Decreased mixed lineage leukemia 1 is involved in endometriosis-related infertility

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Abstract

The aberrant histone methylation patterns contribute to the pathogenesis of endometriosis (EM). Mixed lineage leukemia 1 (MLL1), a histone methyltransferase, is crucial for gene expression by catalyzing the trimethylation of histone 3 lysine 4 (H3K4me3) in gene promoter. This study aimed to explore whether MLL1 is involved in EM-related infertility. The expressions of MLL1 and H3K4me3 were analyzed in the eutopic endometria from EM women with infertility (n = 22) and the normal endometria from EM-free women (n = 22). Mouse EM model was established. The MLL1 and H3K4me3 expression patterns in mice endometria of early pregnancy were also investigated. Immortalized human endometrial stromal cells (iESCs) were cultured and underwent in vitro decidualization. The chromatin immunoprecipitation followed by deep sequencing (ChIP-seq) was performed to find the target gene of MLL1 during decidual process. Results showed that both MLL1 and H3K4me3 decreased in the eutopic endometrium from EM patients compared to that in the normal endometrium. During early pregnancy and the decidual process, MLL1 and H3K4me3 were significantly upregulated in stromal cells. ChIP-seq and ChIP-qPCR found that the cytochrome c oxidase subunit 4I 2 (COX4I2) was directly targeted by MLL1. The dominance of COX4I2-containing enzyme induced the expression of hypoxia-inducible factor-2α (HIF-2α), whose expression in the peri-implantation endometrium is essential for embryo implantation. Further results showed that MLL1 was directly regulated by progesterone (P4) – P4 receptors (PRs). Our study proved that MLL1 was involved in EM-related infertility, which may provide a novel approach to treat the nonreceptive endometrium in EM patients.

Introduction

Endometriosis (EM), defined by the presence of endometrial cells outside the uterus, is one of the most common benign gynecological diseases and afflicts more than 10% of women of reproductive age (Greene et al. 2016). Among patients with EM, approximately 50% have fertility problems and approximately 68% of EM-related infertility is caused by minimal or mild EM (Tanbo & Fedorcsak 2017). Although EM is a major cause of female infertility, the pathogenesis is still unclear. Recent studies revealed that the nonreceptive endometrium is an underlying cause for observed infertility in minimal or mild EM (Marquardt et al. 2019) and epigenetic aberrations have been strongly indicated to contribute to endometrial dysfunction (Monteiro et al. 2014, Houshdaran et al. 2016).
Lysine (K)-specific methyltransferase 2A, also known as mixed lineage leukemia 1 (MLL1), is an important member of histone methyltransferases (HMT) family. MLL1 can specially catalyze the trimethylation of histone 3 lysine 4 (H3K4me3) via its conserved SET domain (Xue et al. 2019). The previous studies found that MLL1 could be recruited to the promoter region of HOXA10 and catalyze H3K4me3 in this region to induce HOXA10 transcription, together with other progesterone (P4)/estrogen signalings to produce a receptive uterus capable of embryo implantation (Yao et al. 2014, Jiang et al. 2017). However, the function of MLL1 in EM remains unclear. Considering the aberrant histone methylation patterns in endometria from EM patients (Monteiro et al. 2014), we speculated that MLL1 was involved in EM development. In the current study, we analyzed the expression of MLL1 before and after EM onset and further focused on the function of MLL1 in regulating endometrial receptivity to dissect the role of MLL1 in EM-related infertility.

Methods

Collection of human endometrial tissues

From July 2018 to June 2020, eutopic endometrial biopsy samples were collected from EM patients with infertility and fertile women undergoing tubal ligation (for each group, n = 22, 8 in proliferative phase, 7 in early secretory phase, and 7 in mid-secretory phase) at the Gynaecology and Obstetrics Department of Zhongnan Hospital of Wuhan University. Ovarian EM lesions and the corresponding eutopic endometrial biopsies were also obtained from patients (n = 10 pairs, proliferative phase). Normal endometria obtained from fertile women were served as the control (n = 10, proliferative phase). Women were all between the ages of 22 and 40. EM was confirmed by pathological results. Women who were laparoscopically free of EM were served as the control. All women had regular menstrual cycles and had no history of hormonal treatment within the previous 3 months. The menstrual cycle phase was confirmed by menstrual history combined with histological results.

Induction of EM in mouse

Five-week-old female C57BL/6 mice were purchased from Charles River Laboratories (Beijing, China). Mice were housed in a facility with a 12 h light:12 h darkness cycle maintained at 25 ± 0.5°C and 50–60% humidity. Before modeling, all mice were adaptively fed for 1 week. The donor mice were injected with estradiol (E2) (3 μg/mouse, s.c.) for 1 week, then the donor mice were euthanized, the two horns were isolated and the endometrial tissue was scraped out and suspended in saline. Equal amounts of the endometrial fragment from one donor mouse were injected into the peritoneum of two recipient mice (0.5 mL/mouse). Mice injected with 0.5 mL saline without endometrial tissue were taken as the control. Mice were kept housing for 3 weeks. Hematoxylin and eosin (HE) staining was performed to confirm the success of modeling (Supplementary Fig. 1, see section on supplementary materials given at the end of this article).

Vasectomy

Vasectomy was performed on 7-week-old male mice. Briefly, mice were anesthetized and a ~0.5 cm incision was made in abdomen to expose the deferent duct. One of the ducts was ligatured with the suture and was cut off using scissors. The same procedure was performed on the other side duct. After the surgery, mice were housed for 1 week to recover.

In vitro fertilization

For oocyte collection, the female mice were superovulated by i.m. injection of gonadotropin (PMSG, 5 IU), followed by i.m. injection of hCG (5 IU) 48 h later to trigger ovulation. Mice were sacrificed and the oocytes were collected by flushing the oviducts. The oocytes were cultured in the KSOM-AA solution (ELITE-MEDIA, China). For sperm collection, 7-week-old male mice were euthanized and the cauda portion of the epididymis was minced and put into the TYH medium (Easycheck, China). The sperm were allowed to swim out for 1 h, then the sperm was collected and incubated at 37°C with 5% CO₂ for another 1 h. After that a droplet (approximately 30 μL) was loaded into the petri dish and 15–20 oocytes were introduced to each droplet. Six hours after the co-incubation, the fertilized eggs were transferred to KSOM-AA medium. The successful fertilization was determined by the presence of the second polar body. In this study, the sperm concentration in TYH medium was ~1.5 × 10⁶/mL.

Cell culture

Immortalized human endometrial stromal cells (iESCs) were purchased from American Type Culture Collection (SC-6000). Cells were authenticated using STRS analysis.
Cells were cultured in 1:1 formula of Dulbecco’s modified Eagle’s medium and Ham’s F-12 medium with no phenol red (DMEM/F-12) (GENOM, China) and containing 10% charcoal treated fetal bovine serum (CLARK Bioscience, USA). Cells were incubated at 37°C with 5% CO2. In this study, the passage number of cells <20.

Cell transfection

siRNAs (Ribobio, China) were used to knock down the expression of target genes. A non-targeting siRNA was used as negative control (NC). The sequences of siRNAs are shown in Supplementary Table 1. Vectors of PGL4.21-COX4I2 were constructed and purchased from the VectorBuilding (Shanghai, China), and the empty vectors were used as the NC. The vectors were amplified in Escherichia coli by being shaken at 37°C for 24 h, then the vectors were extracted using the Plasmid Extraction Kit (TIANGEN, China) according to the manufacturers’ instructions. The transfection was performed using Lipofectamine 3000 (Invitrogen) according to the manufacturers’ instructions.

In vitro decidualization

In vitro decidualization was induced according to the previous report (Xiong et al. 2020). Briefly, cells were cultured in DMEM/F12 containing 2% charcoal treated fetal bovine serum and supplemented with 10 nM E2, 1 mM medroxyprogesterone acetate (P), and 50 μM cAMP (C) (EPC). For the individual treatment with P, E2, and C, each substance was added to the culture medium alone and lasted for 6 days. The culture medium was replaced every other day.

In vivo decidualization

The in vivo decidualization was induced according to the previous report (Fullerton Jr et al. 2017). Briefly, sexually mature female mice were randomly paired with a vasectomized male; the day a plug observed was designated as day 0.5 of gestation (GD 0.5). On GD 4.5, 10 μL of corn oil was injected into the lumen of one of the uterine horns. On GD 7.5, mice were euthanized and the uterus was taken out and weighed immediately. The surgery was performed under aseptic conditions.

Immunohistochemistry

Immunohistochemistry was performed on paraffin-embedded sections. Briefly, the tissue sections were dewaxed and hydrated. After being soaked with 5% BSA for 0.5 h at the room temperature, tissues were incubated separately with MAB against MLL1 (1:200, L19062105, United States Biological, USA), H3K4me3 (1:2000, 9751, Cell Signaling Technology), COX4I2 (1:300, 11463-1-AP, Proteintech Group, China), HIF-1α (1:250, ab114977, Abcam), HIF-2α (1:1000, ab109616, Abcam), PRs (1:1000, 8757, Cell Signaling Technology), and PRB (1:500, 3157, Cell Signaling Technology) overnight at 4°C. Then the secondary antibody (SV-0002, BOSTER Biological Technology, China) was added and incubated for another 0.5 h. All slides were visualized using DAB-Substrate (ZSGB-Bio, China) and photographed using the Aperio ePathology Scanner (Leica). Protein expression was quantified according to the H-score: H-score = Pi(i), where i is the intensity of staining with a value of 1, 2, or 3 (weak, moderate, or strong, respectively) and Pi is the percentage of stained cells for each intensity in the range of 0–100%.

Western blot analysis

Cells were lysed on ice and lysates containing total proteins were collected. Equal amount of proteins from each group were separated by 10% SDS-polyacrylamide gel, then were transferred onto a polyvinyl difluoride (PVDF) membrane. After soaked in 5% BSA, membranes were incubated with one of the following primary antibodies overnight at 4°C: anti-MLL1 antibody (1:200, L19062105, United States Biological, USA), anti-H3K4me3 antibody (1:2000, 9751, Cell Signaling Technology), HIF-1α (1:250, ab1, Abcam), anti-HIF-2α antibody (1:500, ab199, Abcam), anti-COX4I2 antibody (1:1000, 11463-1-AP, Proteintech Group, China), and anti-tubulin antibody (1:2000, 10068-1-AP, Proteintech Group, China). Membranes were then exposed to the secondary HRP-tagged antibodies (1:5000, SA00001-1 or SA00001-2, Proteintech Group) for another 0.5 h at 37°C. The blots were visualized using ECL plus kit (Beyotime, China) and the bands were quantified using Image J (NIH).
Chromatin immunoprecipitation assay (ChIP) and ChIP followed by deep sequencing (ChIP-seq)

ChIP assay was performed using the Simple ChIP Plus Sonication Chromatin IP Kit (56383, Cell Signaling Technology) according to the manufacturer’s instructions. Briefly, cells or tissues were cross-linked, lysed, and sheared by sonication to produce DNA fragments with an average length of approximately 500 bp. One percent of the chromatin fragments were separated and stored at −80°C as the input. For each IP reaction, 50 µg of DNA was immunoprecipitated using antibodies against MLL1 (1:200, A300-374A, BETHYL, USA), or antibodies against H3K4me3 (1:50, 9751, Cell Signaling Technology). Normal rabbit IgG was added as the control. The sequences of the primers used for MLL1 response element in the COX4I2 gene were provided in Supplementary Table 1. ChIP-seq analyses were performed using the ChIP-IT High Sensitivity Kit (Active Motif) on iESC. The model-based analysis of ChIP-Seq (MACS) peak-finding algorithm was used to normalize ChIP against the input control.

RNA isolation, cDNA synthesis, and qPCR

Total RNA was isolated using Invitrogen™ TRIzol™ Reagent (Thermo Fisher Scientific). cDNA was synthesized from total RNA using PrimeScript™ RT Master Mix (Takara). qPCR was performed on the Bio-Rad CFX96 (Bio-Rad Laboratories) using SYBR Premix Ex Taq™ (Takara). The sequences of primers were provided in Supplementary Table 1. The quantity of each transcript was calculated using comparative threshold cycle (Ct) normalized to GAPDH and the relative expression of the target gene was calculated by the 2^ΔΔCt method.

Statistical analysis

In this study, data were all analyzed by SPSS version 22.0 (SPSS Inc.) and GraphPad Prism 6.0 (GraphPad Software). Results were obtained from at least three independent experiments. For continuous variables, data were shown as mean ± S.E.M. Student’s t-test was used to analyze the difference between two groups and one-way ANOVA was used for multiple groups. Categorical variables were analyzed using the Chi-square test. P < 0.05 were taken as statistically significant.

Results

MLL1 and H3K4me3 are decreased in eutopic endometrium after EM onset

Fertility tests showed that both control and EM mice had normal ovulation and fertilization (Supplementary Table 2) but the average of pups/litter and the average number of litters/mouse were significantly lower in EM mice compared to that in control mice (Supplementary Table 3), indicating that the receptivity of endometrium was impaired in EM mice. In normal endometrium, MLL1 was abundant in the secretory phase, especially in the mid-secretory phase. However, in eutopic endometrium from EM patients, MLL1 expression was faint throughout the whole menstrual cycle (Fig. 1A). In mice EM model, MLL1 was upregulated with the extension of gestation time in control mice, however, in EM mice, MLL1 expression was faint throughout the whole early pregnancy period (Fig. 1B). In the estrous cycle, the expression of MLL1 was strong in the phase of metestrus in control mice but was weak through the whole cycle in EM mice (Supplementary Fig. 2). The expression pattern of H3K4me3 was similar to MLL1, highly expressed in human mid-secretory phase endometrium and mouse GD 5.5 endometrium but faint in the endometrium from EM patients and EM mice (Supplementary Fig. 3).

MLL1 is essential for endometrium decidualization

MM102 had no effects on the ovarian morphology and serum hormone level on GD 5.5 (Supplementary Fig. 4). However, compared to the Veh treated uterus, uteri treated with MM102 exhibited significant decidual defect, which was verified by the dysregulation of decidual markers, bone morphogenetic protein 2 (Bmp2) and wingless-related mouse mammary tumor virus integration site 4 (Wnt4), in the endometrium from the uteri treated with MM102 (Fig. 2A). In vitro decidualization showed that MLL1 and H3K4me3 increased significantly during EPC treatment, whereas siMLL1 inhibited the increase of H3K4me3, as well as the decidualization of iESCs, which is evident by the marked decrease of decidual markers, prolactin (PRL) and insulin-like growth factor-binding protein-1 (IGFBP1) (Fig. 2B and C). Besides, the morphological change of iESCs during decidualization was not typical in cells transfected with siMLL1 (Supplementary Fig. 5).
Figure 1
MLL1 is decreased in eutopic endometrium after EM onset. (A) MLL1 is decreased in eutopic endometrium from EM patients with infertility compared to that from EM-free women. (B) The expression of MLL1 in eutopic endometrium is dysregulated in early pregnancy in EM mice. Ten mice in each group. Data were shown as mean ± s.e.m. ***P < 0.001.
Figure 2

MLL1 is essential for endometrium decidualization. (A) The uterine horn treated with MM102 showed decidual defect. (B) MLL1 and H3K4me3 were upregulated in iESCs during EPC treatment, whereas siMLL1 inhibited the upregulation of H3K4me3. (C) Loss of MLL1 resulted in decidual defect in iESCs, which was evident by the decreased expression of PRL and IGFBP1. Three mice in each group. Data were shown as mean ± s.e.m. **P < 0.01, ***P < 0.001.
Loss of MLL1 leads to the dysregulation of cytochrome c oxidase subunit 4I2 (COX4I2)

To identify the target gene of MLL1, we performed ChIP-seq using iESCs with or without EPC treatment. Motif analysis of MLL1 interval sequences using the Cistrome motif database identified several transcription factor motifs, including E26 transformation-specific (ETS) domain, leucine zipper (bZIP) family, helix-loop-helix (bHLH) family, hormone-nuclear receptor (NR) family, and the myeloblastosis (Myb) family (Supplementary Fig. 6). Pathway analysis identified the involvement of the metabolic and the oxidative phosphorylation pathways, which are related to the activation of the COX family (Fig. 3A). GeneGo analysis revealed that a large amount of DNA fragments of COX4I2 were enriched by anti-MLL1 antibody in decidual cells compared to control cells (Fig. 3B). This gene includes conserved MLL1-binding elements (MLL1-BE) in its promoter region (Fig. 3C). To determine whether the recruitment of MLL1 is dependent on the MLL1-BE on COX4I2 promoter, substitutions and deletion mutations of the MLL1-BE were made on pGL4.21-Basic plasmid including COX4I2 gene (Fig. 3D), which was then transfected into iESCs. The results of ChIP assay showed that MLL1-BE on COX4I2 promoter is critical for the recruitment of MLL1 (Fig. 3E). Mouse model showed that COX4I2 was upregulated in the endometrium on GD 5.5 in control mice but its expression was faint in EM mice (Fig. 3F). The same results were observed in human endometrium, expression of COX4I2 increased significantly in normal endometrium of secretory phase, however, its expression in eutopic endometrium from EM patients was faint throughout the whole menstrual cycle (Fig. 3G).

MLL1 attenuation results in the aberrant H3K4me3 level in the promoter of COX4I2 gene

Since MLL1 is mainly responsible for the catalysis of H3K4me3, we next analyzed the H3K4me3 level in COX4I2 promoter. In vitro study showed that the recruitment of MLL1 to the MLL1-BE of COX4I2 was increased during the process of EPC treatment as well as the H3K4me3 level in this region. However, siMLL1 significantly blocked the upregulation of the recruitment of MLL1 to the MLL1-BE as well as the increase of the H3K4me3 level in this region (Fig. 4A). The same results were observed in human endometrium, the recruitment of MLL1 to the MLL1-BE and the H3K4me3 level in this region were both increased significantly in the normal endometrium of secretory phase, but had no significant change in the eutopic endometrium from EM patients in different menstrual cycle phase (Fig. 4B). Besides, in vitro study showed that the knockdown of MLL1 significantly inhibited the increase of COX4I2 during EPC treatment (Fig. 4C).

COX4I2 is essential for stoma decidualization by inducing hypoxia-inducible factor-2α (HIF-2α) expression

COX4I2 was reported to have a decreased oxygen affinity and its dominance may lead to the hypoxia microenvironment of endometrium (Pajuelo Reguera et al. 2020). We next analyzed the expression pattern of HIF-1α/HIF-2α during decidualization. No significant change of HIF-1α expression was found during in vitro decidualization and it expressed faintly in mouse stroma cells during the peri-implantation period (Supplementary Fig. 7), which is consistent to previous report (Daikoku et al. 2003). However, the HIF-2α expression increased significantly during in vitro decidualization and its expression was regulated by MLL1 and COX4I2 (Fig. 5A). Besides, the morphological change of iESCs during decidualization was not typical in cells transfected with siCOX4I2/siHIF-2α (Supplementary Fig. 5). We also observed that the decidual markers decreased markedly in cells transfected with siCOX4I2/siHIF-2α compared to control cells (Fig. 5B), indicating the decidual defect in iESCs without COX4I2/HIF-2α expression. Animal studies showed that HIF-2α protein level decreased significantly in the eutopic endometrium from EM mice on GD 5.5 compared to the endometrium from the control mice (Fig. 5C). Human study also showed that the HIF-2α was dysregulated in the eutopic endometrium from EM patients with infertility compared to that in the normal endometrium from EM-free women (Fig. 5D).

We also analyzed the expression pattern of MLL1/COX4I2/HIF-2α in EM lesion and eutopic/normal endometrium. Results showed that compared to normal endometrium, the expression level of MLL1/COX4I2/HIF-2α in eutopic endometrium and lesion from both human and mouse sources was downregulated (Supplementary Fig. 8).

MLL1 is regulated by P4-P4 receptors (PRs)

To investigate which molecule regulates MLL1 expression, iESCs were cultured in medium containing EPC, E2, P, and C, respectively. Results showed that MLL1 was increased most significantly in cells undergoing EPC and P treatment (Fig. 6A). Besides, PRs inhibitor Mifepristone (HY-13683,
Figure 3
Loss of MLL1 leads to the dysregulation of COX4I2. (A) Pathway analysis identified the involvement of the metabolic and the oxidative phosphorylation pathways. (B) GeneGo analysis revealed that a large amount of DNA fragments of COX4I2 were enriched by anti-MLL1 antibody in decidual cells compared to control cells. (C) COX4I2 includes conserved MLL1-binding elements (MLL1-BE) in its promoter region. (D and E) Substitutions and deletion mutations of the MLL1-BE were made on pGL4.21-Basic plasmid including COX4I2 gene. ChIP assay showed that MLL1-BE on COX4I2 promoter is critical for the recruitment of MLL1. (F) Mouse model showed that COX4I2 in endometrium was upregulated on GD 5.5 in control mice but its expression was faint in EM mice. (G) The expression of COX4I2 increased significantly in normal endometrium of secretory phase, however, its expression in eutopic endometrium from EM patients was faint throughout the whole menstrual cycle. Ten mice in each group. Data were shown as mean ± s.e.m. ***P < 0.001.
MCE, USA) but not ERs inhibitor Fulvestrant (ICI 182780, MCE, USA) significantly blocked the P-induced MLL1 expression (Fig. 6A), suggesting that MLL1 is regulated by P4-PRs-related pathway. Besides, siMLL1 markedly blocked P-induced overexpression of COX4I2 and HIF-2α during EPC treatment (Fig. 6B), indicating that COX4I2 and HIF-2α were regulated by MLL1 via P4-related pathway.

We used the PROMO database to predict the BE of PRs in MLL1 promoter and found 1 potential BE for both PRA and PRB (Fig. 6C). ChIP assay showed that the recruitment of PRs to the PRs-BE of MLL1 was increased significantly in cells with P treatment (Fig. 6D), indicating that MLL1 expression is regulated directly by P4-PRs. We next detected the PRs expression in human endometrium. Results showed that both the total PRs and PRB were decreased in the eutopic endometrium from EM patients with infertility compared to that in the normal endometrium from EM-free women (Fig. 6E).

Taken together, our study revealed that MLL1 expression increased in peri-implantation endometrium under the regulation of P4-PRs. Highly expressed MLL1 binds to the promoter of COX4I2 and promotes its expression. The dominance of COX4I2-containing enzyme induces the expression of HIF-2α, together with other P4/E2 signalings to produce a receptive uterus. However, in the eutopic endometrium from EM patients with infertility, PRs expression was decreased and that leads to the dysregulation of MLL1, which results in the decidual-defect endometrium where the embryo can not implant successfully (Fig. 7).

**Discussion**

The aberrant epigenetic modifications were proved to be involved in the pathogenesis of EM. DNA methylation, histone deacetylation, and histone methylation have shown different patterns in EM compared with normal endometrium (Xiaomeng et al. 2013, Barjaste et al. 2019). As a histone methyltransferase, MLL1 can specially catalyze the H3K4me3, which marks the activation of gene transcription (Liu et al. 2016). In this study, we found that MLL1 expression increased significantly during the early pregnancy, while MLL1 ablation leads to the decidual defect of endometrium, these results indicated that
Figure 5
COX4I2 is essential for stoma decidualization by inducing HIF-2α expression. (A) The expression of HIF-2α in iESCs was increased significantly during EPC treatment, however, in cells transfected with siCOX4I2 or siMLL1, no significant change of HIF-2α expression was observed. (B) The decidual markers decreased markedly in cells transfected with siCOX4I2/siHIF-2α compared to control cells. (C) HIF-2α protein level decreased significantly in the eutopic endometrium from EM mice on GD 5.5 compared to the endometrium from the control mice. (D) The expression of HIF-2α was dysregulated in the eutopic endometrium from EM patients with infertility compared to that in the normal endometrium from EM-free women. Ten mice in each group. Data were shown as mean ± S.E.M. *P < 0.05.
MLL1 is indispensable for the transition of endometrium to a receptive state. HOXA10 was reported as one of the downstream genes of MLL1, whose dysregulation in endometrium is involved in the failure of embryo implantation (Yan et al. 2018). In the present study, we found that COX4I2 was also targeted by MLL1. During the early pregnancy period, COX4I2 was upregulated together with MLL1. In vitro study showed that the upregulation
of COX4I2 during EPC treatment could be blocked by siMLL1. The increased H3K4me3 level in the MLL1-BE of COX4I2 promoter during EPC treatment is another evidence for the interaction between MLL1 and COX4I2.

COX4 is ideally thought to serve as a regulatory factor by interacting with other subunits of the COX enzyme. Its C-terminal part protrudes into the intermembrane space where it interacts with COX2 and helps shape the docking site for cytochrome c (Böttinger et al. 2013). The COX4 subunit is thought to optimize respiratory chain function by controlling expression of its isoforms COX4I1 and COX4I2 (Kocha et al. 2015). The function of these two isoforms is not clear yet. However, a recent study revealed that the COX4I2-containing enzyme had decreased oxygen affinity (Reguera et al. 2020). In this case, the dominance of COX4I2 may result in a hypoxia microenvironment in endometrium. Actually, the hypoxia status has been indicated to be crucial for successful implantation and angiogenesis in endometrium (Okada et al. 2014, Ma et al. 2017, Bagheri et al. 2018), and this may be associated with a key molecule--HIF-2α. HIF is a major transcription factor that responds to hypoxia and induces the expression of hypoxia-related genes such as VEGF and erythropoietin (Macklin et al. 2017). A previous study showed that HIF-1α is primarily expressed in the uterine luminal epithelium during the peri-implantation period, and the expression of HIF-2α is in both the stroma and the epithelium at the same time, whereas HIF-3α is undetectable in the uterus (Daikoku et al. 2003). HIF-2α in peri-implantation endometrium allows trophoblast invasion through detachment of the luminal epithelium and activation of an embryonic survival signal (Matsumoto et al. 2018). In this study, we analyzed the expression pattern of HIF-2α in iESCs and found it increased during in vitro decidualization. Besides, siCOX4I2 blocked the increase of HIF-2α, suggesting that HIF-2α expression in stroma can be regulated by the switch of COX4 isoforms.

Finally, we investigated which pathway was MLL1 regulated by. As we expected, MLL1 was regulated by P4-PRs pathway. After being activated by P4, PRs can bind to the promoter of MLL1 and promotes the transcription of MLL1 directly. However, the expression of PRs is decreased in the eutopic endometrium from EM patients with infertility, which leads to the inactivation of P4-PRs pathway, and further results in the dysregulation of MLL1.

**Conclusion**

In conclusion, this study first explored the function of MLL1 in EM-related infertility and found that the dysregulation of MLL1 leads to the decidual-defect nonreceptive endometrium. These results provide us a novel approach to diagnose and treat the nonreceptive endometrium in EM women.

**Supplementary materials**

This is linked to the online version of the paper at https://doi.org/10.1530/JME-20-0193.

**Declaration of interest**

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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**Ethical approval**

This study was approved by the Ethics Committee of Zhongnan Hospital, Wuhan University (China). Written informed consent was obtained from patients following the principles of the Declaration of Helsinki. All animal experiments were performed following the 3R principle of experimental animals and complied with the ethical regulations approved by the Center for Animal Experiment/Animal Biosafety Level-III Laboratory.
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