International Journal of Biochemistry and Cell Biology 109 (2019) 33–39

Contents lists available at ScienceDirect

International Journal of Biochemistry and Cell Biology

journal homepage: www.elsevier.com/locate/biocel

Histone H3K9 demethylase JMJD1A is a co-activator of erythropoietin expression under hypoxia

Zhantao Tian[a,d,1], Lv Yao[b,e,1], Yongqing Shen[c], Xiaoqiang Guo[a,b,*,2], Xianglin Duan[a,**,2]

[a] Laboratory of Molecular Iron Metabolism, College of Life Science, Hebei Normal University, Shijiazhuang, 050024, Hebei, China
[b] Department of Urology, Peking University Shenzhen Hospital, Institute of Urology of Shenzhen PKU-HKUST Medical Center, Shenzhen, 518036, Guangdong, China
[c] Department of Nursing, Hebei University of Chinese Medicine, Shijiazhuang, 050020, Hebei, China
[d] No.1 Middle School of Yuxian, Yuxian, 075700, Hebei, China
[e] Department of Obstetrics and Gynecology, Sir Run Run Shaw Hospital, Zhejiang University School of Medicine, Hangzhou, Zhejiang 310016, China

ARTICLE INFO

Keywords:
Erythropoietin
Hypoxia-inducible factor
HIF-2α
Histone demethylase
JMJD1A

ABSTRACT

Erythropoietin (EPO) is a secreted hormone that stimulates the production of red blood cells, and the level of EPO is increased under hypoxia. The expression of EPO is regulated not only by the hypoxia-inducible factor (HIF) but also partly through epigenetic modifications, including histone acetylation and methylation. In this study, we report that histone H3K9 demethylase JMJD1 A is regulated by HIF-2α in HepG2 cells under hypoxia. Knockdown or over-expression of JMJD1 A can decrease or increase EPO expression, respectively. JMJD1 A can interact with HIF-2α to form a co-activator complex, which binds to the hypoxia response elements of EPO and increases EPO expression by catalyzing demethylation of H3K9me2, a transcription suppression marker. The results demonstrate that JMJD1 A is a co-activator of EPO expression.

1. Introduction

Erythropoietin (EPO) is a glycoprotein hormone and the principal stimulator of erythropoiesis (Jelkmann, 2011). EPO promotes the proliferation and differentiation of red blood cells, which is important for oxygen transport. Low EPO levels in blood cause anemia; however, excessive levels of EPO cause polycythaemia. Therefore, it is important to accurately regulate EPO levels (Franke et al.,2013).

In hypoxia, EPO is upregulated to adapt to the stressful environment (Haase, 2010). EPO production is controlled at the transcriptional level and is tightly regulated by hypoxia-inducible factors (HIFs) (Haase, 2013). HIFs are specific transcription factors that are composed of an oxygen-sensitive α-subunit (HIF-α) and a constitutive β-subunit (HIF-β). In normoxia, the HIF-α subunit is rapidly hydroxylated by the prolyl hydroxylase domain (PHD) enzyme, whose activity is dependent on oxygen (Maxwell, 2005). The von Hippel–Lindau protein (pVHL) is a component of the multiprotein E3 ligase complex and leads to polyubiquitylation of the hydroxylated HIF-α subunit, which is destroyed by the proteasome (Maxwell, 2005). The HIF-α subunit is not hydroxylated and is thereby stabilized in hypoxia (Pugh and Ratcliffe, 2003). Therefore, the HIF-α subunit can translocate to the nucleus and heterodimerize with HIF-β subunits and then activate the transcription of hypoxic target genes, including *EPO,* upon binding to hypoxia response elements (HREs) (Yoon et al., 2011). EPO is mainly produced in the embryonic liver and adult kidney (Wenger and Hoogewijs, 2010). Several studies have indicated that EPO is mainly regulated by HIF-2α rather than HIF-1α in both the liver and kidney in hypoxia Kapitsinou et al.,2010; Rankin et al., 2007).

EPO expression is also regulated epigenetically, such as DNA methylation and histone post-translational modification (PTM) (Steinmann et al.,2011). Methylation in promoter and 5′-untranslated regions (5′-UTR) of the *EPO* gene represses the expression of EPO (Yin and Blanchard, 2000). Histone PTMs include acetylation, methylation, phosphorylation and ubiquitination, which can be associated with either gene activation or gene repression (Kouzarides, 2007). Histone acetylation is involved in the regulation of EPO expression (Rankin et al., 2007), and histone acetyltransferase (HAT) p300 plays a central role in co-activation in hypoxia-induced EPO (Wang et al., 2010). Whether other histone modifying enzymes, such as demethylase, are involved in the process remains unclear.

* Corresponding author at: Peking University Shenzhen Hospital, No 1120, Lianhua Road, Shenzhen, 518036, China.
** Corresponding author at: College of Life Science, Hebei Normal University, No 20 Nanerhuan East Road, Shijiazhuang, 050024, China.
E-mail addresses: xiaoqiangguo123@163.com (X. Guo), xlduan0311@163.com (X. Duan).
[1] Z, T and L, Y contributed equally to this work.
[2] X, D and X, G are equally responsible for this work.

https://doi.org/10.1016/j.biocel.2019.01.022
Received 5 December 2018; Received in revised form 27 January 2019; Accepted 31 January 2019
Available online 01 February 2019

In this study, we investigated the role of histone H3K9-specific demethylase JMJD1A (also known as KDM3 A and JHDM2 A) on EPO regulation under hypoxia. The results indicate that JMJD1 A is an essential regulator to promote EPO expression and demonstrate that JMJD1 A can bind EPO HREs and catalyze H3K9me2 demethylation to activate gene transcription. Based on these data, we propose that hypoxia-induced JMJD1A can enhance EPO expression as a co-activator of HIF-2α.

2. Materials and methods

2.1. Animal experiment

C57BL/6 male mice (6 weeks old) were purchased from the Experimental Animal Center of Guangdong Province (Guangzhou, China). All animal experiments were performed in accordance with the animal ethical standard. The research protocol was reviewed and approved by the Ethics Committee of Peking University Shenzhen Hospital. The mice were divided into two groups (6 mice/group). The experimental group was treated with hypoxia (5%) for 6 h. The control group was kept under normal conditions. Then, all mice were sacrificed after anaesthesia with sodium pentobarbital. The kidney and liver were immediately taken for subsequent experiments.

2.2. Cell culture and hypoxia treatment

Human embryonic kidney cells (HEK293) and hepatocellular carcinoma cells (HepG2) were purchased from the Cell Resource Center of Shanghai Institutes for Biological Sciences, Chinese Academy of Science. Both cells were maintained in Dulbecco's Modified Eagle Medium (DMEM, Gibco, Grand Island, USA) supplemented with 10% heat-inactivated foetal bovine serum (FBS, HyClone, Logan, USA). Cells were cultured at 37 °C with 5% CO_2. For hypoxia treatment, both HepG2 and HEK293 cells were cultured under hypoxia (1% O_2) or with iron chelator desferrioxamine (DFO) (100 μM) for 24 h. DFO can inhibit prolyl hydroxylases and thus increase HIF-1 and HIF-2 contents.

2.3. RNA interference (RNAi)

HepG2 cells were seeded at 70–80% confluency and transfected with 20 nM JMJD1 A or HIF-2α siRNAs (together with negative control) using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). The knockdown effect of JMJD1 A or HIF-2α was evaluated by qRT-PCR and Western blotting. All siRNAs were synthesized by Genepharma (Shanghai, China), and the sequences were as follows: negative control (si-NC): 5′-UUCUCCGAACGUGUCACGU-3′; JMJD1 A (si-JMJD1 A): 5′-GCACAGUCCUCCAUACGUU-3′; and HIF-2α (si-HIF-2α): 5′-CAGCA UCUUUGAUAGCAGU-3′.

2.4. JMJD1A overexpression

The cDNAs for human JMJD1A were cloned into pcDNA3.0 and then transfected into HepG2 cells with 20 nM JMJD1A and Lipofectamine 2000. The empty plasmid pcDNA3.0 was transfected at the same time as the control. The effect of overexpression was qualified by qRT-PCR and Western blotting.

2.5. Quantitative reverse transcription polymerase chain reaction (qRT-PCR)

RNA was extracted from HepG2 and HEK293 cells using Trizol reagent (No. 10296028, Invitrogen) in accordance with the manufacturer's instructions and qualified by measuring the absorbance at 260 nm. cDNA was synthesized from 1 μg of total RNA with a Fermentas RT system (Thermo Scientific, Wilmington, DE, USA) according to the manufacturer's protocol. Real-time PCR was performed in a 20 μl reaction mixture containing 10 μl of SYBR Premix, 0.5 μM forward and reverse primers, and 1 μl of template cDNA on a LightCycler480 System (Roche, Foster City, CA, USA). The primers of JMJD1 A, HIF2α, EPO and β-Actin were synthesized by Sangon (Shanghai, China), and the sequences are listed in Supplementary table 1.

2.6. Western blotting

HepG2 cells were collected and treated with radioimmunoprecipitation assay (RIPA) buffer containing the protease inhibitor cocktail and phenylmethylsulfonyl fluoride (PMSF). Protein samples (50 ug) were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride (PVDF) membranes. The membranes were saturated with 5% skim milk in TBST (50 mM Tris–HCl, 150 mM NaCl, 0.1% Tween-20) and then incubated with primary antibodies at 4 °C overnight. The primary antibodies used in this study included rabbit polyclonal antibodies to JMJD1 A (No. BNP1-77070, 1:1,000, Novus Biologicals, USA), HIF-2α (No. NB100-122, 1:1500, Novus Biologicals, USA), H3K9me2 (No. 9753, 1:1,500, Cell Signaling, USA), and β-Actin (No. R1207-1, 1:2,500, HuaAn Biologicals, Hangzhou, China). The blots were incubated with HRP-conjugated goat anti-rabbit secondary antibody (No. HA1001-100, 1:5,000, HuaAn Biologicals) for 2 h at room temperature and then exposed to enhanced chemiluminescence substrate (Millipore, Rockford, USA). The results were detected using a film.

2.7. ELISA assay

The cell medium was collected after the cell experiment, and the content of EPO was determined by ELISA (No. D710202, Sangon Co. Shanghai, China). Cell medium and EPO standard solutions were measured, and the EPO concentration was calculated accordingly.

2.8. Co-immunoprecipitation assay

HepG2 cells were incubated under normoxia and hypoxia for 24 h. Cells were washed with PBS, pelleted by centrifugation and re-suspended in lysis buffer supplemented with a protease inhibitor cocktail. Cell lysate was collected by centrifugation, pre-cleared by incubation with protein A-Sepharose Fast Flow (Sigma-Aldrich, St. Louis, MO, USA), and pre-equilibrated with lysis buffer on a rotating platform. Centrifuged supernatants were then collected and incubated with the anti-HIF-2α antibody or IgG overnight. These supernatants were then mixed with protein G-Sepharose Fast Flow beads. The beads were collected by centrifugation and washed and then re-suspended in an equal volume of 5 × SDS loading buffer. Immunoprecipitated proteins with an average weight of 50 μg were separated by 10% SDS-PAGE. Western blotting analysis was performed as described above.

2.9. Chromatin immunoprecipitation (ChIP)

ChIP assays were performed using an EZ-ChIP kit (No. 17-371, Upstate, Millipore, USA). In brief, HepG2 cells were incubated under hypoxia (1% O_2) for 24 h. Then, formaldehyde was added to cells at a final concentration of 1% (w/v) for 10 min at room temperature and supplemented with glycine (125 mM) for another 5 min. After washing with PBS, HepG2 cells were lysed; the supernatant was collected after centrifugation (15,000 g for 10 min at 4 °C) and pre-cleared with protein G agarose for 1 h at 4 °C with shaking. Chromatin was incubated overnight with an average of 4 μg of rabbit polyclonal antibodies against JMJD1 A or HIF-2α or H3K9me2 at 4 °C with shaking, and then, protein G agarose was added, followed by incubation for 1 h. Normal rabbit IgG (1 μg) was used as a negative control. The beads were washed, and protein G agarose–antibody/chromatin complexes were eluted with 1% SDS and 0.1 M $NaHCO_3$ elution buffer. Cross-links of

protein/DNA complexes were reversed by overnight incubation at 65 °C, followed by protein digestion with proteinase K. DNA fragments were purified using spin columns. For quantification of HIF-binding sites, DNA fragments (5 ng) from each input, rabbit IgG, JMJD1 A or HIF-2α or H3K9me2 were detected by qPCR. The primers were designed to amplify the HRE-containing region of the EPO HREs (Supplementary table 2). The fold-enrichment of each HRE in the hypoxia-treated cells was determined using the $2^{-\Delta\Delta CT}$ method.

2.10. Statistical analysis

All performed cell experiments were repeated for at least three times. All data are represented as the means ± SEM from three independent experiments. The differences between two groups were analysed with a two-tailed Student's *t* test. $P < 0.05$ was considered statistically significant.

3. Results

3.1. JMJD1A is transcriptionally regulated by HIF-2α under hypoxia

To understand the role of histone demethylase in EPO regulation, the effect of hypoxia on H3K9me1/2 demethylase JMJD1 A expression was studied. In mice, the expression of *Jmjd1a* was significantly upregulated in liver and kidney after hypoxia treatment (Fig. 1A, $P < 0.05$). In two cell lines HEK293 and HepG2, the mRNA and protein levels of JMJD1A were also significantly increased after hypoxia or DFO treatment (Fig. 1B–E, $P < 0.05$).

Several studies have demonstrated that JMJD1 A expression is dependent on HIF-1α (Krieg et al., 2010; Pollard et al.,2008). Furthermore, our previous study also supports this conclusion (Guo et al., 2012). However, whether JMJD1 A is also regulated by HIF-2α remains to be clarified. Therefore, we performed a HIF-2α knockdown experiment with RNAi to investigate the regulated role of HIF-2α on JMJD1 A. The results indicated that the up-regulation of JMJD1 A expression was significantly inhibited in HIF-2α knockdown cells under hypoxia (Fig. 2A–C, $P < 0.05$). The result demonstrated that JMJD1A is transcriptionally regulated by HIF-2α.

3.2. JMJD1A is involved in EPO expression under hypoxia

In mice, hypoxia can increase the expression of *Epo* in the kidney and liver. Our results showed that EPO expression was also increased in HepG2 cells under hypoxia. However, this amount of *EPO* mRNA was below the detection limit in HEK293 cells before or after hypoxia treatment. These results demonstrated that EPO expression was cell-specific. Therefore, we decided to use HepG2 cells for further experiments. Our results also proved that EPO expression was HIF-2α-dependent. Because both EPO and JMJD1 A are regulated by HIF-2α, we suspect that JMJD1 A itself may also regulate the expression of EPO based on transcriptional activation of JMJD1 A.

To prove our hypothesis, we performed a JMJD1 A RNAi experiment. The results indicated that *EPO* expression was obviously inhibited under hypoxia (Fig. 3A and B, $P < 0.05$). Furthermore, the content of EPO in cell culture medium was also significantly decreased (Fig. 3C, $P < 0.05$). The JMJD1A overexpression experiment showed that the mRNA and protein levels of EPO were significantly increased compared to those of the control group (Fig. 4A–C, $P < 0.05$), which illustrated that JMJD1A plays an important role in the regulation of EPO expression.

H3K9 di-methylation (H3K9me2) is a marker of transcriptional repression (Snowden et al.,2002), and its demethylation is associated with gene activation. Our results indicated that global H3K9me2 levels were not significantly decreased when JMJD1A expression was upregulated under hypoxia (Fig. 1C and D). This result was consistent with previous findings (Beyer et al.,2008; Sar et al.,2009). However, the H3K9me2 content was decreased after ectopic expression of JMJD1A (Fig. 4B), which means that JMJD1 A has demethylase activity.

3.3. JMJD1A interacts with HIF-2α

To further explore the mechanism of JMJD1 A on EPO expression, the interaction between JMJD1 A and HIF-2α was determined with immunoprecipitation experiments. The results indicated that the binding of JMJD1 A and HIF-2α was significantly increased under hypoxia, which was consistent with the upregulation of EPO expression (Fig. 5). Our results also indicated that JMJD1 A knockdown decreased HIF-2α content; however, JMJD1 A overexpression increased HIF-2α levels. These results implied that JMJD1 A might be necessary for HIF-2α protein stability. However, the mechanism is still unknown. One possible explanation is that the binding of JMJD1 to HIF-2α reduces the binding of HIF-2α to PHD enzymes, which inhibits HIF-2α ubiquitination and proteasome degradation.

3.4. JMJD1A binds to EPO HRE and catalyses H3K9me2 demethylation

To prove the direct link between JMJD1 A function and EPO expression, chromatin immunoprecipitation (ChIP) analysis was performed. Three antibodies, HIF-2α, JMJD1 A and H3K9me2, were used to study binding with or evaluate the di-methylation status of histone H3K9 on *EPO* HREs (Fig. S1A and S1B). Our results indicated that the binding of HIF-2α was significantly increased under hypoxia

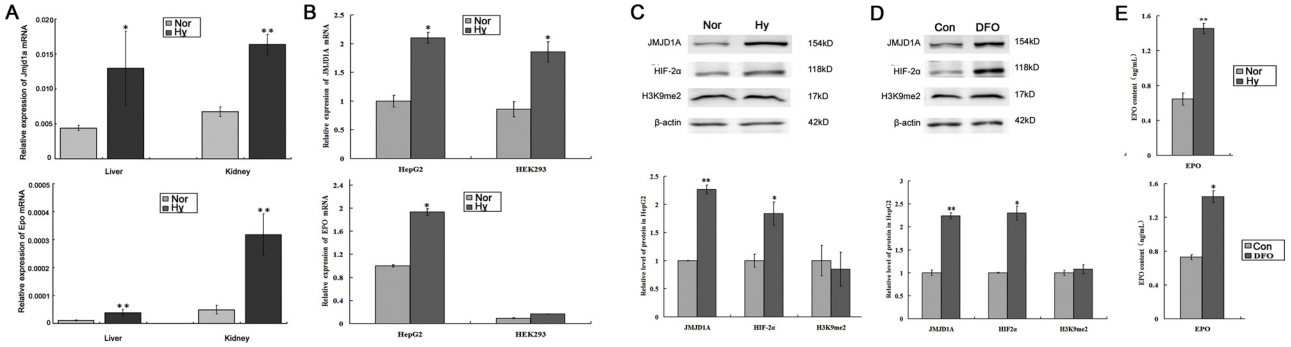

Fig. 1. The expression of JMJD1A and EPO under hypoxia. (A) The expression levels of *Jmjd1a* and *Epo* in mouse liver and kidney after hypoxia treatment. (B) HepG2 and HEK293 cells were cultured under normoxic (21% O_2; Nor) and hypoxic (1% O_2; Hy) conditions for 24 h, and the mRNA levels of *JMJD1A*, *HIF-2α* and *EPO* were determined by qRT-PCR. (C) Western blot analysis for JMJD1A, HIF-2α and H3K9me2 in HepG2 cells under normoxic and hypoxic conditions. (D) Western blot analysis for JMJD1A, HIF-2α and H3K9me2 in HepG2 cells with DFO treatment (DFO, 100 μM) or without control (Con). (E) ELISA assay for EPO in HepG2 cells under normoxic and hypoxic (upper) or control and DFO treatment (lower). All data are presented as the mean ± SEM from three independent experiments. *$P < 0.05$, **$P < 0.01$.

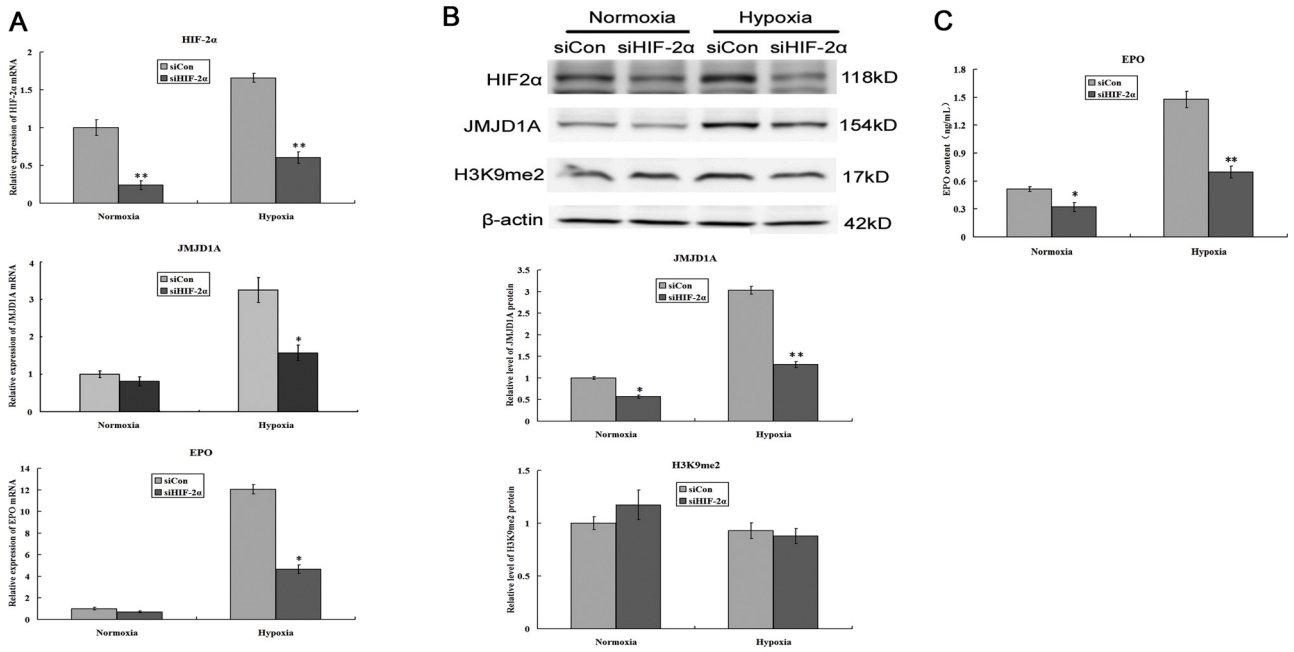

Fig. 2. HIF-2α knockdown inhibits the expression of JMJD1A and EPO. (A) *JMJD1A* and *EPO* mRNA levels in control (siCon) or HIF-2α knockdown (siHIF-2α) HepG2 cells under normoxia (21% O_2) and hypoxia (1% O_2) as determined by qRT-PCR. (B) Western blot analysis for JMJD1A and H3K9me2 in control or HIF-2α knockdown HepG2 cells under normoxia and hypoxia. (C) ELISA assay for EPO in control or HIF-2α knockdown HepG2 cells under normoxia and hypoxia. All data are presented as the mean ± SEM from three independent experiments. $^*P < 0.05$, $^{**}P < 0.01$.

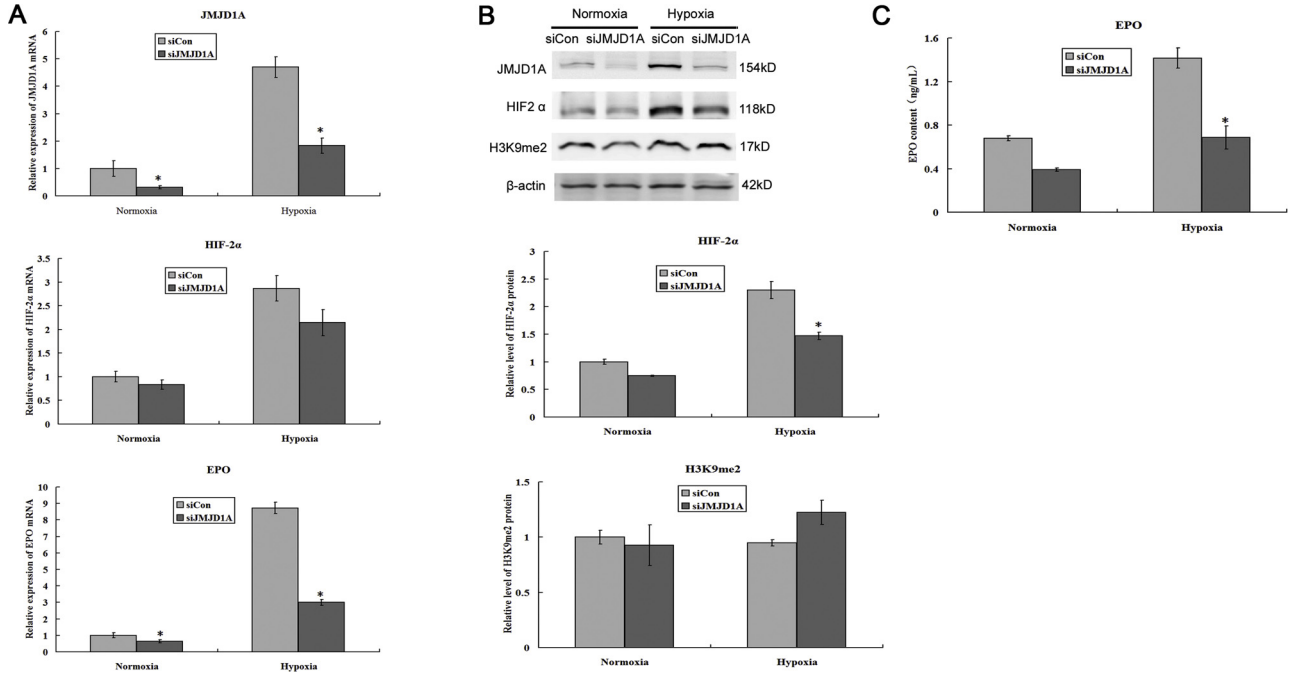

Fig. 3. JMJD1A knockdown inhibits the expression of EPO. (A) *HIF-2α* and *EPO* mRNA levels in control (siCon) or JMJD1A knockdown (siJMJD1A) HepG2 cells under normoxia (21% O_2) and hypoxia (1% O_2) as determined by qRT-PCR. (B) Western blot analysis for HIF-2α and H3K9me2 in control or JMJD1A knockdown HepG2 cells under normoxia and hypoxia. (C) ELISA assay for EPO in control or JMJD1A knockdown HepG2 cells under normoxia and hypoxia. All data are presented as the mean ± SEM from three independent experiments. $^*P < 0.05$.

(Fig. 6A–D), which was consistent with previous reports (Rankin et al.,2007; Xu et al.,2014).

Our results also showed that the binding activity of JMJD1 A to EPO HREs significantly increased, while the H3K9me2 levels decreased in the region. This result implied that JMJD1 A can be recruited to the *EPO* HREs by HIF-2α and mediates H3K9me2 demethylation, which is important for transcriptional activation of *EPO*. Hypoxia did not change the binding of HIF-2α and JMJD1 A or affect H3K9me2 levels in the regions without HRE (Fig. S1C and S1D).

Based on this result, we speculate that the difference of the global and local H3K9me2 levels depends to the binding of JMJD1 A to specific target genes. The binding can induce the activity of demethylase and achieve expression regulation of target genes, which helps to ensure the specificity of JMJD1 A under hypoxia.

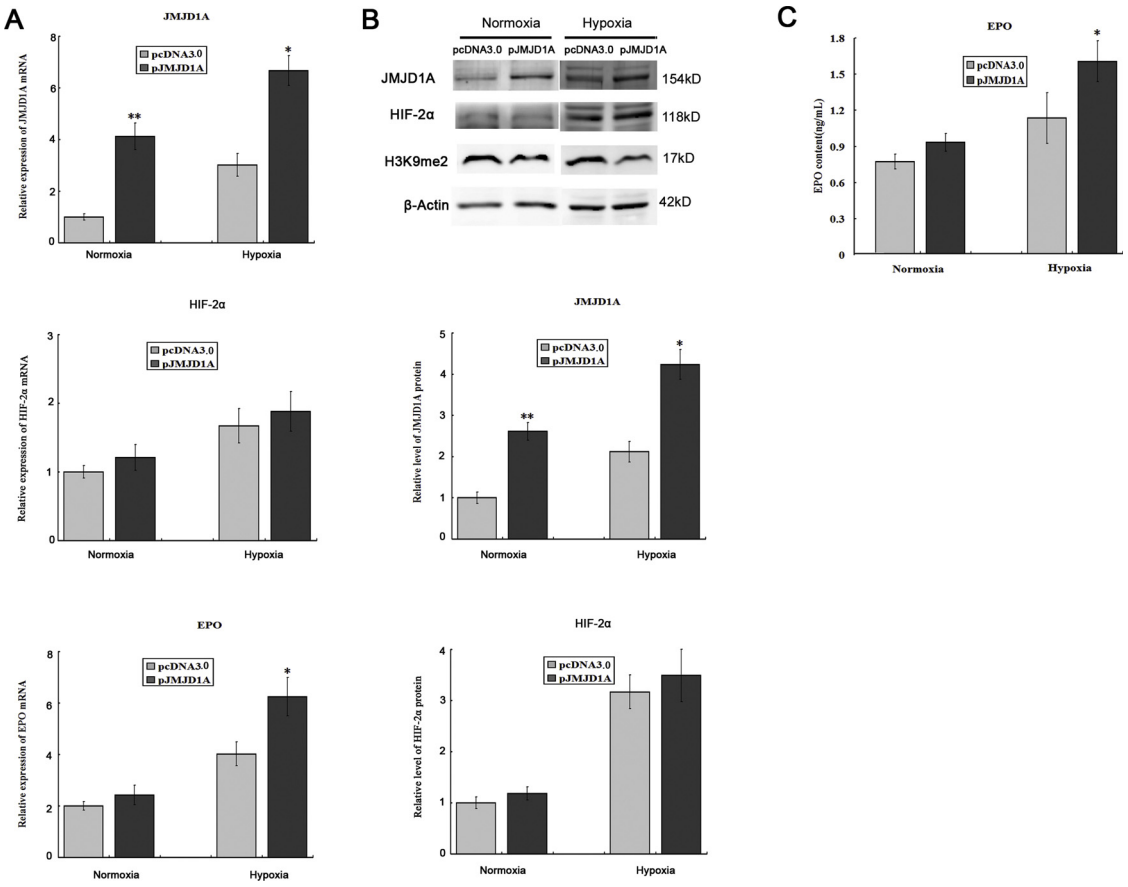

Fig. 4. JMJD1A overexpression increases the expression of EPO. (A) *HIF-2α* and *EPO* mRNA levels in control (pcDNA3.0) or JMJD1A overexpression (pJMJD1A) HepG2 cells by qRT-time PCR. (B) Western blot analysis for HIF-2α and H3K9me2 in control or JMJD1A overexpression HepG2 cells. (C) ELISA assay for EPO in control or JMJD1A overexpression HepG2 cells. All data are presented as the mean ± SEM from three independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$.

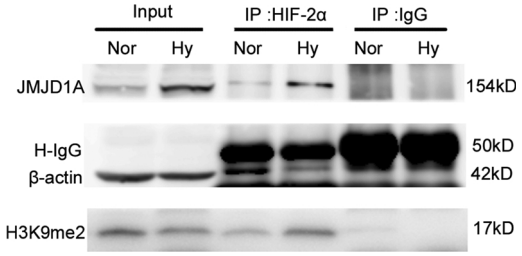

Fig. 5. JMJD1A interacts with HIF-2α. The interaction between JMJD1A and HIF-2α was investigated with a co-immunoprecipitation assay. The HIF-2α antibody was used for immunoprecipitation, and the JMJD1A antibody was used for detection. Immunoglobulin G heavy chain (H-IgG) was used to detect the presence of antibodies used in co-immunoprecipitation. The H3K9me2 antibody was used to detect the binding ability of HIF-2α with chromatin.

4. Discussion

Histone methylation is an important and widespread type of chromatin modification, which is known to influence many biological processes (Greer and Shi, 2012). Histone methylation is a reversible process catalyzed by methyltransferases and demethylases, which determines the precise regulation of gene expression (Dong and Weng, 2013). Di-methylation of lysine 9 of histone H3 (H3K9me2) is a characteristic mark of heterochromatin and transcriptional inhibition (Peters et al., 2003). Therefore, the demethylation of H3K9me2 can achieve transcriptional activation of specific gene.

In this study, we investigated the role of histone H3K9 demethylase JMJD1 A in EPO expression. We have shown that JMJD1 A is essential for EPO expression under hypoxia and that both JMJD1 A and EPO are regulated by HIF-2α. Thus, JMJD1 A is a new regulator of EPO expression, which is important for understanding of adaption mechanism under hypoxia.

EPO was specifically expressed in embryonic liver and postnatal kidney, but not in other organs (Querbes et al.,2012). The pattern of EPO expression suggested that it is related to heterochromatin, in which formation and maintenance are often associated with specific histone PTMs (Hall et al.,2002). Histone methylation influences many biological processes in the context of development and cellular responses by controlling gene expression (Greer and Shi, 2012). H3K9 methylation is essential for heterochromatin formation and gene repression (Audergon et al.,2015). An important example is that fetal haemoglobin (HbF) gene expression also has developmental specificity, and its transcription is regulated by H3K9 methylation (Krivega et al., 2015). Accordingly, we proposed that H3K9 methylation may also be involved in EPO regulation.

There are many histone H3K9 demethylases, including JMJD1 A, JMJD2 A, JMJD2B, and JMJD2C (Kooistra and Helin, 2012). Considering that EPO expression is regulated by hypoxia, we further analyzed several hypoxia-induced H3K9 demethylases, such as JMJD1 A, JMJD2B and JMJD2C (Beyer et al., 2018; Pollard et al., 2008; Xia et al., 2009). One study reported that the activities of the JmjC domain containing histone demethylases required oxygen but that only JMJD1 A maintained its catalytic activity even under severe hypoxia (Lee et al., 2013). Based on these facts, we chose JMJD1 A as the experimental object to study its regulatory effect on EPO expression.

JMJD1 A plays an active role in the coordination of specific transcription factors. JMJD1 A is involved in sperm development by facilitating androgen (AR) receptor-mediated transcription activation

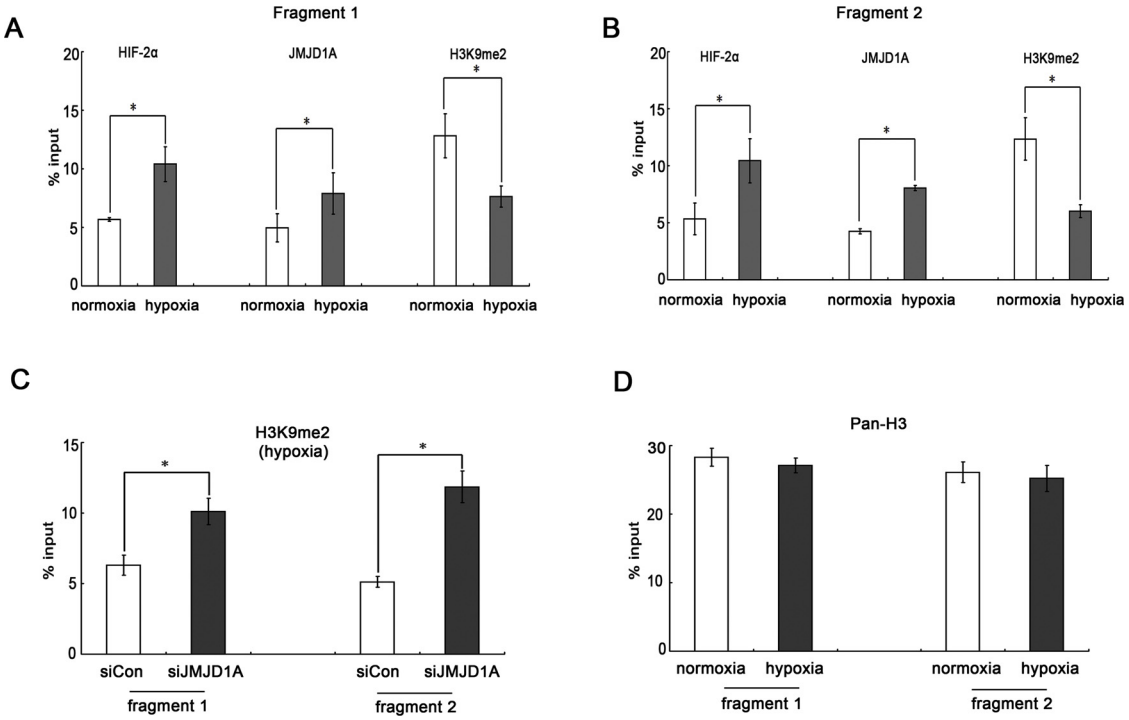

Fig. 6. JMJD1A regulates H3K9 demethylation in the region of *EPO* HREs. ChIP analysis was carried out with antibodies against HIF-2α, JMJD1A, H3K9me2 and pan-H3. (A) The q-PCR results of ChIP DNA fragment 1 (B) The -PCR results of ChIP DNA fragment 2 (C) The q-PCR results of ChIP DNA fragment 1 and 2 with H3K9me2 antibody in control (siCon) or JMJD1A knockdown (siJMJD1A) HepG2 cells under hypoxia (1% O_2). (D) The q-PCR results of ChIP DNA fragments 1 and 2 with pan-H3 antibody under normoxia (21% O_2) and hypoxia. All data are presented as the mean ± SEM from three independent experiments. *$P < 0.05$.

(Yamane et al., 2006). JMJD1 A also plays an important role in regulating the expression of metabolic genes by recruiting peroxisome proliferator-activated receptor α (PPARα) (Tateishi et al.,2009). JMJD1A also participates in cancer development by forming a co-activator complex with HIF-1α (Krieg et al., 2010). In this study, our results demonstrated that JMJD1 A can interact with HIF-2α, which means that JMJD1 A regulated EPO expression by HIF-2α-mediated transcription activation.

In addition to the recruitment of transcriptional activators, JMJD1 A plays a biological role by catalyzing demethylation of the transcriptional repressor H3K9me2. In the process of mouse sex determination, Jmjd1a regulates expression of the sex-determining gene *Sry* by eliminating H3K9me2 marks (Kuroki et al., 2013). Other studies have also shown that JMJD1 A plays a transcriptional activation role partly by demethylating H3K9me2 (Abe et al., 2015; Lee et al., 2013). Our results demonstrated that JMJD1 A can reduce H3K9me2 level in EPO HRE region, which proved that JMJD1 A adopts a similar mechanism in the regulation of EPO expression. One disadvantage of this study is that we have not carried out JMJD1 A inhibitor experiments to provide more direct evidence for EPO expression regulation by JMJD1 A-catalyzed H3K9me2 demethylation. Previous studies have shown that histone acetylation also affects the expression of EPO (Rankin et al., 2007; Xu et al., 2014), and our study further confirmed that histone methylation is also important for EPO expression. Because the methylation and acetylation of histones at the same site H3K9 are mutually exclusive, we suggested that histone H3K9 demethylation and subsequent acetylation may play roles in de-inhibition and re-activation in EPO expression, respectively.

In summary, we propose the hypothesis that JMJD1 A regulates the expression of EPO. Under hypoxia, JMJD1 A was induced by HIF-2α and formed a coactivator complex in the nucleus to increase EPO expression by JMJD1A-mediated H3K9me2 demethylation in EPO HRE (Fig. 7).

In conclusion, we identified a new epigenetic mechanism of EPO expression regulation, by which JMJD1A involved in EPO transcription activation by HIF-2α and H3K9me2 demethylation. These findings have important implications for pharmacological strategy that aims to target JMJD1A for anemia and polycythaemia treatment.

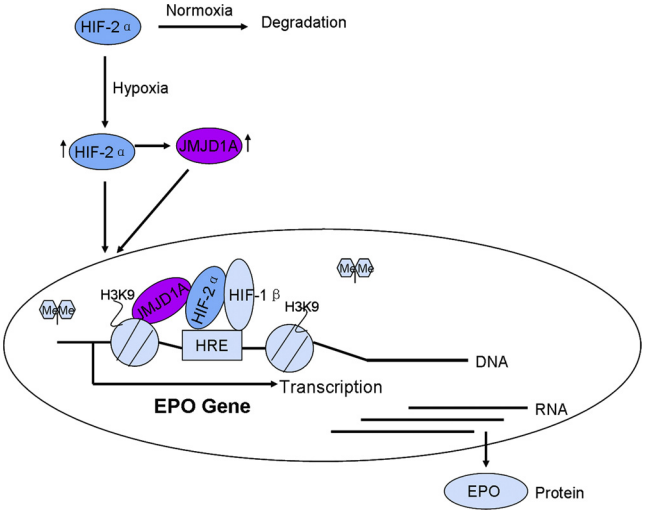

Fig. 7. Proposed model describing the role and mechanism of JMJD1A in the regulation of EPO expression under hypoxia. Under hypoxia, the HIF-2α protein level is increased and induces the expression of JMJD1A. In the nucleus, HIF-2α forms heterodimers with HIF-1β and recruits JMJD1A to EPO HRE, which increases EPO gene transcription by catalyzing H3K9me2 demethylation.

Conflict-of-interest disclosure

The authors declare no competing financial interests.

Authorship

Contribution: X.D. and X.G. designed research, analyzed and interpreted the data, and wrote the manuscript; Z. T., L.Y., X.G and Y.S. performed experiments and analyzed data. All these authors reviewed the manuscript.

Acknowledgment

This work was supported by the Natural Science Foundation of Hebei Province (No H2014205082 and H2016423023).

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.biocel.2019.01.022.

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