Introduction

Transcriptional responses to hypoxia are mainly mediated by hypoxia-inducible factor (HIF), which is a heterodimer consisting of an α subunit (HIF-α) and a β subunit (HIF-β) (1). HIF-α induction is regulated by an oxygen-dependent mechanism, and three HIF-α subunits (HIF-1α, HIF-2α, HIF-3α) have been identified (2). HIF-1α and HIF-2α make a complex with HIF-1β, forming HIF-1 and HIF-2, respectively. HIF-1β is constitutively expressed and also referred to as aryl hydrocarbon receptor nuclear translocator subunits (ARNT). The role of HIF-3α is not clear, but its regulation has been reported to be oxygen-dependent as an HIF-1 target gene and it may modulate hypoxic gene induction (3). Structurally, HIF-3α lacks transcriptional activation domains, and several splice variants of HIF-3α such as mouse inhibitory PAS domain protein (IPAS) and human HIF-3α4 may have an inhibitory effect against other HIFs (4, 5).

In normoxia, HIFα is post-translationally degraded by prolyl hydroxylases (PHDs) (6). The proline residues are hydroxylated by PHDs, which results in binding of von-Hippel-Lindau (VHL) protein. VHL protein targets HIF for polyubiquitination and proteasomal degradation through its role in substrate recognition as part of an E3 ubiquitin ligase complex. In addition to the hydroxylation of the proline residues by PHDs, one asparagine residue in the C-terminal transactivation domain is also hydroxylated by an asparaginyl hydroxylase (FIH1: factor inhibiting HIF-1). Oxygen promotes hydroxylation by FIH-1, which inhibits recruitment of coactivators CBP/p300 and transactivation. In hypoxia, the α-subunit escapes degradation and forms a heterodimer with the β-subunit, which translocates into the nucleus. HIF regulates hundreds of...
hypoxia responsive genes, which are involved in a wide variety of biological functions such as erythropoiesis, angiogenesis, metabolism, defenses against oxidative stress, and survival or death.

HIF-1α is widely expressed in all organs of the body, while HIF-2α is limited to several organs such as vascular endothelium and hepatocytes. Both isoforms have two transactivation domains (N-TAD, N-terminal transactivation domain; C-TAD, C-terminal transactivation domain), which recognize the core sequence -RCGTG- (HRE, hypoxia responsive element) (7, 8).

In addition to direct regulation of gene expression at the transcription level, hypoxia can cause chromatin alteration, such as global deacetylation, histone modification, and DNA methylation. Chromatin conformational change may be reported to modulate target gene expressions (9, 10, 11, 12). Although there have been few reports about the role of HIF-2 in epigenetic regulation, HIF-1 has emerged as a key regulator under hypoxia. In this review, we would like to clarify the chromatin conformational change in the hypoxic condition and the role of HIF-1 as an epigenetic regulator.

2. Histone modification by methyltransferase and demethylases

In 1842, the name epigenetics was first used to describe how genes might interact with their surroundings to produce a phenotype without changing the DNA sequence that influences the development of an organism. In eukaryotes, epigenetic modifications have been identified as DNA methylation and post-translational modifications of histones such as acetylation, methylation, phosphorylation, and ubiquitination. These modifications can affect chromatin environment and gene expression so that an identical gene can be interpreted differently in a temporal and spatial-dependent manner (13 – 16). Various kinds of histone modifiers have been discovered as shown in Fig. 1. Among these modifications, histone methylation and demethylation have been linked to regulatory marks that delineate transcriptionally active and inactive chromatin. These histone methyl marks are reversible and dynamically regulated by site-specific methyltransferases and demethylases. Methylation has been considered to occur on the side chains of lysine (K) and arginine (R) residues. Each residue is mono- (me1), di- (me2), tri-methylated (me3), or unmodified. Most of the histone modifiers such as methyltransferases show significant substrate specificity in epigenetic regulation. While expression of various genes is regulated by histone methylation, the activity of histone methyltransferases and demethylases are also influenced by gene expression, recruitment, coordination with other epigenetic modifiers, and post-translational modifiers.

3. Hypoxia alters chromatin global conformations

The histone tail is modified by acetylase, methylase, ubiquitylase, and demethylase. Many of histone demethylases are up-regulated by hypoxia as shown above. Global histone modification under hypoxia has been reported to be altered under hypoxia. Dimethyl or trimethyl histone H3 lysine 9 (H3K9) increases under hypoxia in HepG2, A549, and Hepa1-6 (17). We confirmed that H3K4me3 levels increased in HUVEC (human endothelial venous endothelial cells) (unpublished data). These findings suggest that chromatin modulation of histone methylation may play a role to adapt to hypoxia via an epigenetic mechanism.

4. Reversible modification of histones

4.1. Histone demethylase or chromatin conformation under hypoxia

Histone demethylase is mainly consisted of two types of enzymes like LSD1 and Jumonji C (JmjC) domain-containing proteins (18). LSD1 is a FAD (flavin adenine dinucleotide)-dependent enzyme, which specifically demethylates histone H3 lysine 4 (K4) and histone H3 lysine9 (K9) (19), and functions as a transcriptional co-repressor (20). On the other hand, JmjC domain-containing histone demethylases are dioxygenases and their activities require Fe (II), ketoglutarate, and oxygen. There are about 30 JmjC domain-containing proteins in humans (9). Among them, the JmjC domain-containing histone demethylases JMJD1A (KDM3A), JMJD2B (KDM4B), and JMJD2C (KDM4C) are known to be induced by hypoxia in a HIF1α-dependent manner (Fig. 2). Wellman et al. found that mRNA level of JMJD1A increased in different organs of rats exposed to hypoxia in vivo (21). They also demonstrated that HIF-1 binds to the specific HRE in the promoter region of JMJD1A under hypoxia in HEK293 (human embryonic kidney cells) and HMEC-1 (human microvascular endothelial cells) and up-regulates the expression of JMJD1A in vitro. In addition to JMJD1A, JMJD2B and JMJD2C were also induced in several cancer cell lines (22, 23). Other JmjC domain-containing proteins may also be up-regulated by hypoxia or HIF. Recent studies using the chromatin immunoprecipitation sequence (ChIP-Seq) approach showed that over half of JmjC family genes were up-regulated by hypoxia (24). Four of them (JMJD1A, JMJD2B, JMJD2C, PLU-1) were reported to be direct HIF-1 targets. Figure 2 shows the correlation between histone modifiers and the HIF pathway under hypoxia.
4.2. JMJD1A (KDM3A)

JMJD1A was cloned as a histone demethylase in 2006 (25). It is a specific demethylase of H3K9me1/2 in vitro. It is expressed in only a few organs including testis and pancreas, and it was originally identified as a male germ–specific transcript (26). Analysis of JMJD1A-knockout mice revealed that JMJD1A is essential for spermatogenesis and positively regulates gene expression of Tnp1 (Transition nuclear protein1) and Prm1 (Protamine 1) via demethylation of H3K9 in the position of these gene promoters (27).

Later studies demonstrated more ubiquitous expression of JMJD1A than originally proposed. Another analysis of a JMDJ1A-knockout mouse has shown that JMJD1A is profoundly associated with regulating metabolic gene expression and normal weight control (28, 29). They confirmed that loss of JMJD1A inhibited β-adrenergic–stimulated glycerol release and oxygen consumption in brown adipose tissue, resulting in obesity and hyperlipidemia in knockout mice. JMJD1A induced by adrenergic stimulation directly regulates expression of PPARα and Ucp1 by demethylating H3K9me2 on the position of the promoter region for PPRE (PPAR responsive element) of PPARα and Ucp1 genes. In addition, JMJD1A plays a role not only as a histone demethylase but also has a transcriptional co-activator function, facilitating the recruitment of PPAR, RXRα, Pgc1α, CBP/p300, and SRC1.

JMJD1A protein has been reported to have an LXXL motif, which is a signature of protein–protein interaction with nuclear receptors (25). JMJD1A interacted with the androgen receptor (AR) in a ligand-dependent manner,
which indicated that JMJD1A acts as a co-activator of AR-mediated transcription. Inhibition of JMJD1A expression in the prostate cancer cell line led to an increase of H3K9me2 in the promoter regions of AR target genes. Thus, JMJD1A has potential roles as a histone demethylase, transcriptional co-activator, and ligand-mediated transcriptional modulator. Molecular mechanisms by which JMJD1A regulates gene transcription under hypoxia require further studies to find their biological functions.

4.3. JMJD2B (KDM4B)

HIF-1 regulates hundreds of genes involved in many biological processes including cancer survival and tumor angiogenesis. Cancer therapy targeting HIF-1 and the hypoxia signaling pathway is a focus of intensive research (30, 31), and histone demethylases can be therapeutic targets of cancer therapy. For example, Yang et al. reported that one of the histone demethylases, JMJD2B, which is up-regulated under hypoxia, drives breast cancer cell proliferation in normoxia and hypoxia and that its inhibition can be a therapeutic strategy in breast cancer patients (32). The past reports have shown that breast cancer is known to be estrogen receptor α (ERα)-positive and that up-regulation of HIF-1α in ERα-positive cancers might promote cancer progression (33). In fact, HIF-1α has positive correlation with an aggressive phenotype of breast cancer with large tumor size, high grade, high proliferation, and lymph node metastasis (33). Yang et al. demonstrated that JMJD2B is regulated by both ERα and HIF-1α and that JMJD2B regulates the expression of cell cycle genes such as CCND1, CCNA1, and WEE1. Those findings provide evidence that JMJD2B is critical for breast cancer cell survival through regulation of cell cycle progression and that JMJD2B is an adverse prognostic factor in hypoxic breast cancers.

4.4. Ezh2 (enhancer of zeste homolog 2)

Another group has recently reported that one of the methyltransferases, Ezh2, a member of the polycomb group (PcG) protein family and suppresses gene expression by methylating its target, is a therapeutic target for prostate cancer (34). Ezh2 catalyzes histone H3 lysine 27 (H3K27) trimethylation, which is a hallmark of gene silencing (35). They demonstrated that microRNA-101 (miR-101) inhibits Ezh2 expression and differentially regulates prostate cancer cells. They also indicated that the expression of miR-101 alters upon androgen treatments and HIF-1α / HIF-1β induction and that miR-101 targets Ezh2 and decreases the invasiveness of prostate cancer cells.

5. Other histone modifiers and hypoxia

Systematic sequencing of clinical samples of clear renal cell carcinoma (ccRCC) has shown the inactivating mutations in two histone modifiers (36). SETD2 is a histone H3 lysine 36 (H3K36) methyltransferase, and JARID1C (KDM5C) is a histone H3 lysine 4 (H3K4) demethylase. They already found that inactivation of UTX (KDM6A), a histone H3 lysine 27 (H3K27) demethylase, resulted in slowing of cancer proliferation (37). They examined 101 cases of ccRCC and identified that 3% of all cases had somatic truncating mutations in SETD2 and JARID1C, while non-clear cell cancers had no mutations in either SETD2 or JARID1C. Additionally, hypoxia related genes in this study are up-regulated in the clinical samples of RCC. SETD2 and JARID1C mutant tumors are all clustered in the hypoxic group. These results suggest that the histone modifiers play important roles in components of the chromatin modification machinery in hypoxic conditions such as human cancer.

6. The role of HIF-1α in epigenetic regulation via histone modifiers

6.1. SIRT1

Under hypoxia, various factors try to keep biological homeostasis. In addition to the HIF-1 target genes modulated by chromatin modifiers, HIF-1α itself is known to be deacetylated at lysine 674 by Sirtuin 1 (Sirt1), which is one of the deacetylases and reported to be redox-sensitive (38). Lim et al. showed that Sirt1 inactivated HIF-1α by blocking p300 recruitment and repressed HIF-1 target genes. They also demonstrated the Sirt1 – HIF-1α interaction in hypoxic mouse tissues and that Sirt1 has negative effects on tumor growth and angiogenesis. HIF-2α is also deacetylated by Sirt1 under hypoxia in the C-terminal of three lysine residues (39). Dioum et al. indicated that Sirt1 activity affected expression of the HIF-2α target gene, EPO (erythropoietin), both in vitro and vivo. They confirmed that Sirt1 was recruited to the enhancer of EPO under hypoxia by using ChIP. They showed that knockdown of Sirt1 by shRNA leads to the decrease of HIF-2α, while overexpression of Sirt1 increased HIF-2α. They concluded that Sirt1 promotes HIF-2 signaling during hypoxia.

6.2. SIRT6

SIRT6 is one of the highly conserved family members of NAD+ dependent deacetylases. Zhong et al. found that knockout mice of SIRT6 die due to lethal hypoglycemia early in life (40). They demonstrated that SIRT6 functioned as a histone H3 lysine 9 (H3K9) deacetylase to
control the expression of multiple glycolytic genes and that SIRT6 behaved as a corepressor of HIF-1. In vitro, SIRT6-deficient cells exhibit increased HIF-1α activity, up-regulated glucose uptake and glycolysis, and decreased mitochondrial respiration, while knockdown of HIF-1α rescues the glucose uptake in SIRT6-deficient ES cells. They demonstrated that HIF-1 recruits SIRT6 to the glycolytic promoters by performing ChIP and that SIRT6 deacetylates histones of these genes, inhibiting the gene expressions under normoxia. Under the hypoxic condition, the promoter regions of the HIF-1 target genes are acetylated because SIRT6 is inactivated. HIF-1 recruits its binding complex, p300, which leads to the transcriptional activity to maintain homeostasis.

6.3. Reptin
Another group has demonstrated that Reptin, a chromatin-remodeling factor, is methylated at a lysine residue (K67) in its N-terminal under the hypoxic condition by the methyltransferase G9a (41). They showed that methylated Reptin binds to the promoter regions of a subset of HIF-1α target genes such as VEGF (vascular endothelial growth factor) and BNIP3 (BCL2/adenovirus E1B 19kDa interacting protein 3), which leads to decreased expression of HIF-1 target genes. They identified the Reptin-dependent and Reptin-independent target gene subsets by using microarray analysis when Reptin is knocked down by shRNA. They clarified that G9a-mediated methylation of Reptin under hypoxic condition inhibits HIF-1 transcriptional activity.

7. Perspectives and discussion for cancer therapeutics, hypoxia, and histone modifiers
These findings have shown a possibility that a series of hypoxia-responsive genes may be regulated by chromatin modulation and that HIF-1α may contribute to the modulation not only by binding to the TSS (transcriptional starting sites) but also by composing the chromatin conformational complex, including deacetylases and methylases. These hypotheses need further studies and may provide evidence for the new mechanism of transcriptional gene regulation.

8. Summary
Hypoxic response to adapt to the environment is regulated both by transcriptional gene activation and epigenetic regulation through modifying the chromatin conformational change. HIF-1 plays an important role to regulate the gene expression in both mechanisms. Further investigations should be able to clarify whether the new epigenetic regulation can lead to finding therapeutic targets for cancer therapy.

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