-1 (FIH-1), leading to blockade of p300 recruitment to the C-terminal transactivation domain of HIF-1α, thereby preventing HIF-1-dependent gene transcription. Under hypoxia, PHDs and FIH-1 lose their enzymatic activity. As such, HIF-1α protein is stabilized and the epigenetic regulators (e.g., p300/CBP, JMJD2C) are recruited to promote HIF-1-mediated transactivation

(5′-A/GCGTG-3′) across the genome to enhance gene transcription [15]. Emerging studies have shown that epigenetic regulators, including epigenetic writers, erasers, and readers, and ATP-dependent chromatin remodelers, are essential for HIF-mediated transactivation (Table 1). In this review, we will discuss the comprehensive regulation of HIF transcriptional activity by different types of epigenetic regulators.

## Regulation of HIF transcriptional activity by epigenetic writers

Acetyltransferases, methyltransferases, protein kinases, and ubiquitin E3 ligases function as epigenetic writers by adding epigenetic marks onto histones, DNA, or RNA. p300/CBP possess the intrinsic histone acetyltransferase activity that induces histone acetylation to relax the chromatin [16]. They bind to the transactivation domain of HIF-α to coactivate HIF-mediated transactivation, and are responsible for expression of about 30–50% of global HIF-1 downstream target genes [17]. Post-translational modifications of HIF-α are known to modulate p300 coactivator functions. Asparagine 803 of HIF-1α is hydroxylated by factor inhibiting HIF-1 (FIH-1) under nonhypoxic conditions, thereby blocking p300 binding to HIF-1α to inhibit HIF-1 transcriptional activity (Fig. 2) [18, 19]. In contrast, *S*-nitrosylation of cysteine 800 enhances p300 recruitment to HIF-1α to increase HIF-1-mediated transactivation [20]. Our previous study showed that the recruitment of p300 to the hypoxia response element is enhanced by pyruvate kinase M2, a HIF coactivator [21]. Several other HIF-1α-interacting proteins, including CITED2, EAF2, protein kinase C zeta, FOXO3a, ORF3, p65, histone deacetylase 4 (HDAC4), HDAC5, and FHL1, are also shown to regulate HIF-1α-p300 interaction, leading to altered HIF-1 transcriptional activity [22–29].

Similarly, the histone acetyltransferase TIP60 was recently reported to enhance HIF-1 transcriptional activity and activate about 25% of HIF-1 downstream target genes in colorectal cancer HCT116 cells [30]. TIP60 is recruited by HIF-1α to the hypoxia response element of the HIF-1 target gene *ANKRD37*. Knockdown of TIP60 decreases RNA polymerase II loading and activation at the promoter of *ANKRD37*. Acetylation of histone H3 at lysine (K) 9 and histone H4 at the promoter of *ANKRD37* is also reduced in TIP60 knockdown HCT116 cells. TIP60 is known to acetylate the N-terminal lysine residues of histone H4 [31], and thus TIP60 may regulate chromatin reprogramming to enhance HIF-1 transcriptional activity.

In addition, histone acetyltransferases directly acetylate HIF-α to modulate HIF transcriptional activity. p300 acetylates HIF-1α at K709 and reduces HIF-1α ubiquitination, leading to increased HIF-1α protein stability [32]. p300/CBP-associated factor (PCAF) also acetylates HIF-1α at K674 and increases transcription of the HIF target genes *BID*, *CA9* and *VEGFA* in human osteosarcoma cells [33, 34]. Jeong et al. showed that the acetyltransferase arrest-defective-1 (ARD1) induces acetylation of K532 of HIF-1α to increase HIF-1α ubiquitination and subsequent protein degradation by the PHD/VHL pathway, which diminishes HIF-1 transcriptional activity [35]. However, ARD1 fails to acetylate HIF-1α in vitro [36, 37]. It was recently suggested that prolyl hydroxylation and K391 methylation are prerequisite for ARD1-mediated K532 acetylation of HIF-1α [38].

Histone methyltransferases also play a critical role in HIF transcriptional activity. An inhibitor of the lysine methyltransferase G9a, BIX01294, increases prolyl hydroxylation of HIF-1α under hypoxic conditions, leading to increased HIF-1α ubiquitination and decreased HIF-1α protein stability in human hepatocellular carcinoma HepG2 cells [39]. Treatment of BIX01294 blocks expression of the HIF target gene *VEGFA*. Pharmacological or genetic inhibition of G9a prevents tumorigenesis in mice [40, 41]. G9a is induced by hypoxia at both transcriptional and post-translational levels in embryonic stem cells and cancer cells and may mediate hypoxia-induced gene repression through increasing dimethyl K9 of histone H3 in cancer cells [41–43]. However, the direct effect of G9a on HIF transcriptional activity remains unknown.

Regulation of HIF activity by the lysine methyltransferase SET7/9 has been the focus of recent studies, but its role is controversial. Liu et al. found that SET7/9 interacts with HIF-1α and blocks the ubiquitin E3 ligase CHIP-mediated degradation of HIF-1α protein in human cancer cells [44]. SET7/9 is also enriched at the hypoxia response elements of a subset of HIF-1 regulated genes *LDHA*, *HK2*, and *PDK1*, and increases transcription of these glycolytic genes in hypoxic cells [44]. In contrast, Xiao and his colleagues recently showed that SET7/9 directly induces monomethylation of HIF-1α at K32 and of HIF-2α at K29, leading to suppression of HIF transcriptional activity without affecting their protein levels [45]. Knockdown of SET7/9 increases HIF-1α binding to the hypoxia response element to promote HIF-1 transcriptional activity and expression of the glycolytic genes in human clear cell renal carcinoma RCC4 cells, thereby increasing glucose uptake and ATP production. SET7/9-mediated monomethylation of HIF-1α at K32 was later confirmed by Kim et al. in vitro and in human cervical carcinoma HeLa cells [46]. SET7/9 also catalyzes dimethylation of HIF-1α at K391 in HEK293T cells [38]. However, these two recent studies showed that SET7/9-mediated HIF-1α methylation decreases HIF-1α protein levels through increasing VHL-HIF-1α interaction and HIF-1α ubiquitination, thereby inhibiting HIF-1 transcriptional activity in transfected cells [38, 46]. Upregulated HIF-1α protein and the HIF-1 target genes *Epo*, *Vegfa*, and *Slc2a1* are observed in knockin mice bearing mutant $Hif1a^{K32A/K32A}$ [46]. These

**Table 1** The functions of epigenetic regulators in HIF activity

| Epigenetic regulators | Functions | Disease phenotypes | References |
|---|---|---|---|
| *Epigenetic writers* | | | |
| p300/CBP | Histone acetylation at the HIF target genes and enhanced gene transcription; | ND | [16] |
| | HIF-1α acetylation at K709 and protein stability | ND | [32] |
| TIP60 | Acetylation of histones H3K9 and H4 at the HIF target genes and enhanced gene transcription | ND | [30] |
| PCAF | HIF-1α acetylation at K674 and protein stability; | ND | [33, 34] |
| | Upregulation of the HIF target genes *BID*, *CA9*, and *VEGFA* | ND | [33] |
| ARD1 | HIF-1α acetylation at K532 and protein destability | ND | [35] |
| SET7/9 | HIF-1α methylation at K32 and K391, protein destability, and inhibition of the HIF-1 target genes *SLC2A1*, *PGK1*, *VEGFA*, *LDHA*, *PDK1*, and *EPO*; | K32 methylation-resistant HIF-1α knockin mice exhibit a haematologic abnormality, enhanced retinal angiogenesis, and increased lung tumor growth and angiogenesis | [38, 45, 46] |
| | HIF-2α methylation at K29 and inhibition of the HIF-2 target genes *PAI1*, *CITED2*, and *POU5F1*; | ND | [45] |
| | Blockade of CHIP-mediated HIF-1α protein degradation and upregulation of HIF-1 target genes *LDHA*, *HK2*, and *PDK1* | ND | [44] |
| PRMT1 | HIF-1α mRNA downregulation; | ND | [47] |
| | Inhibition of the HIF-1 target genes *PFKFB4*, *VEGFA*, *HIG2*, and *BNIP3* | ND | [47] |
| PRMT5 | Increased HIF-1α mRNA translation and *de novo* HIF-1α protein synthesis; | ND | [50] |
| | Upregulation of the HIF-1 target genes *VEGFA*, *LOX*, and *PDK1* | ND | [50] |
| PRMT9 | Decreased *de novo* synthesis of HIF-1α protein; | ND | [49] |
| | Inhibition of the HIF-1 target genes *CA9*, *PDK1*, and *BNIP3* | ND | [49] |
| DNMT3a | DNA methylation of the *EPAS1* gene and decreased HIF-2α levels | Overexpression of DNMT3a reduces renal tumor and glioblastoma growth in mice | [54] |
| *Epigenetic erasers* | | | |
| HDAC1 | HIF-1α protein stability; | ND | [59] |
| | HIF-1α deacetylation at K532 and K709 | ND | [32, 106] |
| HDAC2 | HIF-1α protein stability;<br>HIF-1α deacetylation at K532 | HDAC2 knockdown decreases oral squamous cell carcinoma growth in mice | [57]<br>[57] |
| HDAC3 | HIF-1α protein stability | ND | [59] |
| HDAC4 | HIF-1α protein stability; | ND | [58] |
| | HIF-1α deacetylation at K10/11/12/19/21; | ND | [58] |
| | Enhanced p300 recruitment to HIF-1α; | ND | [29] |
| | Decreased FIH-1 binding to HIF-1α; | ND | [29] |
| | Upregulation of the HIF-1 target genes *VEGFA*, *SLC2A1*, and *LDHA* | ND | [29, 58] |
| HDAC5 | Enhanced p300 recruitment to HIF-1α; | ND | [29] |
| | Decreased FIH-1 binding to HIF-1α; | ND | [29] |
| | Upregulation of the HIF-1 target gene *VEGFA* | ND | [29] |

**Table 1** (continued)

| Epigenetic regulators | Functions | Disease phenotypes | References |
|---|---|---|---|
| HDAC6 | HIF-1α protein stability | ND | [56] |
| HDAC7 | Interaction with HIF-1α, p300, and CBP; | ND | [60] |
| | Increased HIF-1 transcriptional activity and expression of VEGFA and GLUT1 | ND | [60] |
| SIRT1 | HIF-2α deacetylation at K385/685/741 and increased expression of HIF-2-dependent *EPO*; | ND | [65] |
| | HIF-1α deacetylation at K674, blockade of p300 recruitment, and inhibition of the HIF-1 target genes *PDK1*, *VEGFA*, and *CA9*; | Overexpression of SIRT1 reduces fibrosarcoma growth and angiogenesis | [34, 66] |
| | HIF-1α protein stability and upregulation of the HIF-1 target genes *SLC2A1*, *VEGFA*, *MMP2*, *CA9*, and *EPO*; | An SIRT1 inhibitor decreases hepatocellular carcinoma growth and angiogenesis in mice | [63, 64] |
| | HIF-1α protein downregulation in primary vascular smooth muscle cells and inhibition of VEGFA; | SIRT1 attenuates neointima formation, but increases blood flow in wire-injured femoral arteries of mice | [67] |
| | Histone H3K14 deacetylation at the *HIF1A* gene and downregulation of HIF-1α mRNA in MPP+-treated cells | ND | [68] |
| SIRT2 | HIF-1α deacetylation at K709 and protein degradation; | SIRT2 knockdown increases cervical tumor growth and angiogenesis in mice | [69] |
| | Inhibition of the HIF-1 target genes *SLC2A1*, *VEGFA*, and *LDHA* | | [69] |
| SIRT3 | Decreased ROS levels and HIF-1α protein stability; | SIRT3 knockdown increases colon cancer growth in mice | [72] |
| | Inhibition of the HIF-1 target genes *PGK1* and *PDK1* | | [72] |
| SIRT6 | Decreased *de novo* synthesis and stability of HIF-1α protein; | SIRT6 knockout enhances glucose uptake in mice | [71] |
| | Inhibition of the HIF-1 target genes *SLC2A1*, *PFK1*, *LDHA*, and *PDK4* | | [71] |
| SIRT7 | HIF-α protein destability; | ND | [70] |
| | Inhibition of the HIF target genes *EPO*, *SOD2*, and *VEGFA* | | [70] |
| JMJD1A | Demethylation of dimethyl K9 of histone H3 at the HIF-1 target genes; | JMJD1A knockdown reduces colon tumor growth in mice | [81] |
| | Upregulation of the HIF-1 target genes *SLC2A3*,*ADM*,and *GDF15* | | [81] |
| JMJD2A | Demethylation of trimethyl K9 of histone H3 on the *HIF-1A* gene, and HIF-1α mRNA induction; | ND | [91] |
| | Upregulation of the HIF-1 target genes *CA9*, *SLC2A3*, *TWIST1*, *ZEB2*, and *SNAI1* | ND | [91] |
| JMJD2C | Demethylation of trimethyl K9 of histone H3 at the HIF-1 target genes; | JMJD2C knockdown reduces breast tumor growth and metastasis to the lungs in mice | [78] |
| | Upregulation of the HIF-1 target genes *LDHA*, *PDK1*, *SLC2A1*, *LOXL2*, and *L1CAM* | | [78] |
| LSD1 | HIF-1α demethylation at K32 and K391, and increased HIF-1α protein stability and VEGFA expression; | LSD1 promotes breast tumor growth in mice | [38, 46] |
| | RACK1 demethylation at K271 and blockade of RACK1-mediated HIF-1α protein degradation | ND | [82] |
| TET1 | Interaction with HIF-1/2α and coactivation of HIF-mediated GLUT3, HK1, PGK1, PGK2, PKM, LDHA, N-Cadherin, and Vimentin | ND | [86] |

**Table 1** (continued)

| Epigenetic regulators | Functions | Disease phenotypes | References |
|---|---|---|---|
| *ATP-dependent chromatin remodelers* | | | |
| BRG1 | Nucleosome remodeling at the HIF target genes; | ND | [98] |
| | Increased RNA polymerase II recruitment to the HIF-1 target genes; | ND | [98] |
| | Upregulation of the HIF target genes *CA9*, *ANGPTL4*, *EPO*, *SLC2A1*, *PGK1*, *PAI1*, *PTPRB*, *ADM*, and *VEGFA*; | ND | [98] |
| | Increased transcription of *HIF1A* and *EPAS1* | ND | [98] |
| BRM | Nucleosome remodeling at the HIF target genes; | ND | [98] |
| | Increased RNA polymerase II recruitment to the HIF-1 target genes; | ND | [98] |
| | Upregulation of the HIF target genes *CA9* and *EPO* | ND | [98] |
| BAF57 | Increased HIF-1α mRNA expression | ND | [101] |
| ISWI | Increased FIH-1 expression; | ND | [102] |
| | Inhibition of the HIF-1 target genes *BNIP3*, *CA9*, and *EGLN1* | ND | [102] |
| Reptin | Decreased RNA polymerase II binding to the HIF-1 target genes and inhibition of the HIF-1 target genes *PGK1*, *VEGFA*, and *BNIP3*; | Reptin knockdown increases breast tumor growth in mice | [43] |
| | Enhanced HIF-1 transcriptional activity | ND | [30] |
| Pontin | Increased p300 recruitment; | ND | [105] |
| | Upregulation of the HIF-1 target genes *ETS1*, *KDM4B*, and *IGFBP3* | ND | [105] |
| MTA1 | HDAC1 recruitment to decrease HIF-1α acetylation at K532; | ND | [106] |
| | HIF-1α protein stability; | ND | [106] |
| | Upregulation of the HIF-1 target gene *VEGFA* | ND | [106, 107] |

*ND* not determined

mice display elevated erythrocytosis and angiogenesis in the retina as well as increased tumorigenesis [46], suggesting the important physiological and pathological functions of monomethyl K32 of HIF-1α. The discrepancies in regulation of HIF by SET7/9 in these studies remain unknown.

Recent studies showed that the protein arginine methyltransferase (PRMT) regulates HIF-α mRNA stability to alter HIF transcriptional activity. PRMT1 inhibits HIF-1α mRNA transcription through its methyltransferase activity in HeLa cells [47]. Knockdown of PRMT1 increases the transcriptional activity of Sp1 and Sp3, which are known to bind to the *HIF1A* promoter to control its mRNA transcription [48], leading to upregulation of HIF-1α mRNA transcription, thereby enhancing HIF-1 binding to the hypoxia response element and expression of the HIF target genes under hypoxia. Another member of the PRMT family PRMT9 (also named as FBXO11) impairs stability of HIF-1α mRNA to decrease de novo synthesis of HIF-1α protein and its transcriptional activity in human glioblastoma U87MG cells [49]. Interestingly, the ubiquitin E3 ligase activity of PRMT9 is responsible for HIF-1α mRNA destabilization. In contrast, PRMT5 promotes cap-dependent translation of HIF-1α mRNA, leading to increased de novo synthesis of HIF-1α protein in A549 cells [50].

Apart from PRMTs, DNA methyltransferase is also involved in the regulation of HIF-α mRNA transcription. The CpG within the hypoxia response element at the *HIF1A* promoter is highly methylated in human colon tissues, but is hypomethylated in human embryonic tissues and colorectal cancer tissues [51, 52]. Treatment of the DNA methylation inhibitor 5-Aza-2′-deoxycytidine blocks CpG methylation at the *HIF1A* promoter, leading to increased autoregulation by HIF-1 and subsequent its downstream target gene expression [52]. The transcription factor Kaiso binds methylated *HIF1A* promoter and suppresses HIF-1α mRNA expression under hypoxia to impair HIF-1 transcriptional activity [53]. Recently, it was shown that DNMT3a methylates the CpG at the promoter of the *EPAS1* gene and decreases expression of HIF-2α in normal human epithelial cells. DNMT3a is frequently downregulated in primary tumors, which elevates HIF-2α levels to promote tumorigenesis [54]. Methylation of the *HIF3A* gene was found in adipose tissues and correlated with obesity [55], although its DNA methyltransferase has not yet been identified.

## Regulation of HIF transcriptional activity by epigenetic erasers

The deacetylases and demethylases, acting as epigenetic erasers, remove the acetyl and methyl groups from the chromatin, respectively, and are involved in chromatin remodeling and gene regulation. The role of epigenetic erasers in HIF transcriptional activity has been extensively studied. Several HDACs, including HDAC1, HDAC2, HDAC3, HDAC4, and HDAC6, have been shown to increase HIF-1α protein stability to promote HIF-1 transactivation [56–59]. Consistently, HDAC inhibitors (HDACi), including trichostatin A, apicidin, valproic acid, FK228, sodium butyrate, and LAQ824, dose-dependently decrease HIF-1α protein levels in various cell lines [56, 59]. Ubiquitintation of HIF-1α is elevated upon treatment of HDACi or knockdown of HDAC, leading to HIF-1α protein degradation in the 26*S* proteasome and inhibition of HIF-1 transcriptional activity. The PHD/VHL pathway is dispensable for HDACi-mediated HIF-1α protein degradation [56]. Interestingly, deacetylation of HIF-1α by HDAC2 or HDAC4 was suggested to increase HIF-1α protein stability and transcriptional activity [57, 58].

Additional mechanisms underlying HDAC-mediated HIF-1 transcriptional activity have been also proposed. HDAC4 and HDAC5 bind to the inhibitory domain of HIF-1α to compete with FIH-1 for binding to HIF-1α, leading to increased recruitment of p300 to the transactivation domain of HIF-1α [29]. HDAC4 or HDAC5 promotes expression of the HIF-1 target gene *VEGFA* without affecting HIF-1α protein stability. HDAC7 was also shown to physically interact with HIF-1α, p300, and CBP, and enhances HIF-1 transcriptional activity [60].

Sirtuin (SIRT, also known as Sir2 in yeast) was initially characterized as the class III $NAD^+$-dependent HDAC in yeast [61]. This protein is highly conserved from *archaea* to humans. Mammalian SIRT consists of seven family members and their activity is highly regulated [62]. Several environmental stresses including hypoxia reduces intracellular $NAD^+$ levels in mammalian cells, which results in decreased deacetylase activity of SIRT [34]. SIRT1 is the most studied SIRT family member in gene regulation, but its effect on HIF-1 transcriptional activity is still under debate [34, 63–66]. Dioum et al. reported that SIRT1 selectively interacts with HIF-2α and deacetylates HIF-2α to increase its transcriptional activity in human hepatoma Hep3B cells [65]. SIRT1 knockout reduces HIF-2-dependent *Epo* gene expression in vitro and in mice. Distinct to its effect on HIF-2, SIRT1 does not associate with HIF-1α nor affect HIF-1 transcriptional activity [65]. However, the Park group found that SIRT1 is able to bind to HIF-1α and reverses PCAF-induced acetylation of K674 of HIF-1α, leading to blockade of p300 recruitment to the transactivation domain of HIF-1α and subsequent inhibition of HIF-1 transcriptional activity in multiple cancer cell lines [34, 66]. HIF-2 activation by SIRT1 was shown to be cell type-dependent [66]. Forced expression of SIRT1 decreases hypoxia-induced expression of PDK1, VEGFA, and CA9, and impairs tumor growth in xenograft mice [34]. Subsequent studies indicate that SIRT1 indirectly acts on HIF-1 transcriptional activity through HIF-1α protein stability [63, 64]. Genetic or

pharmacological inhibition of SIRT1 increases the acetylation of HIF-1α to decrease stabilization of HIF-1α protein and HIF-1-dependent gene expression in human cancer cell lines. In contrast, SIRT1 inhibits HIF-1α expression in primary vascular smooth muscle cells under hypoxia as well as in the femoral artery of SIRT1 transgenic mice after wire injury [67], suggesting differential regulation of HIF-1 by SIRT1 in the disease context. Finally, SIRT1 was recently shown to deacetylate K14 of histone H3 at the promoter of *HIF1A* gene to attenuate HIF-1α mRNA expression in SH-SY5Y cells treated with methyl-4-phenylpyridinium, a toxic chemical causing symptoms of Parkinson's disease [68].

SIRT2 interacts with and deacetylates HIF-1α at K709 to facilitate PHD2-HIF-1α interaction in HeLa cells, which increases HIF-1α ubiquitination and protein degradation, thereby inhibiting HIF-1 transcriptional activity [69]. Similarly, SIRT7 also destabilizes HIF-1α and HIF-2α proteins and blocks their transcriptional activity [70]. This effect is independent of its deacetylase activity. SIRT6 negatively regulates the de novo protein synthesis and stability of HIF-1α in embryonic stem cells and blocks HIF-1-dependent glucose uptake and glycolysis [71]. Finally, SIRT3 knockdown increases HIF-1α protein levels in human cancer cells and tumorigenesis in mice [72]. SIRT3 is mainly localized in mitochondria and loss of SIRT3 increases reactive oxygen species (ROS) in mouse embryonic fibroblasts. ROS is known to increase HIF-1α protein stability [73]. Elimination of ROS by *N*-acetyl-cysteine abolishes increased HIF-1 transcriptional activity in SIRT3 knockdown cells [72], suggesting that SIRT3 is involved in indirect regulation of HIF-1 activity.

The Jumonji domain (JMJD) containing protein family consists of 32 members in humans and possesses lysine demethylase activity with histone H3 and non-histone proteins as substrates [74–77]. Iron, α-ketoglutarate and $O_2$ are required for their enzymatic activity like other α-ketoglutarate-dependent dioxygenases [74]. Our previous work showed that JMJD2C selectively coactivates HIF-1 in breast cancer cells [78]. JMJD2C demethylates trimethyl K9 of histone H3 at the hypoxia response element to enhance HIF-1 binding to its target genes and subsequent transcription of *LDHA*, *PDK1*, *SLC2A1*, *LOXL2*, and *L1CAM*. Knockdown of JMJD2C reduces breast tumor progression and metastasis in mice. JMJD2C expression is controlled by HIF-1 and HIF-2 [79], and thus JMJD2C represents a positive feedback mechanism amplifying HIF-1 activation in breast cancer. Similarly, JMJD1A (also known as KDM3A) interacts with HIF-1α and demethylates dimethyl K9 of histone H3 at the hypoxia response element of a subset of HIF-1 target genes to enhance their gene transcription, thereby increasing colorectal tumor growth in mice [80, 81]. JMJD1A also activates HIF-1-dependent *SLC2A3* gene transcription to enhance glucose uptake in HUVEC cells [80]. Therefore, the histone demethylases JMJD proteins control HIF transcriptional activity through altering histone methylation at the HIF target genes.

LSD1 is a flavin adenine dinucleotide (FAD)-dependent lysine demethylase. Unlike JMJD proteins, LSD1 indirectly promotes HIF-1 transcriptional activity through altering HIF-1α protein degradation machinery [38, 46, 82]. LSD1 counteracts SET7/9-induced methylation of HIF-1α at K32 and K391 and also inhibit PHD2-mediated prolyl hydroxylation of HIF-1α to decrease HIF-1α ubiquitination and protein degradation [38, 46]. In addition, LSD1 interacts with and demethylates RACK1, which attenuates RACK1 binding to HIF-1α [82]. RACK1 competes with Hsp90 to mediate $O_2$-independent but proteasome-dependent HIF-1α protein degradation [83]. Consequently, inhibition of LSD1 by its inhibitors or small interfering RNAs enhances RACK1-mediated HIF-1α protein degradation [82]. LSD1 potentiates transcription of HIF-1-dependent VEGFA and glycolytic genes in human cancer cells, leading to increased tumor angiogenesis [38, 82]. Interestingly, the FAD levels are reduced during prolonged hypoxia, which diminishes the demethylase activity of LSD1 [82]. Thus, LSD1 represents a molecular mechanism of reduced HIF-1 transcriptional activity during prolonged hypoxia.

The DNA demethylase TET is another subgroup of α-ketoglutarate-dependent dioxygenases and consists of three family members (TET1-3) [84]. TET hydroxylates 5-methylcytosine to generate 5-hydroxymethylcytosine, 5-formylcytosine, and 5-carboxycytosine in the consecutive biochemical reactions [85]. It has been reported that TET1 functions as a HIF coactivator and controls HIF-1-dependent epithelial-mesenchymal transition in breast cancer cells [86]. The protein interaction of TET1 with HIF-1α and HIF-2α, but not its DNA demethylase activity, is critical for HIF activation. Like JMJD protein, TET1 is induced by HIF-1 and HIF-2 in breast cancer cells and provides a positive feedback regulation of HIF in breast cancer [86].

On the other hand, hypoxia causes reprogramming of the chromatin landscape by altering expression and activity of epigenetic erasers. Hypoxia induces expression of HDAC3 and decreases the global levels of acetyl K4 of histone H3 in breast cancer cells [87]. HDAC3 further recruits the methyltransferase WDR5, which increases dimethyl and trimethyl K4 of histone H3 in breast cancer cells under hypoxia [87]. Changes of these histone marks are associated with altered expression of E-cadherin, plakoglobin, N-cadherin, and Vimentin that are involved in epithelial-mesenchymal transition [87]. In addition, the demethylase activity of JMJD proteins such as JMJD3 and JARID1A/B, is impaired under hypoxic conditions, due to limited availability of their substrate $O_2$ [88], which contributes to increased trimethyl K27 and K4 of histone H3, respectively [88, 89]. The global increases of dimethyl and trimethyl K9 of histone H3,

trimethyl K36 of histone H3, dimethyl K79 of histone H3, acetyl K14 of histone H3, and dimethyl arginine 3 of histone H4 are also found under hypoxic conditions [87, 90]. In contrast, hypoxia decreases the global levels of acetyl K5 of histone H4, acetyl K12 of histone H4, acetyl K5 of histone H2A in breast cancer cells [87]. Interestingly, knockdown of JMJD2A increases levels of trimethyl K9 of histone H3 on the *HIF-1A* gene in RKO cells under hypoxia, leading to inhibition of HIF-1α mRNA transcription and subsequent HIF-1 activity [91].

## Regulation of HIF transcriptional activity by epigenetic readers

The role of the epigenetic reader in HIF transcriptional activity is less studied. Emerging studies have elucidated an essential role of bromodomain and extra-terminal (BET) proteins, particularly BRD4, in gene regulation [92, 93]. The epigenetic reader BRD4 recognizes acetylated lysine of histones and recruits the transcription factors and epigenetic regulators to control gene transcription [94]. A recent study found that a BET inhibitor JQ1 downregulates hypoxia-induced global transcriptome by blocking HIF binding to the hypoxia response element in breast cancer cells [95]. Decreased expression of the HIF target genes *CA9* and *VEGFA* is also observed in JQ1-treated breast tumors in mice, leading to inhibition of angiogenesis and tumor growth [95]. Although hypoxia increases BRD4 recruitment to the hypoxia response element of *CA9* and *VEGFA* [95], the direct effect of BRD4 on HIF transcriptional activity is still unclear.

## Regulation of HIF transcriptional activity by ATP-dependent chromatin remodelers

The ATP-dependent chromatin remodelers modulate chromatin structure and assembly and have an important role in gene regulation. Four chromatin remodeler complex families, including SWI/SNF complex, ISWI complex, INO80 complex, and NuRD complex, have been well characterized [96]. Transient ischemia causes chromatin condensation in accompany with decreased cellular ATP levels, which is reversible upon reoxygenation [97]. Although little is known about the role of chromatin remodelers in ischemia-induced chromatin condensation, several ATP-dependent chromatin remodelers have been shown to regulate HIF transcriptional activity. The key components of the SWI/SNF complex, BRG1 and BRM, promote expression of a subset of HIF target genes in human cancer cell lines in an ATPase-dependent manner [98]. Upon hypoxia, BRG1 is recruited by HIF to the hypoxia response elements of *CA9* and *EPO* and induces nucleosome remodeling at these gene promoters [98, 99]. BRG1 and BRM also enhance the recruitment of RNA polymerase II to the *EPO* gene in hypoxic Hep3B cells [100]. On the other hand, knockdown of BRG1 decreases transcription of *HIF1A* and *EPAS1* genes in hypoxic Hep3B cells [98, 101]. The other component of the SWI/SNF complex, BAF57, is also required for HIF-1α mRNA expression [101]. Thus, the SWI/SNF complex regulates HIF transcriptional activity through controlling HIF-α mRNA levels and HIF transactivation machinery.

Unlike SWI/SNF complex, ISWI negatively modulates HIF-1 transcriptional activity through the FIH-1-dependent pathway [102]. Knockdown of ISWI decreases RNA polymerase II binding to the promoter of the *HIF1AN* gene (encoding FIH-1) in hypoxic U2OS cells, which may lead to decreased FIH-1 expression. ISWI selectively inhibits expression of HIF-1 target genes *BNIP3*, *CA9*, and *EGLN1*, but not *BHLHE40*, *EGLN3*, and *SLC2A1* [102]. It was shown that FIH-1 preferentially downregulates expression of the HIF-1 target genes *CA9*, *EGLN3*, *SLC2A1*, but not *BNIP3* [103], suggesting that FIH-1 may be not the only mechanism underlying ISWI-mediated inhibition of HIF-1.

Reptin and Pontin belong to AAA+ ATPases with a similar conserved structure and are present in multiple protein complexes, including the INO80 complex [104]. The data from the Baek group indicate that Reptin and Pontin both are monomethylated by G9a in a hypoxia-dependent manner, and methylated Reptin and Pontin exhibit the opposite functions of HIF transcriptional activity in human breast cancer cells [43, 105]. Reptin is enriched at the hypoxia response elements of a subset of HIF-1 target genes and recruits HDAC1 to decrease RNA polymerase II occupancy at the promoter of these genes, thereby blocking HIF-1-dependent gene transcription and breast tumorigenesis [43]. In contrast, Pontin enhances the recruitment of p300 to the HIF-1 target genes to promote HIF-1 transcriptional activity and breast cancer cell proliferation and mobility in vitro [105]. Pontin's coactivator function may be independent of the INO80 complex as its ATPase dead mutant still enhances HIF-1-mediated transactivation [105]. This scenario has been also suggested in a recent study, showing that Reptin and Pontin are present in the TIP60 complex to coactivate HIF-1 and knockdown of INO80 has no significant effect on HIF-1 transcriptional activity in Drosophila S2 cells [30].

Metastasis-associated protein 1 (MTA1) is a component of the NuRD complex and mediates loading of the NuRD complex on the genome through interacting with transcription factors. MTA1 was shown to bind to the C-terminus of HIF-1α and recruit HDAC1 to decrease K532 acetylation of HIF-1α [106]. MTA1 counteracts ARD1′s effect to increase HIF-1α protein stability [106]. Overexpression of MTA1 potentiates HIF-1 transcriptional activity and VEGFA expression in human cancer cells, leading to

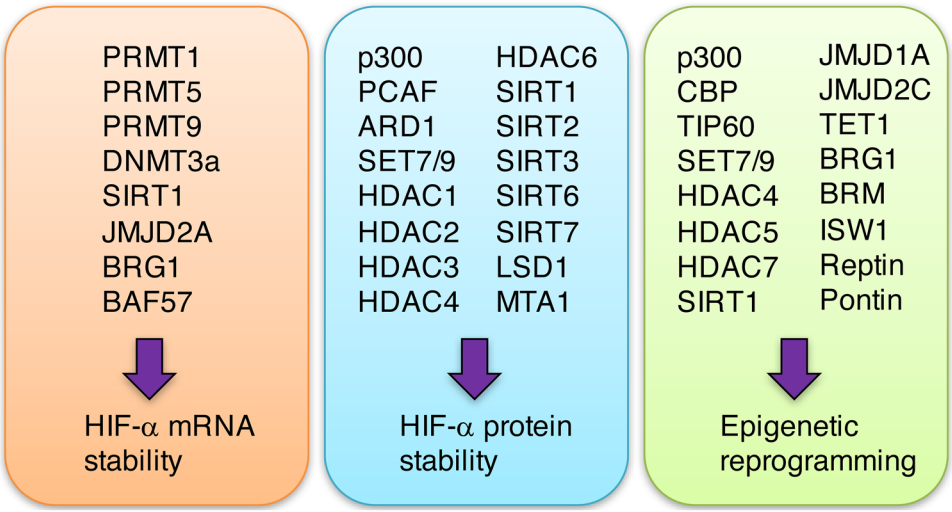


**Fig. 3** Mechanisms of HIF transcriptional activity by epigenetic regulators. Epigenetic regulators modulate HIF transcriptional activity through controlling HIF-α mRNA or protein stability or chromatin reprogramming. A list of epigenetic regulators within three subgroups is shown

increased angiogenesis [106, 107]. MTA1 is upregulated by HIF-1 in human breast cancer cell lines under hypoxia [106]. The LSD1-c-Myc axis also activates MTA1 mRNA transcription [38]. LSD1 additionally increases MTA1 protein stability by blocking ubiquitin-dependent proteasomal degradation of MTA1 [38].

## Concluding remarks

HIF governs the transcriptional program of hypoxic responses and mediates physiology and pathogenesis of many human diseases, particularly cancers. Accumulating studies have shown that epigenetic regulators play a key role in HIF-mediated transactivation (Table 1). Multiple epigenetic regulators cooperate to modulate HIF transcriptional activity through controlling chromatin reprogramming or HIF-α mRNA or protein levels (Fig. 3). Importantly, epigenetic regulators determine the specificity of the transcriptional activity of HIF-1 and HIF-2 and their downstream target genes. Therefore, understanding of epigenetic regulation of HIF activity may uncover the fundamental mechanisms of human physiology and disease progression and provide new targets and approaches for treatment of human diseases.

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**Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no conflict of interest.

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