

Role of Hypoxia-Inducible Factors in Epigenetic Regulation via Histone Demethylases

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Eukaryotic chromatin is subject to multiple posttranslational histone modifications such as acetylation, methylation, phosphorylation, and ubiquitination. These various covalent modifications have been proposed to constitute a “histone code,” playing important roles in the establishment of global chromatin environments, transcription, DNA repair, and DNA replication. Among these modifications, histone methylation specifies regulatory marks that delineate transcriptionally active and inactive chromatin. These histone methyl marks were considered irreversible; however, recent identification of site-specific histone demethylases demonstrates that histone methylation is dynamically regulated, which may allow cells to rapidly change chromatin conformation to adapt to environmental stresses or intrinsic stimuli. Of major interest is the observation that these histone demethylase enzymes, which are in the Jumonji gene family, require oxygen to function and, in some cases, are induced by hypoxia in an HIF α -dependent manner. This provides a new mechanism for regulation of the response to hypoxia.

Key words: hypoxia; HIF α ; histone demethylase; epigenetics

Introduction

Physiological or pathological hypoxia is well-recognized to initiate a gene transcription program of adaptation via hypoxia-inducible factor (HIF), the master regulator of oxygen homeostasis. HIF is a heterodimer consisting of alpha and beta subunits, whose activity is dependent on the availability of HIF α . Three HIF α subunits, including HIF-1 α , HIF-2 α , and

HIF-3 α , have been identified.¹ The role of HIF-3 α is not clear, but it can function as an inhibitor of transcription since it lacks the transcriptional activation domain.² HIF-1 α or HIF-2 α complexes with HIF-1 β (ARNT), forming the competent transcription factor HIF-1 and HIF-2, respectively. HIF α induction is mainly regulated by an oxygen-dependent mechanism.³ In normoxia, HIF α is posttranslationally modified by prolyl hydroxylases (PHDs).⁴ The proline hydroxylation of HIF α results in binding of von Hippel–Lindau (VHL) protein, a component of an E3 ubiquitin ligase complex that targets HIF α for ubiquitination and proteasomal degradation.⁴ In addition to the prolyl hydroxylation by PHDs, one asparagine

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residue in the C-terminal transactivation domain is also hydroxylated by an asparaginyl hydroxylase, termed “factor inhibiting HIF-1 (FIH1).”⁴ Hydroxylation by FIH1 blocks the binding of transcriptional cofactors, such as p300/CBP, to the transactivation domain, thereby suppressing the transcriptional activity of HIF in normoxia. The PHDs and FIH1 are Fe(II)- and 2-oxoglutarate-dependent dioxygenases and their activities require the presence of molecular oxygen.⁴ Therefore, hydroxylation of HIF α by PHDs and FIH1 is inhibited in hypoxia, and HIF α is thus stabilized and activated. The stabilized HIF α undergoes nuclear translocation, dimerizes with HIF-1 β , and associates with transcriptional co-activators, such as p300/CBP and SRC1, to activate the gene transcription. Despite their structural similarity, HIF-1 α and HIF-2 α play different roles in embryogenesis and exert differential functions in cells.¹

Recent studies suggest that hypoxia can cause chromatin alterations, such as global deacetylation, and increase in H3K9me3/me2.⁵ Increased H3K4me3 levels and decreased H3K27me3 levels were also observed at promoters of hypoxia-regulated genes.⁵ These findings suggest that, besides HIF-mediated gene transcription, modulation of histone methylation via an epigenetic mechanism is another device that cells use to adapt to hypoxia. Interestingly, the Jumonji C (JmjC) domain-containing histone demethylases JMJD1A and JMJD2B are induced by hypoxia in an HIF α -dependent manner.⁶⁻⁸ We further confirmed that JMJD1A in breast cancer cell MCF7 and glioblastoma cell U87 is upregulated by hypoxia in an HIF α -dependent manner (Fig. 1). JMJD1A can be induced by overexpression of HIF-1 α or HIF-2 α , while loss of HIF-1 α or HIF-2 α causes reduction of JMJD1A. These data indicate that JMJD1A is commonly regulated by HIF-1 α and HIF-2 α . Nevertheless, we have also found that JMJD1A induction is dependent on HIF-1 α alone in another breast cancer cell line T47D (Fig. 1F), reflecting a cell-type specific effect. These

recently identified HIF targets shed new light on the regulatory mechanisms of HIF α , which might tune hypoxic response via an epigenetic regulation mediated by histone demethylases.

Histone Demethylases Induced by Hypoxia

Histone lysine demethylase is composed of two families, including LSD1 and JmjC domain-containing proteins.⁹ LSD1 is a flavin adenine dinucleotide (FAD)-dependent enzyme,¹⁰ while most JmjC domain-containing histone demethylases are dioxygenases and phylogenetically similar to FIH1, whose activities require Fe(II), α -ketoglutarate, and oxygen;⁹ hence, potentially, their enzyme activities could be affected by oxygen tension. There are about 30 JmjC domain-containing proteins in human beings (Fig. 2). Although JMJD1A and JMJD2B have been demonstrated to be HIF targets, other JmjC domain-containing proteins may also be upregulated by hypoxia or HIF. Based on the published gene expression profile data, we identified that seven additional JmjC domain-containing proteins, including JMJD1B, JMJD2C, JMJD6, PLU-1, SMCX, RBP2, and KIAA1718, could be upregulated by hypoxia (Fig. 2).

A recent study using chromatin immunoprecipitation (ChIP) sequencing approach also found that 17 out of 22 JmjC family genes were upregulated by hypoxia,¹¹ four of which (including PLU-1, JMJD1A, JMJD2B, JMJD2C) were direct HIF-1 targets. The biological functions of these histone demethylases in hypoxia are still unknown. As JmjC domain-containing histone demethylases have already been reviewed,^{9,12-14} here we only focus on the HIF-induced JmjC proteins and their potential functions in hypoxia.

JMJD1A

JMJD1A (DKFZp686A24246, DKFZp686P07111, JHDM2A, JHMD2A, JMJD1, KDM3A, KIAA0742, TSGA) is a

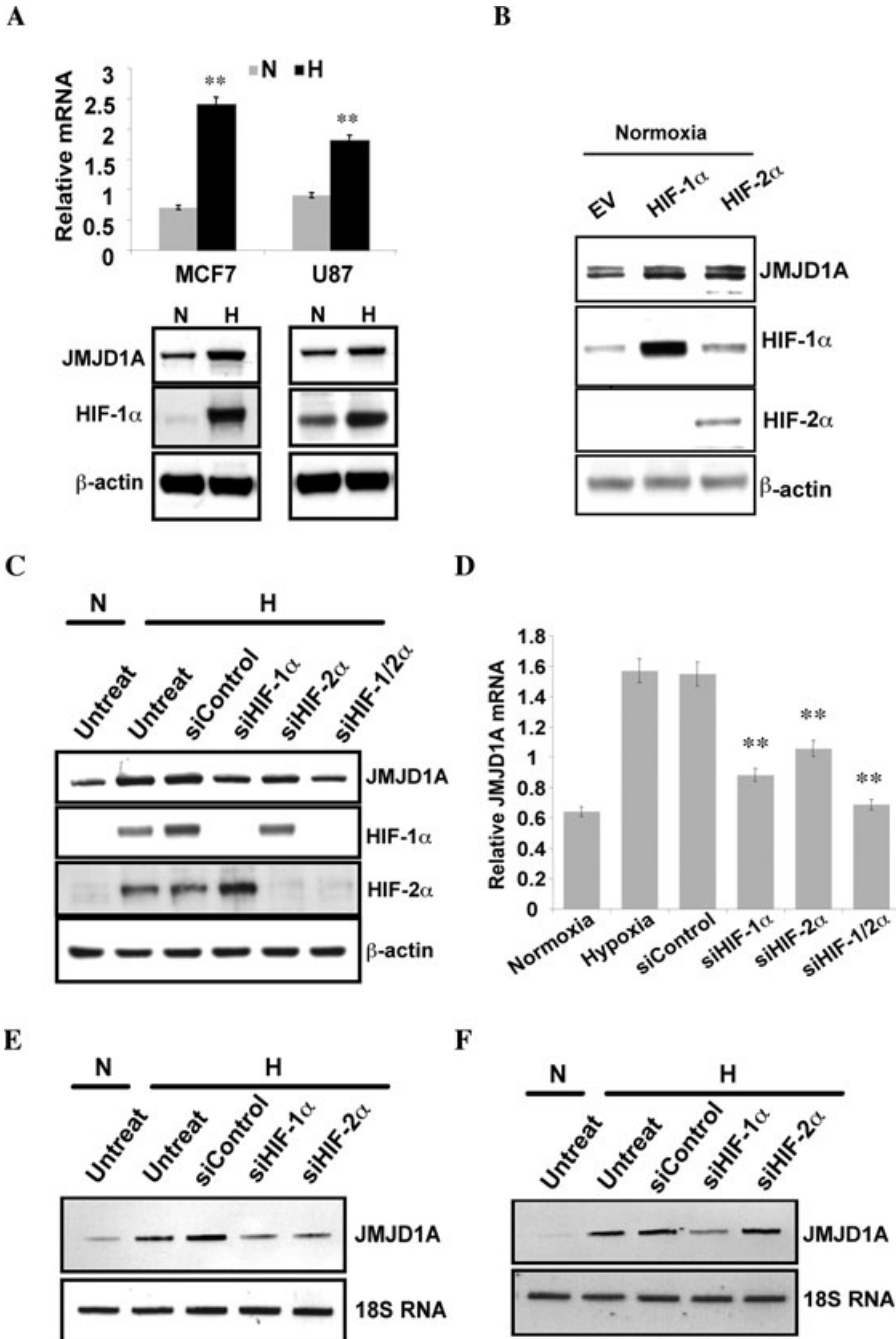


Figure 1. HIF α -dependent induction of JMJD1A. **(A)** MCF7 and U87 cells were incubated in hypoxia for 16 h. Quantitative real-time PCR (*top panel*) and Western blotting (*bottom panel*) were used for assessment of JMJD1A expression. **(B)** PC3 cells that highly express HIF-1 α or HIF-2 α were used to assess the JMJD1A expression with indicated antibodies. **(C, D)** MCF7 cells were transfected with siRNAs against HIF-1 α and HIF-2 α . JMJD1A protein and mRNA levels were assessed by Western blotting (C) and quantitative real-time PCR (D), respectively. (Student's *t* test ***P* < 0.01.) **(E, F)** U87 cells and T47D cells were transfected with siRNAs against HIF-1 α and HIF-2 α . JMJD1A mRNA levels were assessed by PCR; 18S RNA was used as the loading control.

specific H3K9me2/me1 demethylase,¹⁵ which was originally identified as a male germ-specific transcript¹⁶ and is therefore most prominently expressed in testes.¹⁵ JMJD1A has been shown to interact with ER71,¹⁷ a transcription factor that is expressed in the testes of adult mice and during embryogenesis and impairs the ability of ER71 to activate transcription from the matrix metalloproteinase-1 promoter,¹⁷ suggesting that JMJD1A may play a role in male germ cell development. Indeed, a genetrap mouse model shows that JMJD1A is essential for spermatogenesis by in that it positively regulates gene expression of transition nuclear protein (*Tnp1*) and protamine 1 (*Prm1*) via demethylation of H3K9 marks from these genes promoters.¹⁸

Using a knockout mouse the same research group demonstrated that JMJD1A also plays an essential role in regulating metabolic gene expression and normal weight control.¹⁹ They found that loss of JMJD1A disrupted β -adrenergic-stimulated glycerol release and oxygen consumption in brown fat, decreased fat oxidation and glycerol release in skeletal muscles and resulted in obesity and hyperlipidemia in mice.¹⁹ Mechanistically, JMJD1A induced by adrenergic stimulation directly regulates expression of peroxisome proliferator-activated receptor α (PPAR α) and *Ucp1* by removing H3K9me2 marks on PPAR responsive element (PPRE) of *PPAR α* and *Ucp1* genes. Moreover, upon β -adrenergic activation, JMJD1A also can facilitate the recruitment of PPAR and RXR α and their co-activators Pgc1 α , CBP/p300, and SRC1 to PPRE of *Ucp1* gene, indicating that JMJD1A not only serves as a histone demethylase but also exerts transcriptional co-activator function.

Another study shows that JMJD1A is associated with the cardiac and smooth muscle cell (SMC)-specific transcription factor myocardin and the related proteins MRTF-A and MRTF-B²⁰; binds to SMC-specific gene promoters; and regulates TGF β -mediated activation of these genes, suggestive of a role of JMJD1A in SMC differentiation. Knockdown of JMJD1A

attenuates TGF β -induced upregulation of endogenous SM myosin heavy chain expression, concomitant with increased H3K9me2 at the SMC differentiation marker gene promoters and with inhibition of MRTF-A-dependent transactivation of the SMC-specific transcription.²⁰

JMJD1A has been implicated in regulation of self-renewal of embryonic stem (ES) cells.²¹ The ES cell transcription factor Oct4 positively regulates JMJD1A and JMJD2C expression. Depletion of JMJD1A leads to ES cell differentiation, which is accompanied by a reduction in the expression of ES cell-specific genes and an induction of lineage marker genes.²¹ JMJD1A demethylates H3K9me2 at the promoter regions of pluripotency-associated genes, including *Tcl1*, *Tefcp211*, and *Zfp57*, and it positively regulates the expression of these genes.²¹ One study indicates that JMJD1A can also promote ES cell-induced reprogramming of somatic cells by enhancing re-activation of Oct4.²² JMJD1A has been found to be a STAT3 downstream gene in mouse ES cells.²³ STAT3 activation is believed to be important for the maintenance of pluripotency by Leukemia Inhibitory Factor (LIF), further suggesting that JMJD1A might be a critical signaling molecule underlying the maintenance of pluripotency in ES cells.

Although the role of JMJD1A induction in hypoxia remains unknown, implications of hypoxia and HIF in regulation of embryonic stem cells and progenitor cells suggest that JMJD1A might be involved in maintenance or differentiation of hypoxic stem cells or hypoxic cancer stem cells. To test this hypothesis, we cultured HCT116 colon cancer cells as spheroids, dispersed them into single cells, and then sorted them using FACS according to the markers of CD133 and CA9, which represent stem cell marker and hypoxia markers, respectively. We found that three populations of cells, CD133⁺ CA9⁺, CD133⁺, and CD133⁻ cells, differentially express JMJD1A with highest levels in CD133⁺ CA9⁺ populations and lowest levels in CD133⁻ populations (Fig. 3).

Clinical Studies of JMJD1A

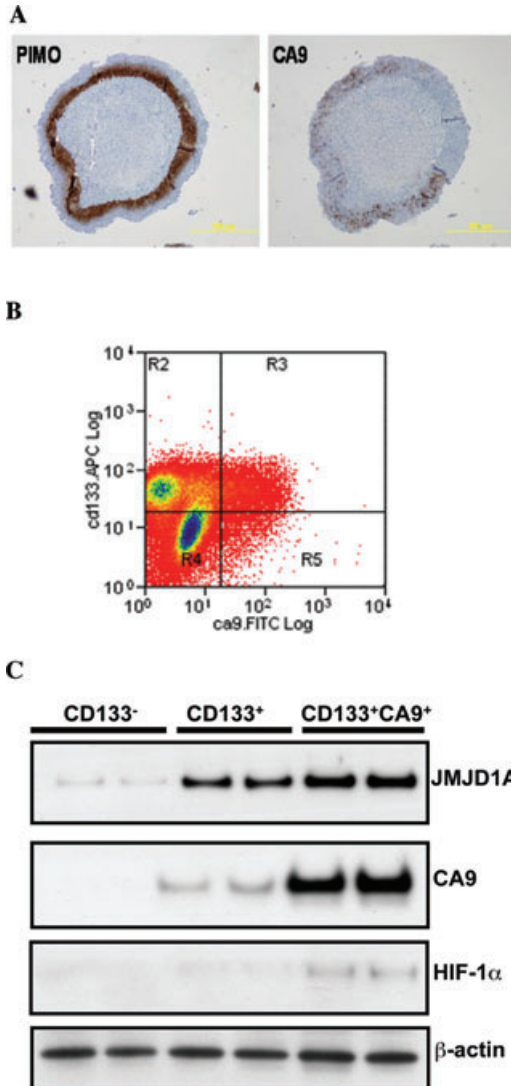


Figure 3. JMJD1A is more highly expressed in CD133⁺CA9⁺ HCT116 colon cancer cells. **(A)** HCT116 colon cancer cells are spheroid showing hypoxia regions stained after incubating spheroids with pimonidazole (PIMO) which is matched with staining for carbonic anhydrase IX (CA9). **(B)** Cells derived from the spheroids were sorted by FACS according to CD133 and CA9 markers. **(C)** Western blotting shows that JMJD1A is highly expressed in CD133⁺ cancer cells and more highly in hypoxic CD133⁺ cancer cells.

These data suggest that cancer cells with stem cell-like properties can highly express JMJD1A, especially for those hypoxic stem-like cancer cells.

Yamane *et al.* has shown that JMJD1A is highly expressed in prostate cancer and has been involved in demethylation of H3K9me2 of androgen receptor (AR) target genes including *PSA*, *NKX3.1*, and *TMPRSS22*,¹⁵ suggesting that JMJD1A might play an important role in prostate cancer development. We have found that JMJD1A is expressed in human cancers such as glioblastoma and breast cancer *in vivo*, and expression is associated with the hypoxic marker CA9 (Fig. 4). Nonetheless, tissue microarray analysis reveals that there is no significant change in JMJD1A expression between various normal tissues and tumors overall (data not shown), suggesting that moderate upregulation of JMJD1A expression might be limited to the most hypoxic areas expressing HIF α . As hypoxia or HIF each plays important roles in cancer metastasis and resistance in chemotherapy and radiotherapy, whether JMJD1A is involved in these processes remains to be studied.

Enzyme Activity of JMJD1A in Hypoxia

JMJD1A protein bears an LXXLL motif that is a signature of protein–protein interaction with nuclear receptors. Thus, JMJD1A was found to interact with the AR in a ligand-dependent manner.¹⁵ Inhibition of JMJD1A expression in the prostate cancer cell line LnCaP led to an increase in H3K9me2 in a subset of AR target genes.¹⁵ These results show that JMJD1A acts as a co-activator of AR-mediated transcription. But whether and how JMJD1A regulates gene expression in hypoxia remains to be elucidated.

As JMJD1A is a dioxygenase whose activity requires the presence of oxygen, hypoxia might limit its enzymatic function. However, a recent study reveals that 1% O₂ does not inhibit the histone demethylase function of JMJD1A⁸; even 0.2% O₂ does not completely inhibit its enzymatic activity,⁸ suggesting that, like FIH1, JMJD1A can tolerate relatively low oxygen. This observation raises an important question:

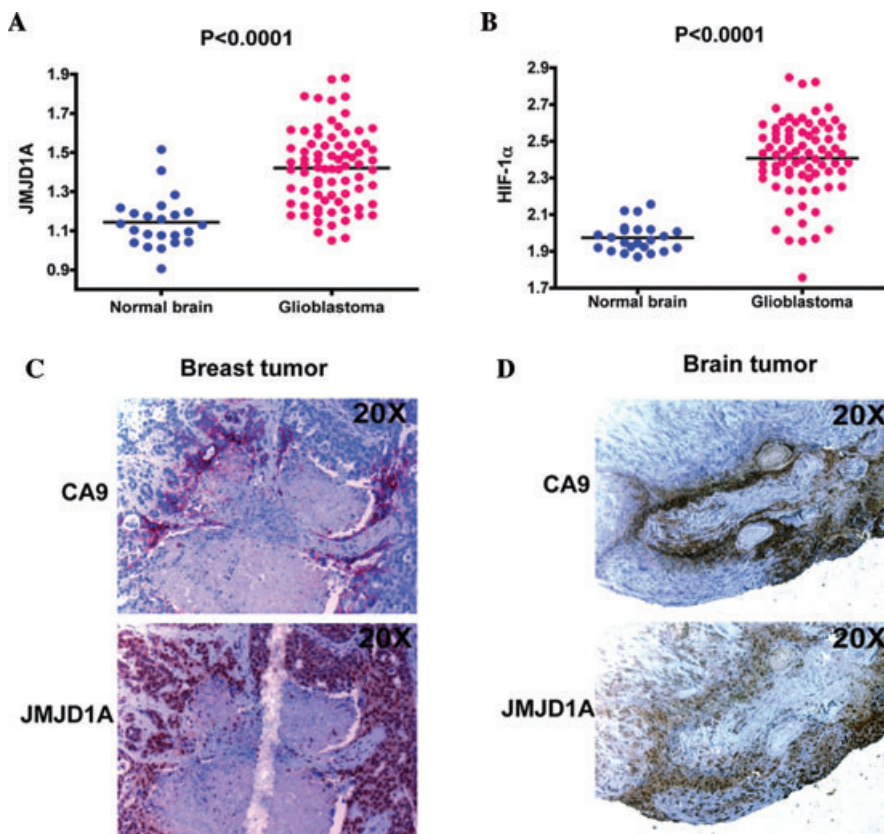


Figure 4. JMJD1A expression is associated with hypoxia in cancer. (**A, B**) JMJD1A and HIF-1 α data from the Sun *et al.* study⁵³ were reanalyzed using the Prism program. (**C, D**) JMJD1A expression in breast cancer and glioblastoma samples were analyzed using immunohistochemistry (magnification $\times 20$).

Does JMJD1A exert its function in hypoxia via a histone demethylase-dependent manner or just act as a transcriptional co-activator (or co-repressor in other circumstances) via interaction with other transcription factors such as nuclear receptors in an enzyme-independent way? Probably both mechanisms can be implicated in hypoxia since its histone demethylase function is not inhibited. Hairless, another member of JMJD1 subfamily, which has no reported histone demethylase activity, can exert as a transcriptional co-repressor for various nuclear receptors including vitamin D receptor (VDR), thyroid hormone receptor (THR), and retinoic acid receptor-related orphan receptors (ROR).^{24,25} Therefore, JMJD1A could also potentially regulate gene transcription in hypoxia independent of its histone demethylase activity.

Identification of JMJD1A target genes in hypoxia is necessary to answer these questions. Further analysis using ChIP would allow us to assess whether histone methylation status can be modulated by JMJD1A in hypoxia. ChIP-sequencing, or ChIP-on-chip, may greatly facilitate the global identification of JMJD1A targets.

Considering that JMJD1A can regulate gene expression and can be induced in hypoxia, one might expect that JMJD1A could modulate transcriptional activities of HIF-1. However, we used qRT-PCR to assess about 20 HIF-regulated genes and found that none of them can be significantly affected by JMJD1A knockdown in MCF7 cells (data not shown), suggesting that either JMJD1A only regulates a small subgroup of genes that are associated

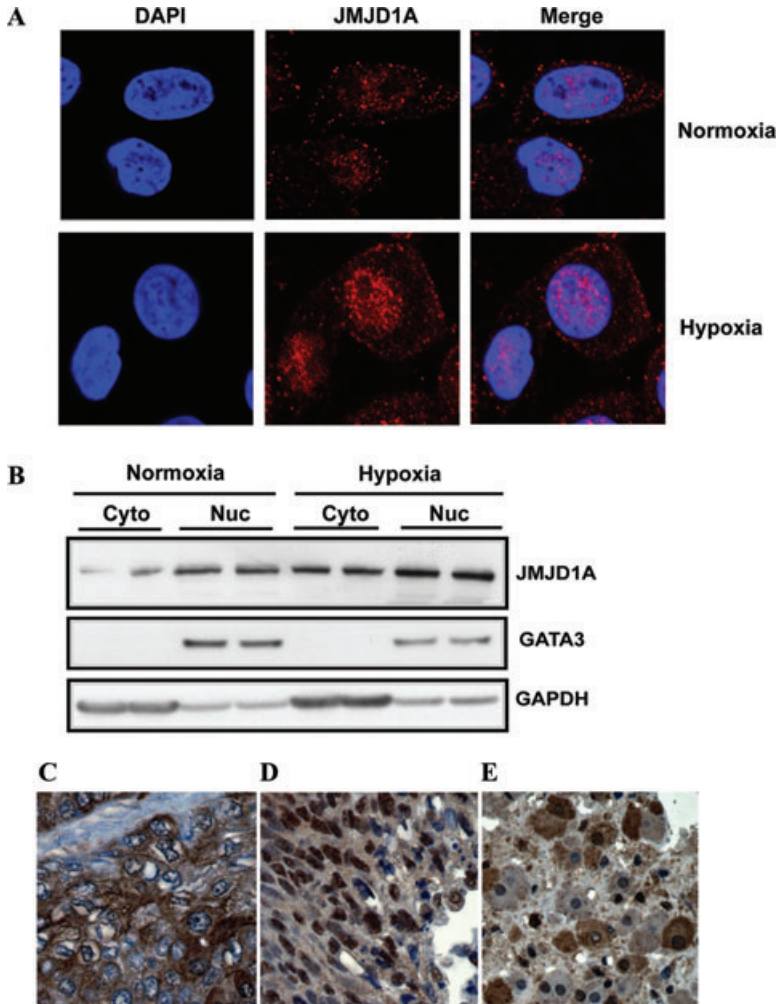


Figure 5. Subcellular localization of JMJD1A. **(A, B)** MCF7 cells were incubated in hypoxia for 16 h. Immunofluorescence (A) and subcellular fractionation (B) were used to assess the localization of JMJD1A. **(C, D, E)** Immunohistochemistry analysis of JMJD2B in tumor sections, which shows predominant localization in cytoplasmic, nuclear, or both compartments.

with HIF that we failed to select or JMJD1A separately regulates a specific group of genes that are distinct from HIF1-regulated genes.

Subcellular Localization of JMJD1A

The ascribed histone demethylase function of JMJD1A may make us expect that JMJD1A should be dominantly localized in nucleus to modulate histone methyl marks. Surprisingly, we have found that in a large proportion of normal tissues and clinical tumor samples, JMJD1A is localized in both cytoplasm and

nucleus and this is also confirmed in cancer cells although JMJD1A can be dominantly localized in nucleus or cytoplasm (Fig. 5). Hypoxia (1% O₂) did not change the cytoplasmic distribution of JMJD1A in MCF7 cells. These observations raise another important question: Does JMJD1A have physiological functions in cytoplasm either in normoxia or hypoxia? Although it remains to be answered for this question, subcellular localization of JMJD1A is probably an important mechanism to regulate its function. We want to further extend this question; does JMJD1A possess

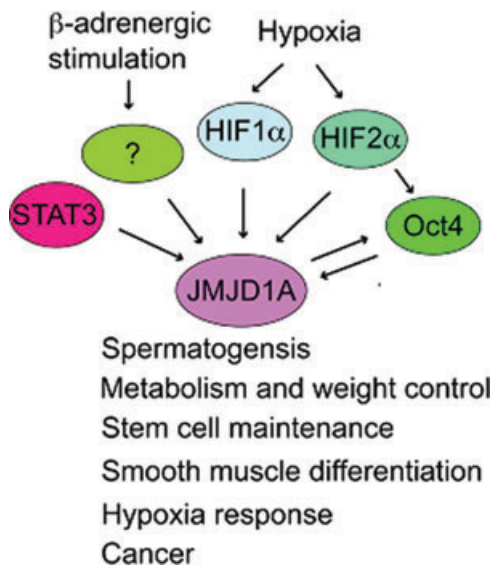


Figure 6. Potential roles of JMJD1A.

histone-independent demethylase function either in nucleus or cytoplasm? Recent studies have shown that lysines on non-histone proteins such as TAF10²⁶ and p53²⁷ can be methylated. G9a, the histone H3 lysine methyltransferase can be automethylated.^{28,29} There is no question that these methylations can be reversed by corresponding demethylases. Indeed, lysine methylation of p53 can be removed by LSD1.³⁰ Identification of non-histone targets of JMJD1A using proteomics could help to answer this question.

In summary, JMJD1A is important for spermatogenesis, metabolism regulation, self-renewal of stem cells, and possibly involved in smooth muscle cell differentiation (Fig. 6). JMJD1A is also involved in cancer and hypoxic stress; however, the role of JMJD1A in hypoxia is still unknown, and further studies need to be conducted to examine the biological functions of JMJD1A in hypoxia and to dissect the molecular mechanism by which JMJD1A regulates gene transcription in hypoxia.

JMJD2

The JMJD2 subfamily consists of four members, including JMJD2A, JMJD2B, JMJD2C,

and JMJD2D, which are capable of removing H3K9me3/me2 and H3K36me3/me2 marks.^{31–33} Amongst these four members, JMJD2B and JMJD2C have been shown to be upregulated in hypoxia^{7,8} and their hypoxic induction is HIF-dependent as evidenced by ChIP-sequencing results.¹¹ We have found that JMJD2B induction is HIF-1α-dependent (Fig. 7), and JMJD2B expression is associated with hypoxia marker CA9 in brain tumors (Fig. 7). The biological functions of JMJD2 are not very clear. Enforced expression of JMJD2B and JMJD2C significantly decreases global H3K9me3 and H3K9me2 levels and delocalizes HP1, suggesting that JMJD2 family proteins are capable of reorganizing heterochromatin.^{31,32} Like JMJD1A, when over-expressed in hypoxia, JMJD2B protein is still capable of removing H3K9me3 mark,⁸ indicating that JMJD2B can exert its demethylase function in hypoxia.

JMJD2C, together with JMJD1A, plays an important role in self-renewal of stem cells.²¹ Again, it is very possible that JMJD2C may be involved in maintenance or differentiation of hypoxia-regulated stem cells and stem-like cancer cells. JMJD2C was found to be amplified in squamous cell carcinoma.^{34,35} A recent aGCH study also shows that JMJD2B and JMJD2C are amplified in medulloblastomas,³⁶ suggesting that JMJD2B and JMJD2C have oncogenic functions. Consistent with this, JMJD2A, JMJD2B, and JMJD2C are overexpressed in cancer, and inhibition of JMJD2A and JMJD2C affects cellular growth.³¹ However, how JMJD2 exactly regulates cell proliferation remains to be elucidated. A functional interaction between JMJD2C and the AR in prostate carcinomas has been reported recently.³⁷ JMJD2C can bind to the AR and acts as an essential co-activator of AR-induced transcription, suggesting that JMJD2C plays an important role in prostate cancer progression.

Whetstine *et al.* demonstrated that depletion of the *Caenorhabditis elegans* JMJD2 homolog, CeJMJD2, resulted in a global increase of H3K9me3 levels, localized H3K36me3 to

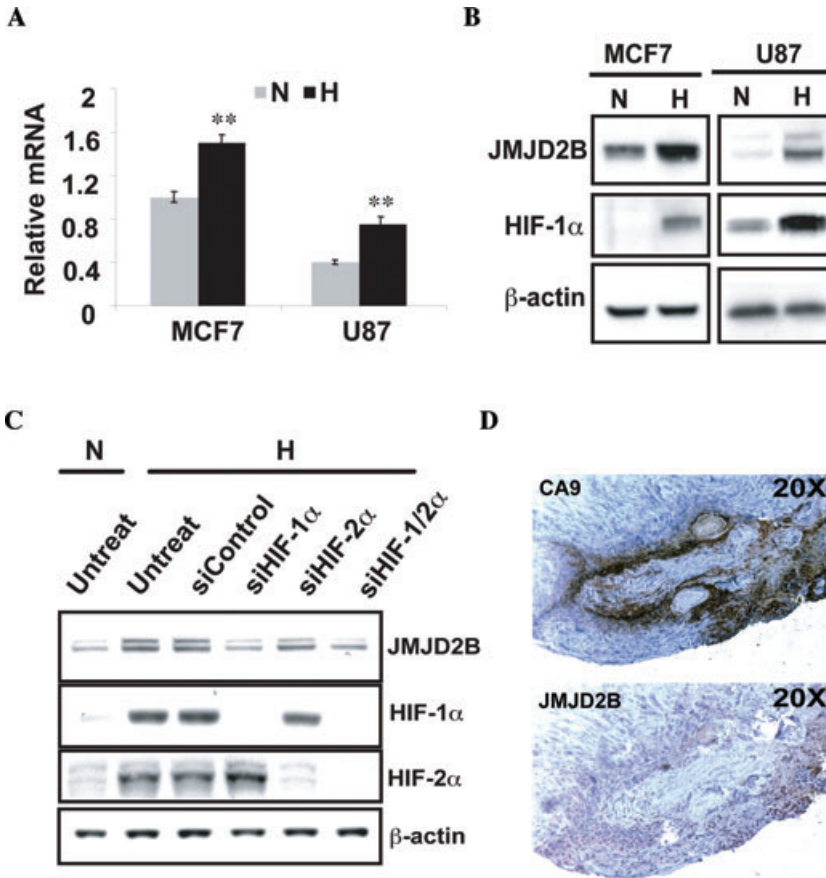


Figure 7. HIF-1 α -dependent regulation of JMJD2B. **(A, B)** MCF7 and U87 cells were incubated in hypoxia for 16 h. Quantitative real-time PCR (A) and Western blotting (B) were used for assessment of JMJD2B expression. (Student's *t* test ** *P* < 0.01.) **(C)** MCF7 cells were transfected with siRNAs against HIF-1 α and HIF-2 α . JMJD2B protein were assessed by Western blotting. **(D)** JMJD2B expression in glioblastoma samples were analyzed using immunohistochemistry (magnification $\times 20$).

meiotic chromosomes, and activation of p53-dependent germline apoptosis.³³ p53 has been shown to play an important role in apoptosis in hypoxic tumor cells as apoptosis is significantly reduced when tumors express mutant p53.^{38,39} Therefore, whether JMJD2B and JMJD2C are entangled in p53-mediated apoptosis in response to hypoxia is another key question.

Genomic amplification of JMJD2B and JMJD2C in medulloblastoma is mutually exclusive,³⁶ suggesting that JMJD2B and JMJD2C may regulate the same signaling pathway. However, the fact that hypoxic induction of JMJD2B and JMJD2C can occur in the same cancer

cells suggests that JMJD2B and JMJD2C may not completely function redundantly but have specific targets. How JMJD2B and JMJD2C regulate, in coordination, gene transcription in response to hypoxia needs to be answered. Similarly, the hypoxic induction of JMJD1A and JMJD2C, both of which are involved in self-renewal of ES cells and interaction with AR in prostate cancer cells, raises an interesting question: Do JMJD1A and JMJD2C cooperatively work to regulate gene expression, or could both be recruited to the same gene promoter, which is AR-regulated? Considering that JMJD1A and JMJD2C demethylate H3K9me2/me1 and H3K9me3/me2, respectively, it is possible

that both enzymes are sequentially or together recruited to the same loci to exert their histone demethylase function or transcriptional activities.

H3K36me3/me2 is associated with transcriptional elongation and suppresses inappropriate transcription within the body of genes,⁴⁰ while the H3K9me3/me2 is associated with transcriptional repression and heterochromatin. Therefore, in one way, JMJD2 would demethylate H3K36me3/me2 and inhibit transcription elongation; in another way, JMJD2 would promote gene transcription by demethylating H3K9me3/me2. Understanding how JMJD2 integrates the milieu of chromatin structure that specifically activate or suppress gene transcription in hypoxia remains a challenge.

PLU-1

PLU-1 (KDK5B, JARID1B) is a member of histone lysine demethylase 5 (KDM5) family,¹³ which specifically demethylates H3K4me3/me2 and therefore plays a transcriptional repressor role.^{41,42} PLU-1 expression is mainly restricted to testes in normal adult tissues.^{43,44} However, high expression of PLU-1 is also seen in the murine pregnant mammary gland and embryonic mammary bud,⁴⁵ suggesting that PLU-1 is involved in development and differentiation of mammary gland. Consistent with the role in mammary glands, PLU-1 is highly expressed in 90% of invasive ductal breast carcinomas regardless of HER2 status, although PLU-1 was identified in a screen for genes regulated by HER2.^{44,46} PLU-1 is able to promote breast cancer cell proliferation by facilitating G1/S phase transition.⁴¹ Loss of PLU-1 by knocking down gene expression suppresses tumor growth of mammary carcinoma cells in nude mice.⁴¹ PLU-1 has also been associated with prostate cancer.⁴² PLU-1 is associated with AR and regulates its transcriptional activity.⁴²

A recent *in vitro* study suggests that PLU-1 may play a role in the choice between prolifera-

tion and differentiation during development.⁴⁷ Transient overexpression of PLU-1 in ES cells decreases the expression of cell fate modulator genes such as *Egr1*, *p27Kip1*, and *BMI1*.⁴⁷ PLU-1 is able to reduce the terminally differentiated cells and increase proliferating progenitors via blocking of the upregulation of cell lineage markers and maintenance of cyclins, thereby allowing cells to escape differentiation and remain uncommitted.⁴⁷ Microarray analysis reveals that PLU-1 suppresses *BRCA1*, metallothionein genes and genes involved in regulation of M phase of the mitotic cell cycle.⁴⁸ PLU-1 can directly interact with histone deacetylases (HDACs), such as HDAC4.⁴⁹ HDAC4 and PLU-1 are co-expressed in the pregnant and involuting mammary gland, and both are silenced at lactation and expressed in breast cancers.⁴⁹ PLU-1 also interacts with brain factor-1 (BF-1) and paired box 9 (PAX9) transcription factors via a conserved sequence motif.⁵⁰ Mutation of this motif in BF-1 and PAX9 abolished PLU-1 co-repression activity.⁵⁰ Both BF-1 and PAX are known to interact with members of the Groucho co-repressor family, potentially supporting a role for PLU-1 in Groucho-mediated transcriptional repression. Despite the importance of PLU-1 in breast cancer, the hypoxic role of PLU-1 is unknown and the relationship of hypoxic induction of PLU-1 and mammary gland differentiation or breast cancer progression has not been studied yet.

Conclusions

The findings of hypoxic induction of histone demethylases have important implications in cancer biology. As some JmjC histone demethylases, such as JMJD2B and JMJD2C, have oncogenic functions, and have been shown to be amplified in cancers,^{35,36} HIF α -mediated induction of these oncogenes further confirms that HIF α plays an important role in cancer progression. The JmjC histone demethylases are dioxygenases, whose activities require

Fe(II) and 2OG. These enzymes can be targeted by small compounds, for example, via competing with Fe(II) or 2-OG, binding or displacing methyl peptide from the pocket that accommodates the histone methyl marks. The crystal structure of JmjC domain of JMJD2A has been solved,^{51,52} the catalytic core of histone demethylase has very high homology to JMJD2B and JMJD2C. This may accelerate the development of small molecules against JMJD2B and JMJD2C.

Since hypoxia can induce multiple histone demethylases, what are the biological consequences for the interactions in hypoxia? The roles of the histone methyltransferases, which may exert opposite effects, have not been widely studied yet. Elucidation of epigenetic regulation via histone methylation and demethylation in response to hypoxia may provide new insight for understanding of how cancer cells evolve under environmental stress and provide rationale for development of novel cancer therapeutic drugs against the methyltransferases or histone demethylases.

Acknowledgments

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Conflicts of Interest

The authors declare no conflicts of interest.

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