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ORIGINAL ARTICLE

Knockdown of long non-coding HOTAIR enhances the sensitivity to progesterone in endometrial cancer by epigenetic regulation of progesterone receptor isoform B

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Abstract

Purpose Progesterone, particularly medroxyprogesterone acetate (MPA) has been mainly used for young endometrial carcinoma (EC) patients with conservative treatment. However, its treatment benefits are limited by insensitivity or acquired resistance. In this study, we aim to investigate the effect of long non-coding RNA HOTAIR on progesterone sensitivity in endometrial cancer, as well as the underlying mechanisms.

Methods The expression of HOTAIR was detected by quantitative real-time PCR. The impact of MPA on the endometrial cancer cells was examined by MTT, colony formation, apoptosis-related protein detection and flow cytometry. Chromatin immunoprecipitation (ChIP) assay was performed to detect the regulatory mechanism between HOTAIR and progesterone receptor B (PRB). We further confirm the function of HOTAIR in vivo though xenograft tumor assay.

Results We found that HOTAIR inversely correlated with PRB expression in endometrial carcinoma. Knockdown of HOTAIR promoted the MPA sensitivity by upregulating PRB, which can be largely reversed by PRB downregulation. Moreover, inhibiting LSD1, a HOTAIR-associated protein that removed activating H3K4me2 chromatin marks, induced PRB expression and promoted apoptosis induced by MPA. We further showed that silencing HOTAIR strengthened the H3K4me2 occupation on the promotor of PRB.

Conclusions Our findings provide compelling evidence that HOTAIR and LSD1 collaboratively repress PRB expression and thus mediate progesterone sensitivity in endometrial carcinoma cells. HOTAIR is a potential predictor for progesterone response in EC and down-regulated expression of HOTAIR might be an effective strategy for overcoming progesterone resistance.

Keywords Endometrial cancer · HOTAIR · Progesterone sensitivity · Epigenetic modification

Introduction

Endometrial cancer (EC) is the fifth most common female cancer, and the occurrence rate of EC is ascending worldwide [1]. Most cases occur in postmenopausal women, but

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20% of patients are diagnosed before menopause, and 5% of those diagnosed cases are in women < 40 years of age [2]. The endometrium is particularly sensitive to steroid hormones, and long-term exposure to estrogens unopposed by progesterone or progestin may be a predisposing factor of endometrial cancer. Hormonal therapy, such as medroxyprogesterone acetate (MPA), has been applied in those patients who desire fertility and have well-differentiated early-stage EC, or those patients with recurrent or advanced stage EC [3, 4]. Unfortunately, due to primary or acquired resistance, more than 30% of well-differentiated adenocarcinoma patients did not respond to progesterone [5–7]. However, the precise mechanism underlying progesterone resistance is not understood, and improving the sensitivity of progesterone therapy has been a challenge.

Progesterone responsiveness is mediated primarily through its receptors, progesterone receptor A (PRA) and progesterone receptor B (PRB), members of the steroid hormone receptor superfamily of ligand-activated transcription factors. Both PRA and PRB are derived from two different promoter usages in a single gene progesterone receptor (PGR). PRB differs from PRA by the extra 164 amino acids it contains at the amino terminus [8]. Accumulating evidence indicates that PRB, but not PRA, highlights a pivotal role in the pathogenesis of EC [9–11]. Furthermore, studies have led to the proposition that downregulating expression of PRB in EC cells could account for progesterone treatment failure [12–14]. Nonetheless, there is a slightly new strategy put forward to maintain or enhance PRB function.

With the advent of high-throughput DNA sequencing, long non-coding RNAs (lncRNAs), which is greater than 200 nt in length, step onto the stage of functional genomics. LncRNA are involved in complicated biological processes through diverse mechanisms in various cancers [15–17]. Regarding the field of multi-drug resistance of tumors, several recent studies identified lncRNAs as the key mediator [18, 19]. HOX transcript antisense intergenic RNA (HOTAIR) has been serving as an oncogene and an adverse prognostic factor in various tumors including EC [20]. HOTAIR recruits and binds with the polycomb repressive complex 2 (PRC2) and lysine-specific demethylase 1 (LSD1), which enhances histone 3 lysine 27 (H3K27) trimethylation, and decreases histone 3 lysine 4 (H3K4) dimethylation, respectively, to epigenetically regulate target gene expression [21]. In addition, HOTAIR produces an effect on target gene expression by competitive binding to miRNAs [22, 23]. Recent studies have demonstrated the crucial functions of HOTAIR in tumor chemoresistance [24–26], but whether HOTAIR takes part in progesterone resistance in EC remains unknown. Interestingly, it was reported that the elevated H3K4 methylation and the decreased level of histone 3 lysine 9 (H3K9) methylation could activate transcription of the methylated and silenced PRB gene [27]. In light of the indistinct relevance, we were prompted to investigate whether HOTAIR plays a part in progesterone response in endometrial cancer.

In the present study, we for the first time determined a critical effect of HOTAIR expression on insensitivity of endometrial cancer cells to progesterone, and provided mechanistic insights into the epigenetic regulation of PRB expression by HOTAIR/LSD1. In doing so, we proposed a novel signaling pathway 'HOTAIR/LSD1/PRB/apoptosis' that attenuates the sensitivity of EC cells to progesterone, and define this pathway as a crucial targetable mediator of PRB-dependent progesterone insensitivity in endometrial cancer.

Materials and methods

Patients and tissue samples

Endometrial cancer ($n=20$) and normal endometrial ($n=20$) tissues were collected from patients who had undergone surgical resections or biopsies at the Department of Obstetrics and Gynecology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, between February 2014 and February 2018. At least two pathologists evaluated all specimens according to the World Health Organization (WHO) guidelines. The Ethics Committees of Union Hospital, Tongji Medical College, Huazhong University of Science and Technology approved this study, and then the patients' permission was obtained before surgery. The tissues were immediately frozen and stored in liquid nitrogen until use.

Cell culture and chemical reagent treatment

Human endometrial cancer cell lines Ishikawa, HEC-1A, HEC-1B and AN3CA were purchased from American Type Culture Collection (ATCC). The cells were cultured at 37 °C with Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum under a humidified atmosphere of 5% CO_2. Medroxyprogesterone acetate (MPA) was purchased from Sigma-Aldrich (St. Louis, MO, USA) and dissolved in ethanol with concentrations of 20 mmol/L (mM). The inhibitor of LSD1-GSK2879552 was purchased from MCE (Medchem Express, Monmouth Junction, NJ, USA) and dissolved in DMSO as stock solution at a concentration of 5 mM.

Cell transfection

Lentiviruses containing HOTAIR-shRNA and non-targeting scrambled shRNA (shNC) were purchased from Genechem (Shanghai, China). PRB-siRNA and a scramble non-targeting siRNA (siNC) were purchased from GenePharma (Shanghai, China). The effective HOTAIR-shRNA sequence was as follows: 5′- GGGAGTACAGAGAGAATA A-3′. The effective PRB-siRNA sequence was as follows: 5′-CCACUGAUCAACCUGUUAATT-3′. Stable transfection with HOTAIR knockdown lentiviruses was proceeded by the instructions. Transient transfection of siRNA was performed using Lipofectamine 2000 transfection reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol.

Cell viability assays

A total of 5000 cells in 100 µl per well were seeded onto 96-well plates and exposed to different concentrations (0, 5 µM,10 µM, 25 µM, 50 µM, 100 µM) of MPA. After incubation for 48 h, 20 µl MTT (Sigma, USA) was added and incubated for another 4 h. The medium was then replaced with 150 µl of dimethyl sulfoxide (Sigma, USA) to dissolve the MTT formazan for 15 min. Absorbance at 570 nm was then surveyed by an automatic microplate reader (Thermo Scientific, Waltham, MA, USA).

Colony formation assay

A total of 400 cells per well were seeded into 3.5 cm^2 dishes and incubated with MPA for 48 h and then allowed to grow for two weeks. Cells were fixed with methanol for 10 min and stained with 0.1% crystal violet, where colonies with cell number equal to or more than 50 were counted manually.

Reverse transcription and quantitative real-time PCR

Total RNA of tissues and cells were extracted by TRIzol (Invitrogen, Carlsbad, California, USA), according to the manufacturer's protocol, and reverse transcripted into cDNA using a Reverse Transcription Kit (TaKaRa, Otsu, Japan). qRT-PCR reactions were performed using Biosystem StepOne Plus PCR system (ABI, USA) and SYBR Green Real-time PCR Master Mix (TaKaRa, Otsu, Japan). Primer sequences used are listed in Supplementary Table 1. Target gene expression was quantified relative to the expression of GAPDH using the comparative Ct ($2^{-\Delta\Delta Ct}$) method.

Western blot analysis and antibodies

Proteins from whole cell lysates were separated using 10% sodium dodecyl sulfate–polyacrylamide gel (SDS–PAGE) and transferred to polyvinylidene difluoride membranes. The membranes were blocked and incubated with specific primary antibody against PR (1:500 dilution, Santa Cruz Biotechnology, CA, USA), BAX (1:1000 dilution, ABclonal, Woburn, MA, USA), Bcl-2 (1:1000 dilution, ABclonal, Woburn, MA, USA), LSD1 (1:1000 dilution, ABclonal, Woburn, MA, USA), H3K4me2 (1:1000 dilution, CST, USA), and GAPDH (1:10,000 dilution, Proteintechin, USA) in 4 °C overnight. After incubation with peroxidase-labeled species-specific secondary antibodies (1:8000, Affbiotech), protein bands were visualized by ECL substrate (Bio-Rad) using Image Lab Software in Molecular Imager® ChemiDocTM XRS+ (Bio-Rad).

Flow cytometry

Cells were digested with trypsin without EDTA, washed twice with cold PBS and then harvested (1×10^6 cells/ml) in 1 × binding buffer. Next, 100 µl of the cells were stained, combined with 5 µl APC-Annexin V and 5 µl 7-AAD, according to the protocol of the Annexin V-APC/7-AAD Apoptosis Detection Kit (BD Biosciences, Shanghai, China). Samples were cultured for 15 min at room temperature, in the dark. 400 µl of 1 × binding buffer was added to each tube before flow cytometry (FACScan, Becton Dickinson, Mountain View, CA, USA).

Nude mouse xenograft cancer model

All animal studies were performed in accordance with the protocols approved by the Tongji Medical College's Animal Care and Use Committee. BALB/C nude mice (Beijing Vital River, China) were bred and housed in a standard pathogen-free laboratory environment. Xenografts were initiated by subcutaneous injection of approximately 1×10^7 HEC-1A cells in 100 µL PBS into the right flank of Balb/c nude mice. Two weeks following the implantation, MPA was administered by intraperitoneal injection at a dose of 100 mg/kg every 3 days; this lasted for 3 weeks. The average tumor weight of xenografts was recorded and tumor samples were partially embedded in paraffin for histopathological analysis of PRB, BAX and Bcl-2.

Chromatin immunoprecipitation (ChIP)

The ChIP assay was carried out according to the manufacturer's instructions for the EpiQuik Chromatin Immunoprecipitation (ChIP) Kit (Epigentek, Farmingdale, NY, USA). In brief, samples were sonicated to gain DNA fragments between 200 and 500 bp in length. The chromatin was then immunoprecipitated using H3K4me2 (ABclonal, USA). Normal IgG was used as the negative control. qRT-PCR was then conducted with primer sets (Supplementary Table 1) targeting the H3K4me2 binding sites within the PRB promoter.

Statistical analysis

All statistical analyses were performed using SPSS 19.0 statistics sofware. Numerical data was presented as mean ± SD. Student's *t* test and one-way analysis of variance were used for two- and multi-group comparisons, respectively. $P<0.05$ were considered significant.

Results

HOTAIR inversely correlates with PRB expression in endometrial cancer

To determine whether HOTAIR plays a role in endometrial cancer cell progesterone resistance, the relationship among HOTAIR and PRB expression in EC tissues and cell lines was analyzed. As shown in Fig. 1a, b, HOTAIR expressed significantly higher and PRB expressed observably lower in EC tissues than normal endometrial tissues. Intriguingly, an inverse correlation between HOTAIR and PRB expression was observed in EC tissues (Fig. 1c). Moreover, high HOTAIR and low PRB mRNA levels were found in HEC-1A, AN3CA, and HEC-1B cell, while low HOTAIR and high PRB levels were observed in Ishikawa cell (Fig. 1d, e). Also, there was an obviously negative correlation between HOTAIR and PRB expression in these cell lines (Fig. 1f).

HOTAIR downregulation enhances endometrial cancer progesterone sensitivity in vitro

To illustrate the biological role of HOTAIR in EC progesterone sensitivity, shRNA-mediated HOTAIR knockdown in Ishikawa and HEC-1A cell was performed. Three shRNAs (shHOTAIR1, shHOTAIR2 and shHOTAIR3) were used, and shHOTAIR1 was chosen in the subsequent experiments for the strongest knockdown efficacy (Fig. 2a, d). Interestingly, PRB exhibited enhanced mRNA and protein expression after HOTAIR knockdown (Fig. 2b, c, e, f), suggesting that PRB may be a potential target gene of HOTAIR. In a follow-up experiment, Ishikawa and HEC-1A cell transfected with shNC or shHOTAIR were treated with ethanol (MPA) or MPA for 48 h. It is well-established that increased cell viability and reduced apoptosis are key mechanisms that contribute to the failure of hormonotherapy. MTT results showed that the cell viability was significantly decreased in the shHOTAIR group compaired with shNC group, as evidenced by the comparison of IC50 values between them (Fig. 3a, b). We next used the IC50 value of MPA in shHOTAIR group as the following treatment dose. Colony formation assay exhibited a dramatic decline survival fraction of EC cells in shHOTAIR group compared to shNC group with MPA application (data not shown). BAX and Bcl-2 mediate pro-apoptosis and anti-apoptotic responses, respectively. In the shHOTAIR group, cells showed an increased expression

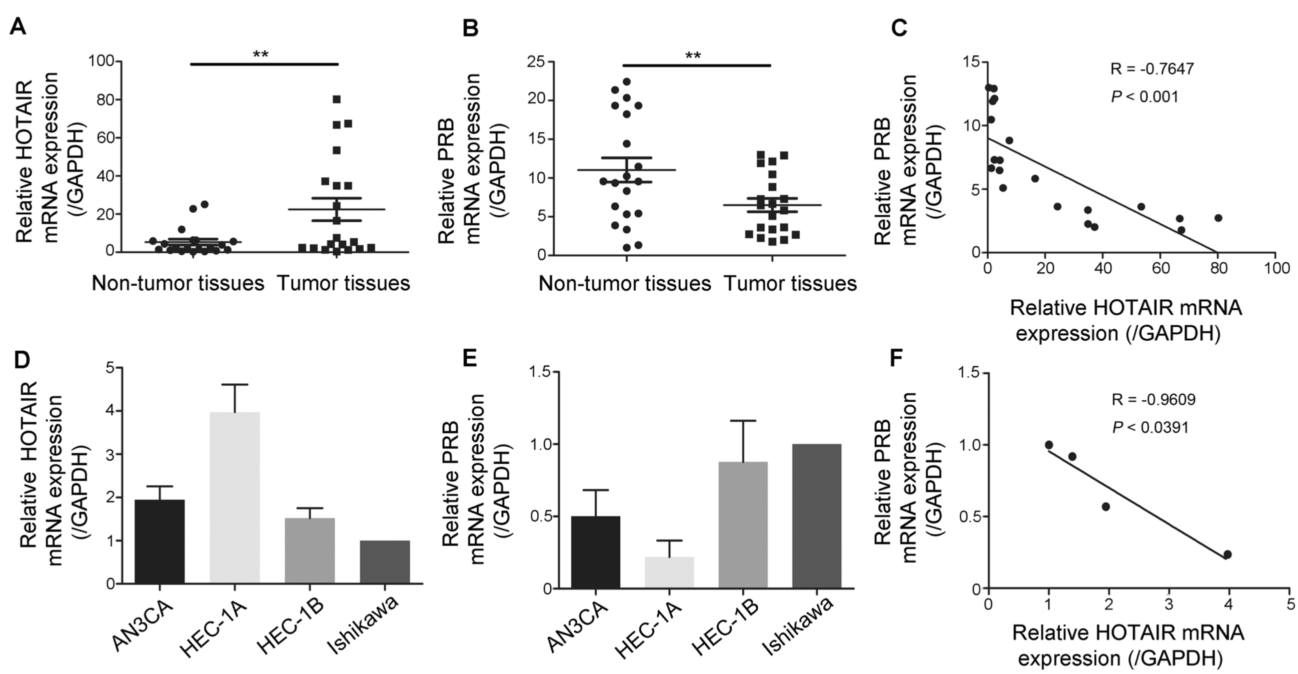

Fig. 1 HOTAIR inversely correlates with PRB expression in endometrial cancer. The mRNA expression of HOTAIR (**a**) and PRB (**b**) in 20 paired EC tissues and normal endometrial tissues were performed by qRT-PCR; **c** Negative correlation between HOTAIR and PRB expression in EC tissues; The mRNA expression of HOTAIR (**d**) and PRB (**e**) in four EC cell lines were performed by qRT-PCR; **f** Negative correlation between HOTAIR and PRB expression in four EC cell lines. Error bars: mean ± SD. $^{**}P<0.01$

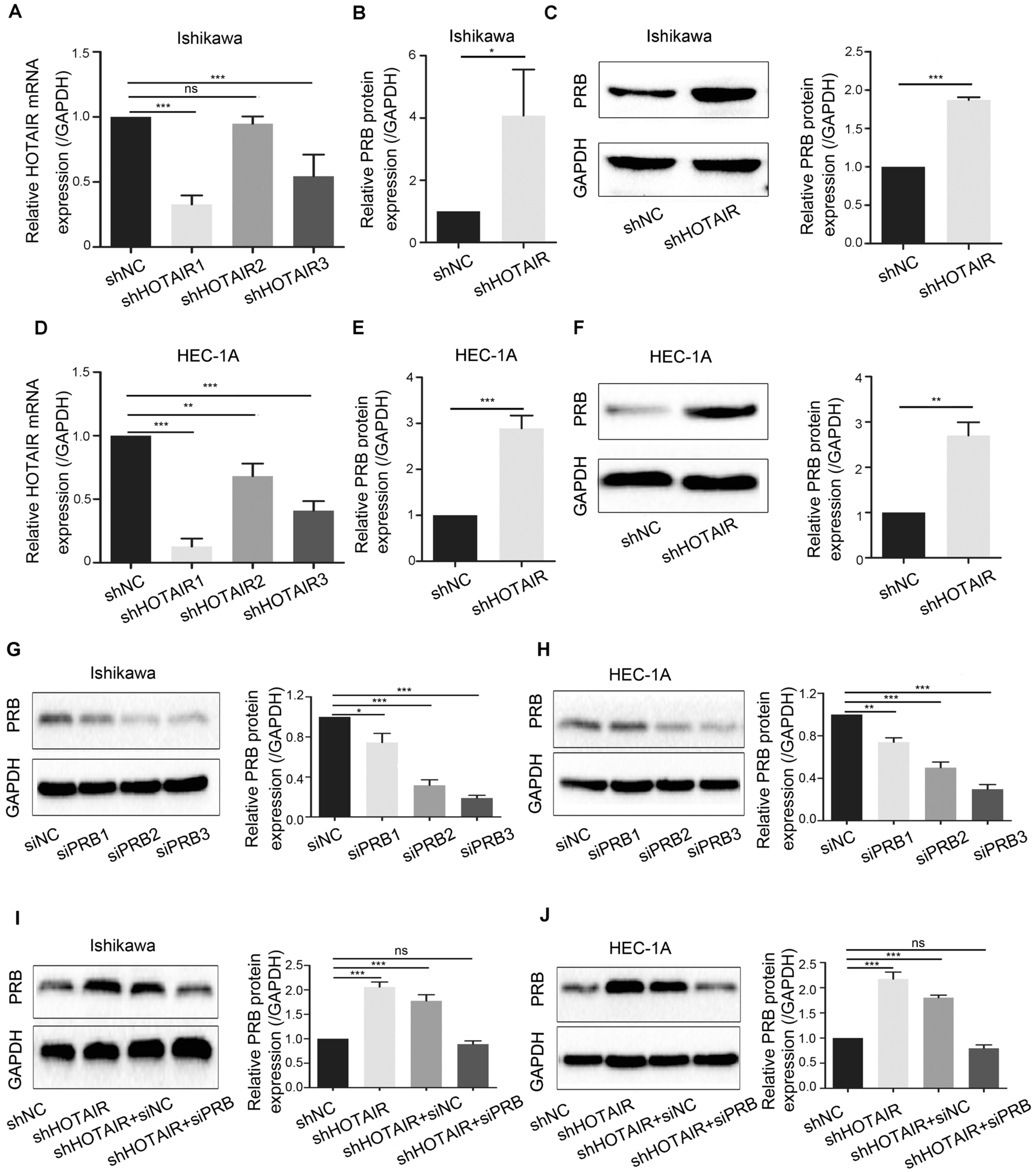

Fig. 2 Knockdown of HOTAIR promotes PRB expression. Efficacy of HOTAIR knockdown by shRNAs was determined by qRT-PCR in Ishikawa cell (**a**) and HEC-1A cell (**d**); the mRNA expression of PRB was determined by qRT-PCR in Ishikawa cell (**b**) and HEC-1A cell (**e**) after HOTAIR knockdown; the protein expression of PRB was determined by Western blot in Ishikawa cell (**c**) and HEC-1A cell (**f**) after HOTAIR knockdown; efficacy of PRB knockdown by siRNAs was determined by Western blot in Ishikawa cell (**g**) and HEC-1A cell (**h**); the protein expression of PRB was determined by Western blot in Ishikawa cell (**i**) and HEC-1A cell (**j**) after cotransfection with shHOTAIR and siPRB. Error bars: mean ± SD. *$P<0.05$, **$P<0.01$ and ***$P<0.001$

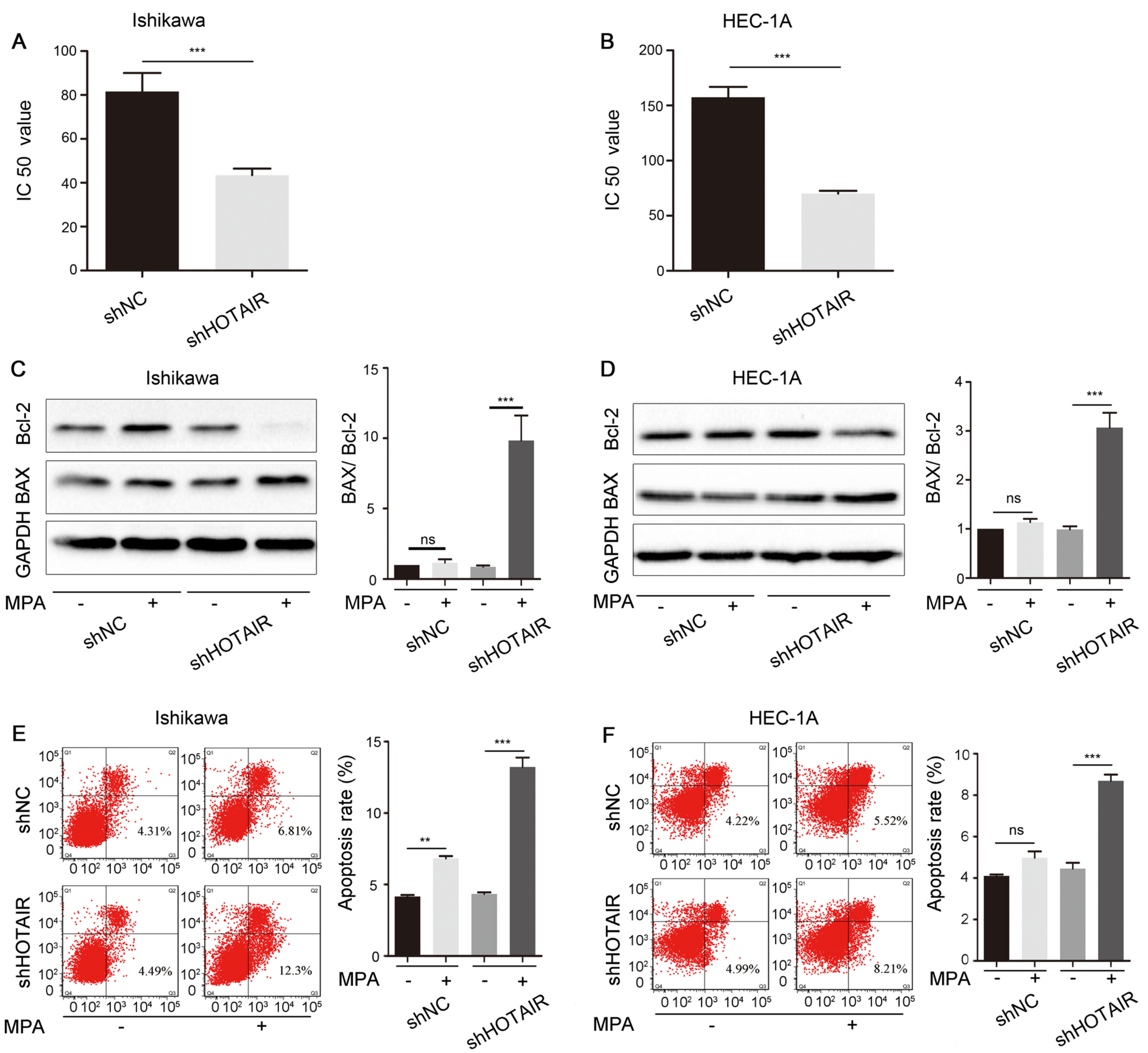

Fig. 3 Down regulation of HOTAIR enhances endometrial cancer sensitivity to MPA in vitro. IC50 values of Ishikawa cell (**a**) and HEC-1A cell (**b**) were examined by MTT assay; the protein expression of BAX and Bcl-2 was detected through Western blot in Ishikawa cell (**c**) and HEC-1A cell (**d**); cell apoptosis rate was detected through flow cytometry in Ishikawa cell (**e**) and HEC-1A cell (**f**). Error bars: mean ± SD. *$P<0.05$, **$P<0.01$ and ***$P<0.001$. *ns* non-significance

of BAX, whereas there was a remarkable decreased expression of Bcl-2 after being exposed to MPA (Fig. 3c, d). The changes of the apoptosis-related protein expression were greater than shNC group, and this variation could be enhancive when presented as BAX/Bcl2 ratio, suggestive of a distinct apoptotic effect when shHOTAIR is combined with MPA treatment. Similarly, a noticeably increased apoptosis rate in the HOTAIR-shRNA transfection combined with MPA administration group was exhibited in flow cytometric analysis (Fig. 3e, f). Collectively, we concluded that HOTAIR knockdown significantly increased EC cell progesterone sensitivity.

HOTAIR downregulation enhances endometrial cancer progesterone sensitivity in vivo

To investigate the role of the HOTAIR in endometrial cancer progesterone sensitivity in vivo, we established a subcutaneous graft tumor model using HEC-1A cells in nude mice. Since there were no significant differences in vitro between shNC and shHOTAIR group without MPA administration, we treated both groups of mice with MPA. As expected, the implanted tumors generated from shHOTAIR group grew dramatically slower than those generated from shNC group after MPA treatment. Compared to shNC group, the tumor weight was reduced approximately 70% in shHOTAIR group (Fig. 4a). The immunohistochemistry showed that PRB, as well as BAX protein expression were evidently increased, while Bcl-2 decreased in shHOTAIR group (Fig. 4b). Our results demonstrated that HOTAIR knockdown leads to PRB induction in vivo as displayed in vitro, associated with increased progesterone sensitivity.

Knockdown of HOTAIR increases progesterone sensitivity via PRB in EC cells

As there was an underlying negative correlation between HOTAIR and PRB, we hypothesized that HOTAIR promotes progesterone resistance through suppressing PRB. To validate this hypothesis, the EC cells were transiently transfected with PRB-siRNA and the siPRB3 was chosen as the subsequent effective sequence (Fig. 2g, h). The PRB expression was reduced again when cells were co-transfected with shHOTAIR and siPRB (Fig. 2i, j). As expected, the cell viability decline caused by HOTAIR knockdown was partially reversed by inhibition of PRB (Fig. 5a, b). In addition, the silencing of PRB attenuated the BAX/Bcl-2 ratio, which was increased caused by shHOTAIR with MPA treatment (Fig. 5c, d). Furthermore, knockdown of PRB weakened the effect of shHOTAIR on MPA-induced apoptosis of EC cells (Fig. 5e–h). These results indicated that repression of PRB was required for HOTAIR-mediated progesterone resistance in EC cells.

HOTAIR epigenetically silences PRB transcription through LSD1-mediated H3K4me2 demethylation

HOTAIR has been proven to regulate genes through binding LSD1, which is able to demethylate H3K4me2 on the promotor of target genes and silences gene transcription [28]. We investigated the effects of pharmacological inhibition of LSD1 using GSK2879552 [29] to further explore the molecular mechanisms by which HOTAIR regulates PRB transcription. When using GSK2879552 (2 μM), the LSD1 expression was inhibited, whereas PRB expression was substantially increased (Fig. 6a, b). A combination of GSK2879552 and MPA induced a noteworthy apoptosis compared to single MPA or GSK2879552 treatment (Supplementary Fig. 1a–d), similar to the effect of shHOTAIR combined with MPA treatment. Moreover, LSD1 inhibition strengthened the H3K4me2 expression in EC cells (Fig. 6c, d), hinting the function of LSD1 in regulating H3K4me2 occupation on PRB promotor. Interesting, downregulation of HOTAIR did not affect the expression of LSD1, while H3K4me2 expression was increased (Fig. 6e, f). Next, we performed ChIP assays to confirm the effects of HOTAIR on the H3K4me2 occupation on PRB promoter. The total DNA of EC cell was broken into small fragments by ultrasonic apparatus, and the broken efficiency was shown in Fig. 6g. The primers #1, #2, #3, #4, #5 and #6 are located in the promoter region of PRB. As shown in Fig. 6h, HOTAIR knockdown markedly increased H3K4me2 binding to the PRB promoter in EC cells. Overall, these results suggest a model that HOTAIR binds with LSD1 to demethylate the H3K4me2 on PRB promotor, thus inhibiting PRB transcription and decreasing progesterone sensitivity (Fig. 6i).

Discussion

Over the past decades, accumulated evidence has emphasized significance of lncRNAs in human cancers. LncRNAs have been shown to participate in biological processes through various mechanisms. HOTAIR is one of the well-documented lncRNAs with a length of 2158 bp [30]. Recent studies have revealed that HOTAIR was capable of inducing tumor multi-drug resistance. For example, silencing of HOTAIR decreased crizotinib resistance of non-small cell lung cancer cells via decreasing ULK1 phosphorylation [24]. In hepatocellular carcinoma, knockdown of HOTAIR sensitized the cell to cisplatin through suppressing the STAT3/ABCB1 signaling pathway [25]. Besides chemotherapy drugs, HOTAIR was also proven to

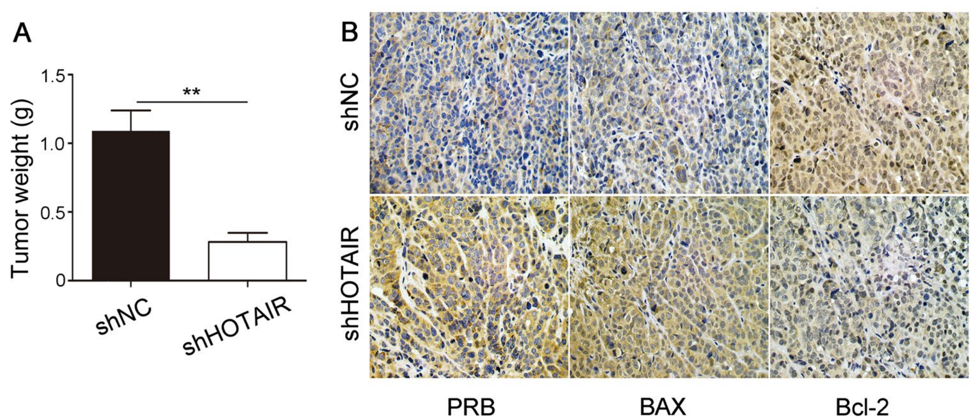

Fig. 4 HOTAIR downregulation enhances endometrial cancer sensitivity to MPA in vivo. Mice were injected subcutaneously of HEC-1A cells transfected with shNC or shHOTAIR and intraperitoneally with MPA ($n=5$). **a** Weights of xenograft tumors were measured; **b** The immunohistochemical analysis of PRB, BAX and Bcl-2 in xenograft tumors (original magnification at ×400). Error bars mean ± SD. $^{**}P<0.01$

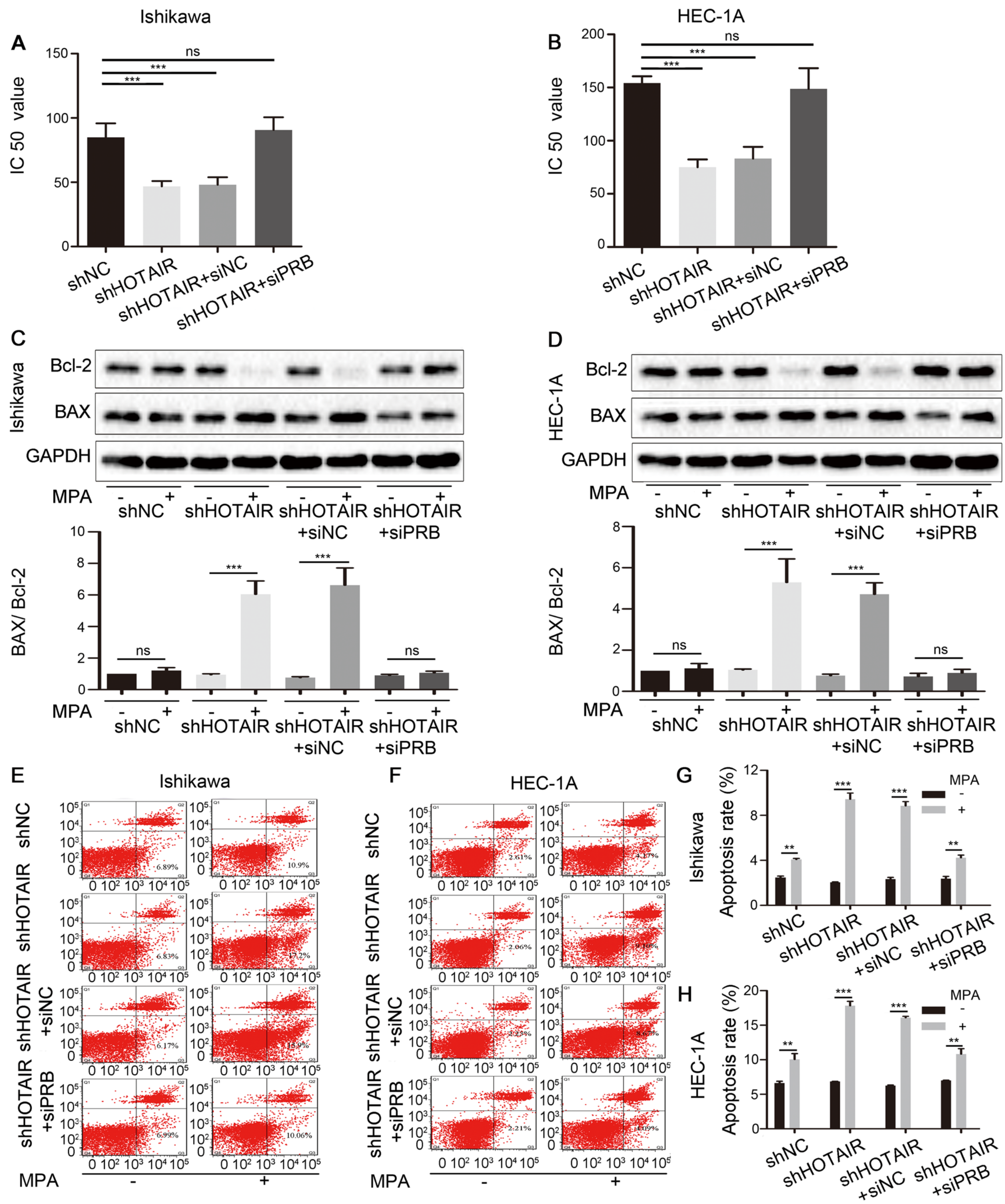

Fig. 5 Knockdown of HOTAIR decreases cell proliferation with MPA treatment via PRB in EC cells. IC50 values of Ishikawa cell (**a**) and HEC-1A cell (**b**) were examined by MTT assay; the protein expression of BAX and Bcl-2 was detected through Western blot in Ishikawa cell (**c**) and HEC-1A cell (**d**); Cell apoptosis rate was detected through flow cytometry in Ishikawa cell (**e**) and HEC-1A cell (**f**). Error bars mean ± SD. $*P<0.05$, $**P<0.01$ and $***P<0.001$. *ns* non-significance

participate in the development of hormone therapy medicine resistance. Xue et al. found that HOTAIR promoted estrogen receptor activation in cells with a lack of estrogen, thus driving tamoxifen resistance [31]. In castration-resistant prostate cancer, HOTAIR functioned as a biomarker of enzalutamide resistance [32]. These studies are consistent with our findings that HOTAIR overexpression led to cancer cell drug resistance.

Progesterone plays an important part in EC therapy. It is likely that the primary barrier of progesterone resistance is the absence or the downregulation of PRB in cancer cells. Progesterone could induce miR-152 expression

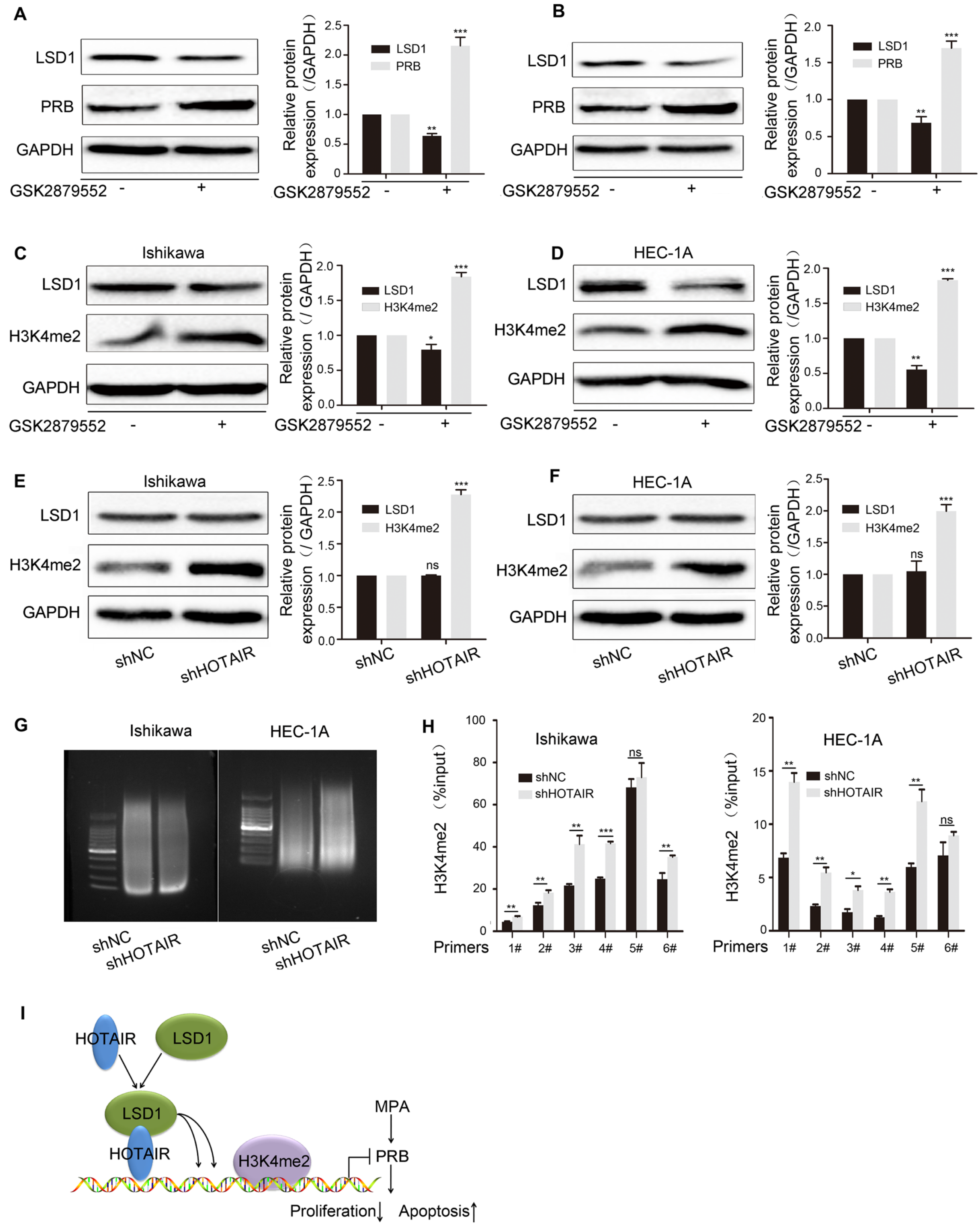

Fig. 6 HOTAIR epigenetically silences PRB transcription through LSD1-mediated H3K4me2 demethylation. The protein expression of LSD1 and PRB was detected through Western blot in Ishikawa cell (**a**) and HEC-1A cell (**b**) with GSK2879552 treatment; the protein expression of LSD1 and H3K4me2 was detected through Western blot in Ishikawa cell (**c**) and HEC-1A cell (**d**) with GSK2879552 treatment; the protein expression of LSD1 and H3K4me2 was detected through Western blot in Ishikawa cell (**e**) and HEC-1A cell (**f**) with HOTAIR knockdown; **e** the efficiency of DNA broken fragments was detected by DNA gel electrophoresis; **f** Ishikawa and HEC-1A cell were transfected with shNC or shHOTAIR followed by ChIP and qRT-PCR for the quantitative detection of H3K4me2 on the PRB promoter. The results were normalized to the input; **g** schematic model of HOTAIR/LSD1-mediated H3K4me2 and PRB expression in EC cells. Error bars: mean ± SD. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$. *ns* non-significance

through activating PRB, and eventually lead to miR-152 inhibited proliferation in EEC by downregulating WNT-1 [33]. Another study indicated that hyperactive Akt signaling reduced PRB transcriptional activity, resulting in an impaired progesterone response [34]. Therefore, it is considerable to find the related mechanism to enhance functional PRB expression to further optimize the responsiveness of progesterone therapy. Our results showed that silencing HOTAIR could significantly induce the expression of PRB in EC cells. Knockdown of HOTAIR promoted the sensitivity of endometrial cancer cells to MPA by upregulating PRB, which can be largely reversed by PRB downregulation.

Gene expression largely hinges on the chromatin level through epigenetic modifications, such as DNA methylation, histone deacetylation, and histone methylation. Evidence has increasingly shown that lncRNAs, such as SNHG1 [35] and SH3PXD2A-AS1 [36], can recruit or dissociate proteins or RNAs, to indirectly perform biological functions in life activities. In previous studies, HOTAIR has been demonstrated to work as a scaffold with the PRC2 and LSD1 complex to regulate target genes. LSD1 is the first discovered demethylase, which specifically demethylates mono- and di-methylated H3K4 and H3K9, and induces transcriptional repression [37, 38]. The enrichment of H3K4me2 at promoters reflects a positive effect on transcriptional activity. We found both downregulation of HOTAIR and LSD1 led to the activation of PRB expression, and promoted apoptosis induced by MPA. We further confirmed that HOTAIR knockdown increased H3K4me2 occupancy at the PRB promoter.

In conclusion, knockdown of HOTAIR enhanced PRB transcription by recruiting LSD1, causing H3K4me2 demethylation at the PRB promoter, which led to the proliferation inhibition and apoptosis activation in endometrial cancer cells with MPA treatment. Those achievements contribute to the mechanism research of progesterone resistance, and might provide a novel potential therapeutic target for endometrial cancer.

Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants and animals were in accordance with the ethical standards of Union Hospital, Tongji Medical College, Huazhong University of Science and Technology research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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