



LncRNA MALAT1 inhibits apoptosis of endometrial stromal cells through *miR-126-5p-CREB1* axis by activating *PI3K-AKT* pathway

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Abstract

Endometriosis is a common, chronic and painful disease in women, whose pathogenesis remains not entirely clear. Long non-coding RNA (lncRNA) MALAT1 participates in the development of endometriosis. This study further investigated the regulation of MALAT1-miR-126-5p-CREB1 axis in the pathological process of endometriosis. MALAT1, miR-126-5p, and CREB1 levels in human endometrial stromal cells (HESCs) were detected by quantitative reverse transcription polymerase chain reaction (RT-qPCR). Protein levels were determined by Western blotting. Cell viability and apoptosis was assessed by MTT assay and annexin V-FITC staining, respectively. The interactivity between miR-126-5p and MALAT1 (or CREB1) was assessed by dual luciferase reporter system. Knockdown of MALAT1 or CREB1 restrained proliferation and induced apoptosis as confirmed by upregulating cleaved caspase-3 and Bax, and down-regulating Bcl-2 in HESCs, while inhibition of miR-126-5p presented the opposite results. Moreover, silencing of MALAT1 triggered apoptosis of HESCs via targeting miR-126-5p. In addition, miR-126-5p directly regulated CREB1 expression via binding to its 3' non-coding region. Finally, miR-126-5p inhibitor-mediated apoptosis inhibition was restrained by CREB1 silencing via inactivation of PI3K-AKT pathway in HESCs. Taken together, our study firstly demonstrates that MALAT1 regulates apoptosis of HESCs through miR-126-5p/CREB1 axis mediated PI3K/AKT pathway. Our findings explained the pathogenesis of endometriosis and offered promising therapeutic option for endometriosis.

Keywords Endometriosis · Apoptosis · lncRNA MALAT1 · miR-126-5p · CREB1

Abbreviations

HESCs	Human endometrial stromal cells
lncRNAs	Long non-coding RNAs
miRNA	MicroRNA
CREB1	Cyclic AMP response element-binding protein 1
DMSO	Dimethylsulfoxide
WT	Wild type
MUT	Mutant
UTR	Untranslated regions
RT-qPCR	Quantitative reverse transcription polymerase chain reaction

Introduction

Endometriosis is a common benign disease and has similar biological features to malignant tumor, including malignant proliferation, apoptosis inhibition, invasiveness, easy to recurrence and malignant transformation, etc. [1, 2]. Endometriosis means that the endometrial glands and stroma infiltrate in other place outside the uterus. The morbidity of endometriosis is 10–15% in bearing aged women, and 20–40% in sterility women [3]. This disease may cause dysmenorrhea, chronic pelvic pain, or infertility, which not only impairs life quality of patients, but also aggravates their psychology burden. However, the pathogenic mechanisms of endometriosis are still ambiguity. Growing evidence has indicated that inducing apoptosis of human endometrial stromal cells (HESCs) is an effective therapeutic approach for endometriosis [4, 5].

Long non-coding RNAs (lncRNAs), composed of over 200 nucleotides without encoding for proteins, possess multiple biological functions. At present there are few investigations about lncRNAs in endometriosis. A previous research

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suggested that lncRNA-H19 facilitated the growth and migration of ectopic endometrial cells via regulating miR-124-3p-ITGB3 axis [6]. Besides, lncRNA AC002454.1 was verified to be upregulated in endometriosis and facilitated the development of endometriosis via enhancing proliferative and invasive abilities of eutopic endometrial cells [7]. Furthermore, recent research demonstrated that lncRNA metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) expression was increased in ectopic endometrial tissues and participated in the progression of endometriosis [8, 9]. However, the detailed mechanisms of MALAT1 in regulating endometriosis still need to be deeply expounded.

lncRNA is well recognized as a sponger of microRNA (miRNA) to indirectly regulate gene expression. There is mounting evidence to prove that miRNAs regulate the occurrence and progression of endometriosis. For example, the course of endometriosis was slowed down by miR-205-5p via targeting ANGPT2 [10]. Besides, a recent study indicated that miR-488 affected the biological function of endometrial glandular endometrial cells, and inhibited the development of endometriosis [11]. MiR-126-5p expression was demonstrated to be declined in endometriosis, which enhanced the migration and invasion of endometrial cells through regulating BCAR3 expression [12]. Nevertheless, the biological function and regulatory mechanisms of miR-126-5p in endometriosis remain unclear.

Cyclic AMP response element-binding protein 1 (CREB1) is an oncogene and takes part in the malignant progression of various cancers. Knockdown of CREB1 restrained growth and induced apoptosis of acute lymphoblastic leukemia cells [13]. Li et al. indicated that CREB1 was overexpressed in renal cancer and promoted the tumorigenesis of renal cell carcinoma [14]. More importantly, the expression of CREB1 was found to be increased in eutopic endometrial tissues of endometriosis patients [15]. Therefore, we speculated that CREB1 might regulate the biological function of HESCs. In addition, bioinformatics analysis suggested that miR-126-5p might bind to MALAT1 and CREB1, so MALAT1 may affected the growth and apoptosis of HESCs via regulating miR-126-5p-CREB1 axis.

In this study, the effect of MALAT1 on apoptosis and growth of HESCs via miR-126-5p-CREB1 axis was investigated for the first time, which uncovered the underlying mechanisms of MALAT1-miR-126-5p-CREB1 axis in endometriosis progression.

Materials and methods

Tissue collection

Human endometrium was collected from patients with endometriosis at the Second Affiliated Hospital of Nanchang

University. All patients signed written informed consent and the experiment was carried out in accordance with the Declaration of Helsinki Principles and approved by the Ethics Committee of the Second Affiliated Hospital of Nanchang University.

Isolation of HESCs and treatment

Primary HESCs were isolated by reference to a study [16]. In short, the endometrium tissues were cut into about 1 mm³, added with 0.1% type II collagenase, and incubated for 1 h to digest. Afterward, the tissue fragments were eliminated by filtration with 100- μ m and 40- μ m nylon mesh, and then centrifuged at 500 g for 5 min. After resuspension, the cells were cultured with Ham's F12/DMEM (1:1) containing 10% fetal bovine serum (Gibco,) and 1 \times penicillin–streptomycin–Fungizone (Lonza, Walkersville, MD, USA), at 37°C and 5% CO₂ in an incubator.

Cell transfection

The small interfering RNA (siRNA) for lncRNA MALAT1 (si-MALAT1), si-CREB1, negative control siRNA (si-NC), miR-126-5p mimics, NC mimics, miR-126-5p inhibitor, and NC inhibitor were obtained from RIBOBIO (Guangzhou, China). The lncRNA MALAT1 cDNA was inserted into pcDNA3.1 vector to establish lncRNA MALAT1 expression plasmid. The HESCs at 70–80% confluence were transfected with the above segments using Lipofectamine 2000 (Thermo Fisher, Waltham, MA, USA).

Quantitative reverse transcription polymerase chain reaction (RT-qPCR)

RNAsimple Total RNA Kit (TIANGEN, Beijing, China) was applied for total RNA isolation from HESCs. After reverse transcription to cDNA with ReverTra Ace® qPCR RT Master Mix (Root biological technology limited company, Shanghai, China), the expression of lncRNA MALAT1 and CREB1 was detected by Realtime PCR Master Mix (TOYOBO, Osaka, Japan) and normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH). MiR-126-5p level was assessed by A TaqMan miRNA assay kit (Thermo Fisher) and normalized to U6 small RNA. 2^{- $\Delta\Delta$ Ct} method was performed to calculate the target gene expression. The primer sequences are listed in Table 1.

Western blotting

The HESCs were lysed in RIPA buffer (Beyotime) added with 1 \times protease inhibitor cocktail (Roche, Mannheim, Germany) to extract total proteins. After quantification, equivalent amounts of proteins were resolved by sodium dodecyl

Table 1 Oligonucleotide primer sets for real-time PCR

Name	Sequence (5'–3')	Length
MALAT1 F	CCACATCACGGCTGTTCTTGTA	22
MALAT1 R	GCATTGTGTCGGCTGGTAATT	21
CREB1 F	CCCAGCCATCAGTTATTCAG	20
CREB1 R	GAGTTGGCACCGTTACAGTG	20
GAPDH F	AGTACCAGTCTGTTGCTGG	19
GAPDH R	TAATAGACCCGGATGTCTGGT	21
miR-126-5p F	CTCAACTGGTGTTCGTGGAGTCGGC AATTCAGTTGAGCGCGTA	42
miR-126-5p R	ACACTCCAGCTGGGCATTACTTTT GGTA	31
U6 F	CTCGTTCGGCAGCACA	17
U6 R	AACGCTTCACGAATTTGCGT	20

MALAT1, metastasis associated lung adenocarcinoma transcript 1; CREB1, cyclic AMP response element-binding protein 1; GAPDH, housekeeping gene glyceraldehyde-3-phosphate dehydrogenase

sulfate polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes (Sigma, Saint Louis, MO, USA). Then, 5% skim milk was adopted for blocking. Afterward, the membranes were reacted with primary antibodies against cleaved caspase-3 (1:1000, bsm-33199 M, Bioss, Beijing, China), Bax (1:1000, bsm-33283M, Bioss), CREB1 (1:500, 12208-1-AP, Proteintech, Rosemont, IL, USA), Bcl-2 (1:1000, 26593-1-AP, Proteintech), p-AKT (1:500, bs-0876R, Bioss), AKT (1:1000, bsm-33282 M, Bioss), p-PI3K (1:1000, bs-5587R, Bioss), PI3K (1:1000, 67017-1-Ig, Proteintech), and GAPDH (1:1000, 10494-1-AP, Proteintech), at 4 °C overnight. Subsequently, the matched secondary antibody was added to the membranes. The protein bands were developed using a BeyoECL Plus kit (Beyotime).

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

Briefly, HESCs were seeded in 96-well plate (2000 cells per well). After incubation at 37 °C for various time points, HESCs were reacted with 100 µL MTT solution. Finally, 100 µL dimethylsulfoxide (DMSO) was added after reacting for 4 h. The optical absorbance was detected at 490 nm by a plate reader (Eppendorf, Hamburg, Germany).

Apoptosis analysis by flow cytometry

The apoptosis of HESCs was evaluated by Annexin V-FITC/PI apoptosis kit (MultiSciences, Hangzhou, China). Briefly, the collected HESCs were resuspended in binding buffer and added with Annexin V-PI solution. Then, the HESCs were incubated for 15 min without light. The percentage of apoptotic cells was then immediately detected on a flow cytometry (Thermo Fisher Scientific).

Dual luciferase reporter system

The sequences of MALAT1 containing the predictive or the mutant miR-126-5p binding sites were inserted into dual luciferase vector to construct wild type (WT)-MALAT1 or mutant (MUT)-MALAT1 dual luciferase reporter plasmid. Similarly, the WT or MUT fragments of CREB1 3' untranslated regions (UTR) were inserted to construct WT-CREB1 or MUT-CREB1 dual luciferase reporter plasmid. Then the constructed dual luciferase reporter plasmids, and miR-126-5p mimics, or miR-126-5p inhibitor, were co-transfected into HESCs using Lipofectamine 2000. The relative luciferase activity was determined at 48 h after the transfection.

Statistical analysis

The experimental data are expressed as mean ± standard deviation and analyzed by GraphPad Prism 8 software. One-way analysis of variance followed by Tukey's test was used to compare data among multiple groups. P value less than 0.05 was considered to be statistically significant.

Results

Knockdown of lncRNA MALAT1 promotes apoptosis of HESCs

To evaluate the functional roles of lncRNA MALAT1 in HESCs, we knocked down MALAT1 by transfection with si-MALAT1. As illustrated in Fig. 1a, MALAT1 level was down-regulated by nearly 60% in HESCs transfected with si-MALAT1. Silencing of MALAT1 led to proliferation inhibition of HESCs as assessed by MTT assay (Fig. 1b). As presented in Fig. 1c, knockdown of MALAT1 increased apoptotic rate by about 14% in HESCs. Additionally, cleaved caspase-3 and Bax levels were upregulated, whereas Bcl-2 level was downregulated by MALAT1 knockdown (Fig. 1d). These results suggested that silencing of MALAT1 induces apoptosis of HESCs.

lncRNA MALAT1 directly interacts with miR-126-5p in HESCs

Next, the interaction between lncRNA MALAT1 and miR-126-5p was determined. The predicted binding sites for MALAT1 and miR-126-5p were illustrated in Fig. 2a. Afterward, the HESCs were co-transfected with WT or MUT MALAT1 and miR-126-5p mimics, or miR-126-5p inhibitor. As shown in Fig. 2b, the relative luciferase activity in WT MALAT1 group was obviously reduced by enhancing miR-126-5p expression, while enhanced by silencing

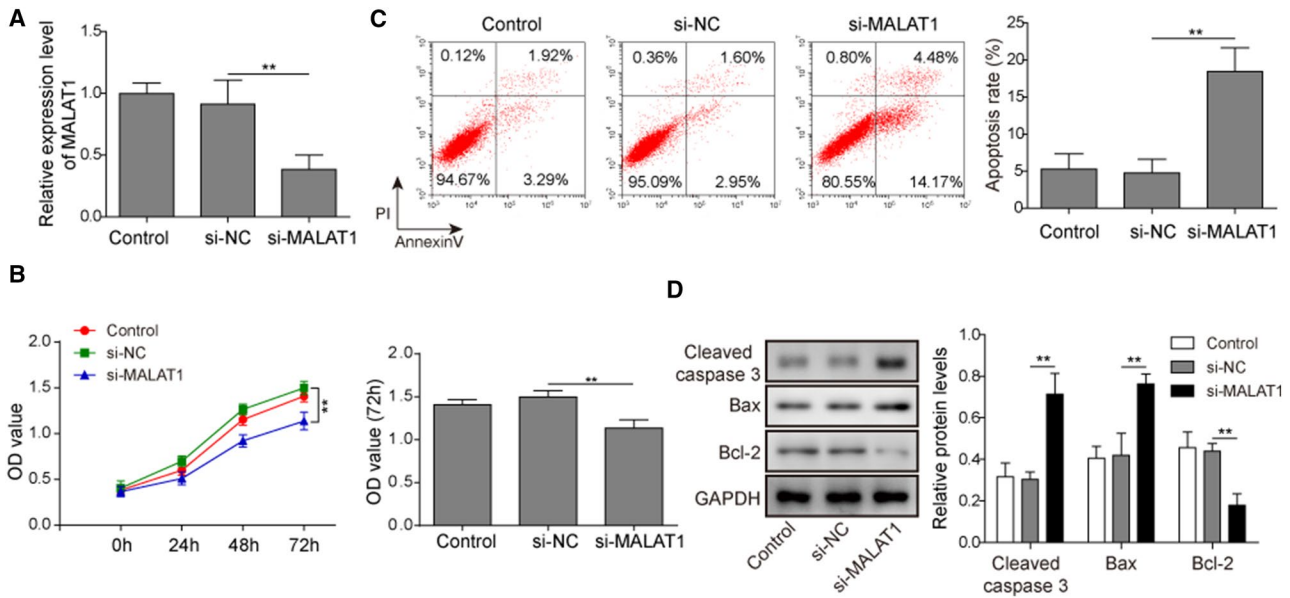


Fig. 1 Knockdown of lncRNA MALAT1 promotes apoptosis of HESCs. ESCs were transfected with si-NC or si-MALAT1. **a** The level of MALAT1 in HESCs was assessed by RT-qPCR. **b** The viability of HESCs was assessed using MTT assay. **c** HESC apoptosis

was detected by AnnexinV/PI staining using flow cytometry. **d** The levels of apoptosis-related proteins in HESCs were determined by western blotting. All results were shown as mean ± standard deviation ($n=3$). ** $P < 0.01$ versus designated group

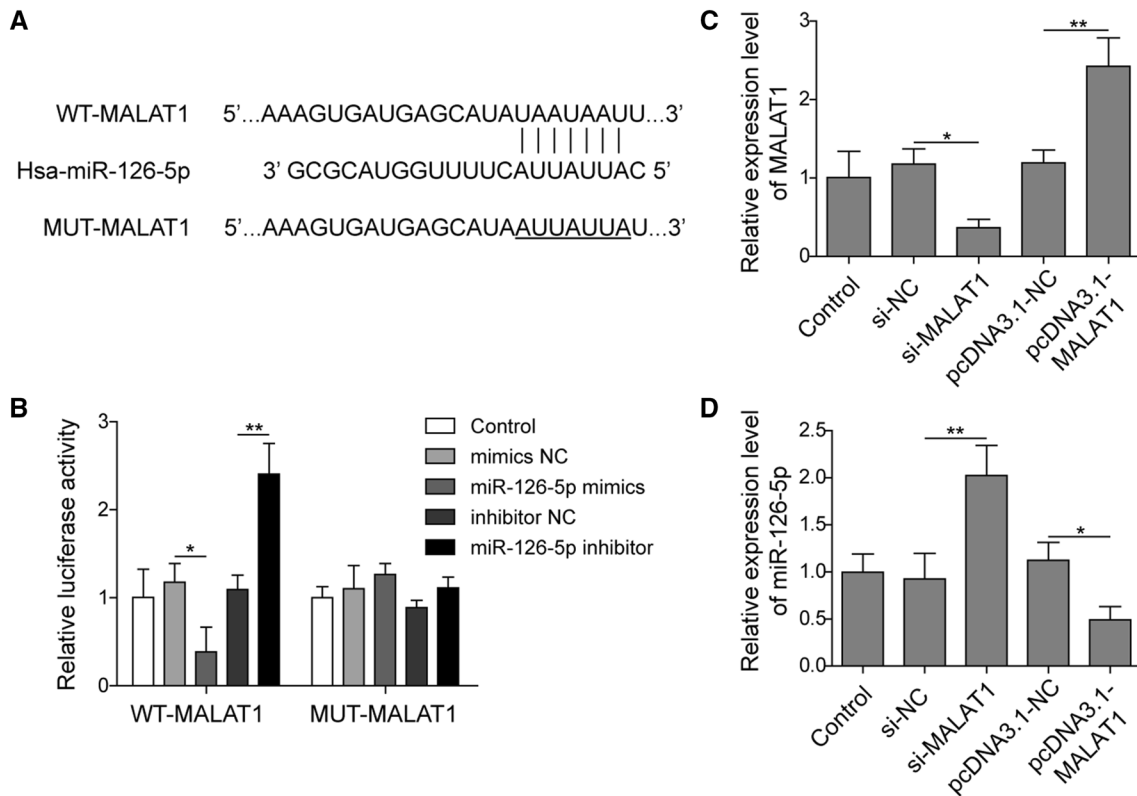


Fig. 2 LncRNA MALAT1 directly interacts with miR-126-5p in HESCs. **a** The binding sequences for miR-126-5p in MALAT1 were shown. **b** Dual luciferase reporter system was adopted to evaluate the interaction ability between miR-126-5p and MALAT1. MALAT1

(**c**) and miR-126-5p (**d**) expression was evaluated by RT-qPCR. All results were shown as mean ± standard deviation ($n=3$). * $P < 0.05$, ** $P < 0.01$ versus designated group

miR-126-5p expression. Whereas, the above changes were not found in MUT MALAT1-transfected cells. In addition, the effect of lncRNA MALAT1 on miR-126-5p expression was presented in Fig. 2c. Overexpression or silence of MALAT1 in HESCs was verified by RT-qPCR. Knockdown of MALAT1 resulted in increased miR-126-5p level, while enforced MALAT1 expression remarkably decreased miR-126-5p expression (Fig. 2d). The above findings indicated that MALAT1 binds to miR-126-5p and negative regulates its expression in HESCs.

lncRNA MALAT1 silencing induces HESC apoptosis via regulating miR-126-5p

Next, we explored whether MALAT1 affected the apoptosis of HESCs via regulating miR-126-5p. As illustrated in Fig. 3a, overexpression of miR-126-5p inhibited the proliferation of HESCs, while miR-126-5p inhibitor facilitated the proliferation and reversed si-MALAT1-induced anti-proliferation in HESCs. Moreover, the percentage of apoptotic HESCs was lessened by miR-126-5p inhibitor and MALAT1 silencing-induced apoptosis was suppressed by miR-126-5p knockdown, whereas the apoptosis of HESCs was promoted by miR-126-5p mimics (Fig. 3b). Similarly, the increased cleaved caspase-3 and Bax levels, and decreased Bcl-2 level induced by si-MALAT1 were all counteracted by miR-126-5p inhibitor (Fig. 3c). Therefore, miR-126-5p was involved in the regulation of apoptosis by MALAT1 in HESCs.

miR-126-5p directly regulates CREB1 expression in HESCs

The interaction between miR-126-5p and CREB1 in HESCs was further studied. The binding sequences for CREB1 and miR-126-5p were illustrated in Fig. 4a. In addition, miR-126-5p mimics lowered the relative luciferase activity of WT CREB1, whereas miR-126-5p inhibitor augmented that of WT CREB1. We did not find differences in MUT CREB1 groups (Fig. 4b). Furthermore, overexpression or knockdown of miR-126-5p in HESCs was confirmed (Fig. 4c). As we expected, CREB1 expression was repressed by miR-126-5p overexpression, while reinforced by miR-126-5p inhibition in HESCs (Fig. 4d). Thus, CREB1 expression was directly regulated by miR-126-5p in HESCs.

miR-126-5p regulates apoptosis of HESCs though CREB1-mediated PI3K/AKT pathway

Furthermore, we determined whether miR-126-5p affected HESC apoptosis via regulating CREB1 expression. The MTT results indicated that knockdown of CREB1 repressed cell proliferation, and counteracted miR-126-5p

inhibitor-induced proliferation promotion effect on HESCs (Fig. 5a). Silencing of CREB1 enhanced the apoptotic rate, and suppressed miR-126-5p inhibitor-mediated anti-apoptotic effect (Fig. 5b). Additionally, si-CREB1 transfection raised cleaved-caspase-3 and Bax levels, but reduced Bcl-2 level, which was reversed by treatment with miR-126-5p inhibitor (Fig. 5c). We further investigated whether PI3K/AKT pathway involved in miR-126-5p/CREB1 axis-mediated apoptosis in HESCs. Silencing of miR-126-5p activated p-PI3K and p-AKT, which was abrogated by knockdown of CREB1. Nevertheless, IGF-1-mediated PI3K/AKT pathway activation rescued the inhibitory effect of si-CREB1 on the phosphorylation of PI3K and AKT (Fig. 5d). Besides, administration with IGF-1 was found to offset the anti-proliferative and pro-apoptotic effect of si-CREB1 (Fig. 5a–c). Collectively, all these results suggested that silencing of CREB1 restrained miR-126-5p inhibitor-induced apoptosis inhibition via activating PI3K-AKT pathway in HESCs.

Discussion

Endometriosis is a commonly encountered disease that may lead to paramenia, painful menstruation, infertility, and even ovarian carcinoma [17]. Therefore, expounding the pathogenesis of endometriosis is significant for diagnosis and treatment. The biological properties of endometriosis are considered to be similar to malignant neoplasm [18]. The apoptosis resistance and increased proliferation of endometrial stromal cells confer on the occurrence and development of endometriosis [19]. This study indicated that silencing of MALAT1 restrained growth and enhanced apoptosis of HESCs via regulating miR-126-5p/CREB1 axis. PI3K/AKT pathway implicated in the regulatory mechanisms of MALAT1 in HESCs.

The pathological mechanism of endometriosis is quite complex. More recent investigations have supported that lncRNAs may be implicated in pathological progress of endometriosis [20, 21]. Of these, lncRNA MALAT1 has been documented to overexpress in multiple malignant tumors, and serves as an oncogene to promote growth and metastasis of cancer cells [22]. Importantly, MALAT1 expression was verified to be increased in ovarian endometriosis tissues, and loss of MALAT1 suppressed growth and migration of HESCs [23]. Our results were consistent with the above study and showed that MALAT1 down-regulation induced apoptosis and decreased viability of HESCs.

It has been recognized that lncRNA may function as sponger to reduce its target miRNA expression. For example, MALAT1 regulated biological functions of renal cell carcinoma cells via decreasing miR-203 expression [24]. Fang et al. showed that hypoxic-ischemic injury-induced apoptosis of hippocampal neurons was modulated by MALAT1

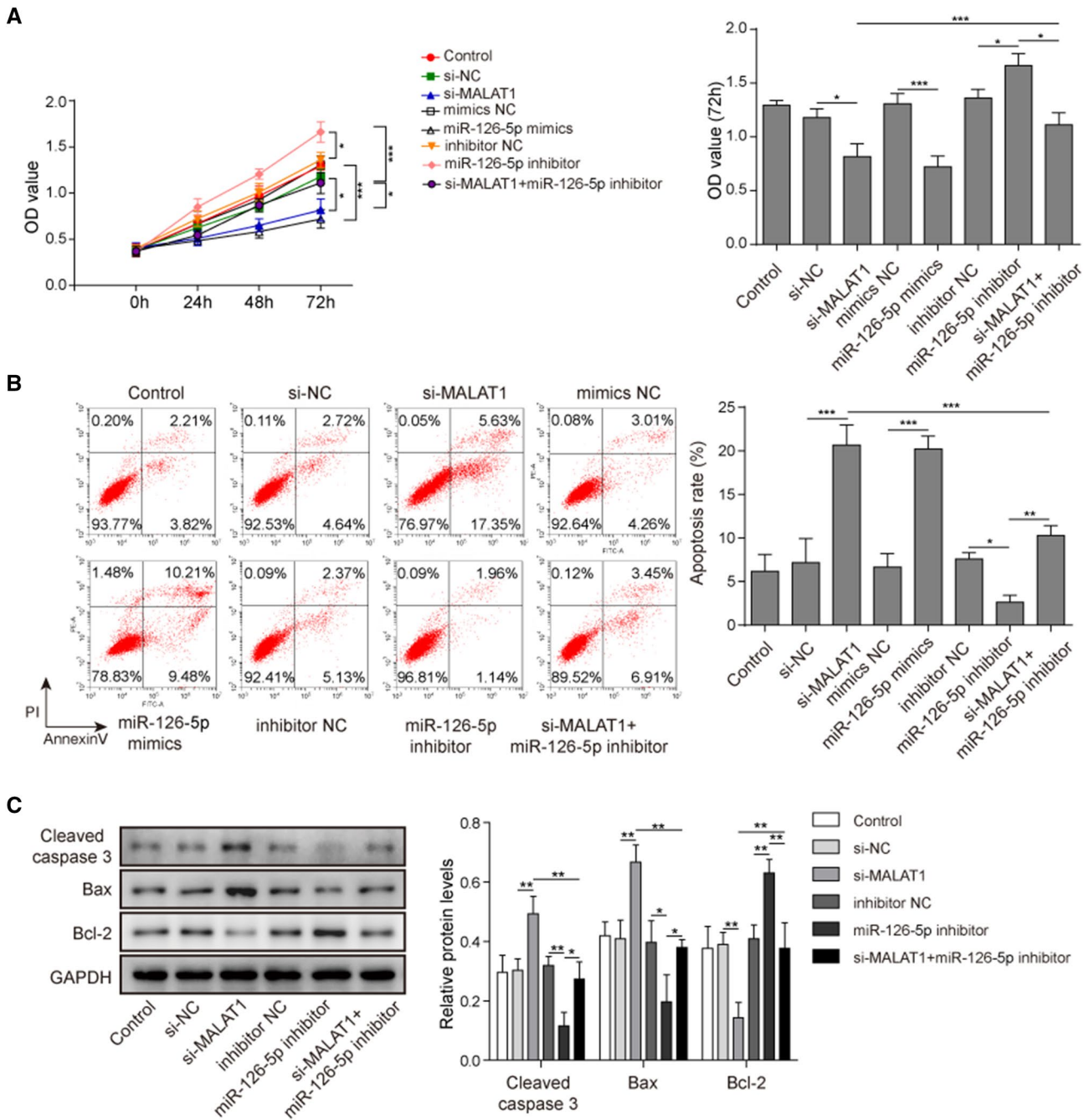


Fig. 3 LncRNA MALAT1 silencing induces HESC apoptosis via regulating miR-126-5p. HESCs were transfected with miR-126-5p mimics, si-MALAT1 or miR-126-5p inhibitor. **a** The viability of HESCs receiving different transfections was determined by MTT. **b** Apoptosis after transfection with si-MALAT1, miR-126-5p inhibitor

or combination of both was assessed by AnnexinV/PI staining using flow cytometry. **c** The levels of apoptosis-related proteins in HESCs were evaluated by western blotting. All results were shown as mean ± standard deviation ($n = 3$). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ versus designated group

via sponging miR-429 [25]. A declined expression of MiR-126-5p in endometriosis patients stimulated the migration of endometrial epithelial and stromal cells [12]. More interestingly, a recent study indicated that MALAT1 accelerated angiogenesis in colorectal cancer via targeting miR-126-5p [26]. Our data also demonstrated that MALAT1 targeting

modulated miR-126-5p expression in HESCs. Additionally, miR-126-5p inhibitor promoted growth and restrained apoptosis, and even counteracted si-MALAT1-induced apoptosis and proliferation inhibition in HESCs. These results suggested that silencing of MALAT1 inhibited growth and induced apoptosis of HESCs via regulating miR-126-5p.

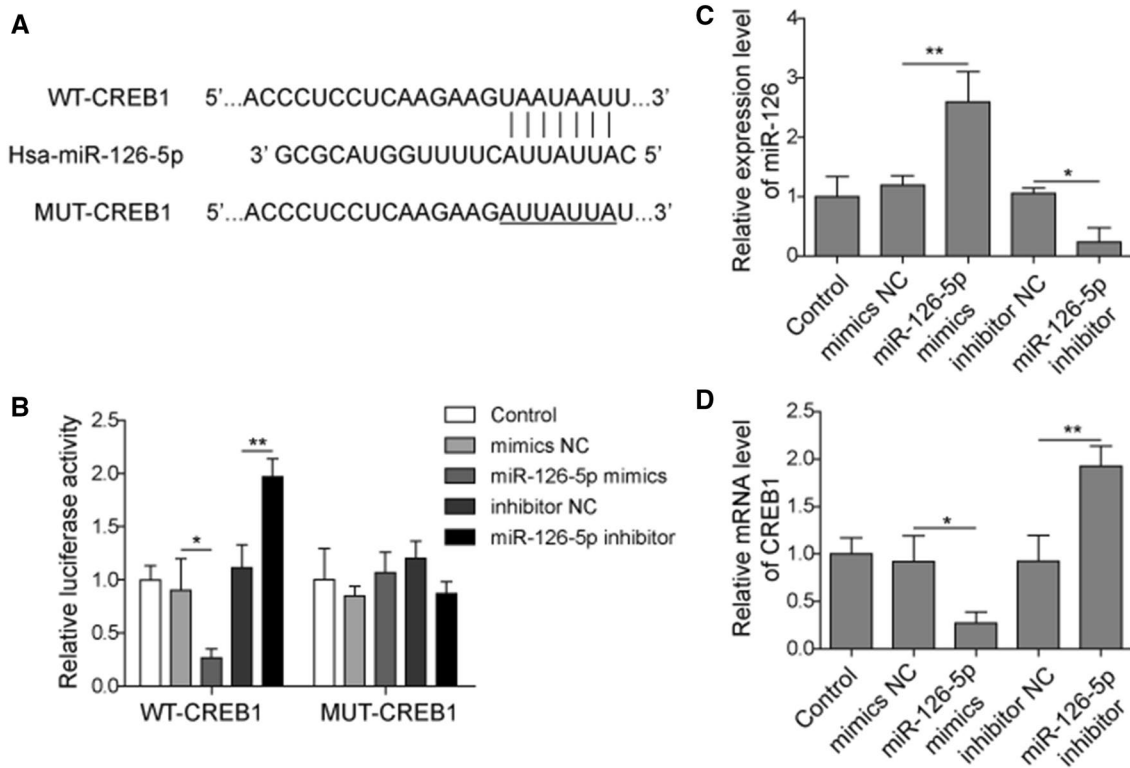


Fig. 4 MiR-126-5p directly regulates CREB1 expression in HESCs. **a** The binding sites for miR-126-5p in CREB1 were shown. **b** Dual luciferase reporter system for determining the interaction ability between miR-126-5p and CREB1. miR-126-5p (c) and

CREB1 (d) levels were detected by RT-qPCR. All results were shown as mean \pm standard deviation ($n=3$). * $P<0.05$, ** $P<0.01$, *** $P<0.001$ versus designated group

It's widely recognized that miRNA silences the target gene expression via binding to its 3' non-coding region. For instance, Lima et al. found that miR-126-5p influenced the progression of non-small cell lung carcinomas via direct binding to the 3' non-coding region of Malate Dehydrogenase 1 and suppressing its activity [27]. miR-126-5p represses the apoptosis by targeting SetD5 during retinal vasculature development [28]. According to our results, miR-126-5p negatively regulated CREB1 expression through binding to its 3' non-coding region in HESCs.

CREB1 is considered as a potent oncogene that plays crucial roles in the malignant behavior of tumor cells [29]. The up-regulation of CREB1 closely relates to the progression and prognosis of tumors. For example, higher expression of CREB1 indicated a poor prognosis of patients with colorectal cancer, and accelerated the malignant progress of colorectal cancer cells [30]. A study by Kong et al. suggested that increased CREB1 expression facilitated the growth of gastric adenocarcinoma cells [31]. In addition, the enhanced expression of CREB1 has been shown to be implicated with the bad outcome of breast cancer [32]. Recently, the expression of CREB1 has been found to be increased in the eutopic endometrial tissues, as compared with normal endometrial tissues [15]. Therefore, we speculate that CREB1 might

regulate the development of endometriosis. According to our results, silencing of CREB1 induced apoptosis and suppressed proliferation, and reversed miR-126-5p inhibitor-mediated apoptosis inhibition in HESCs. A great deal of evidence indicates that the PI3K/AKT pathway participates in the malignant progression in various tumors [33, 34]. A previous study indicated that silencing of CREB1 inhibited the epithelial-mesenchymal transition via repressing the phosphorylation of AKT in bladder cancer cells [35]. Interestingly, AKT can also induce the phosphorylation of CREB1 to mediate its activation [36]. In this study, we found that si-CREB1 restrained the phosphorylation of PI3K and AKT, and treatment with PI3K-AKT pathway activator IGF-1 reversed CREB1 knockdown-induced apoptosis and growth suppression in HESCs. Taken together, miR-126-5p-CREB1-PI3K-AKT axis participated in MALAT1-mediated regulation of proliferation and apoptosis in HESCs.

Our study uncovered the clinical significance of MALAT1 in contributing to the development of endometriosis. RNA interference has the great application potential in clinical treatment. In the future, MALAT1 may be used as a molecular target to benefit diagnose, prognosis, or even therapeutics and complement existing treatments.

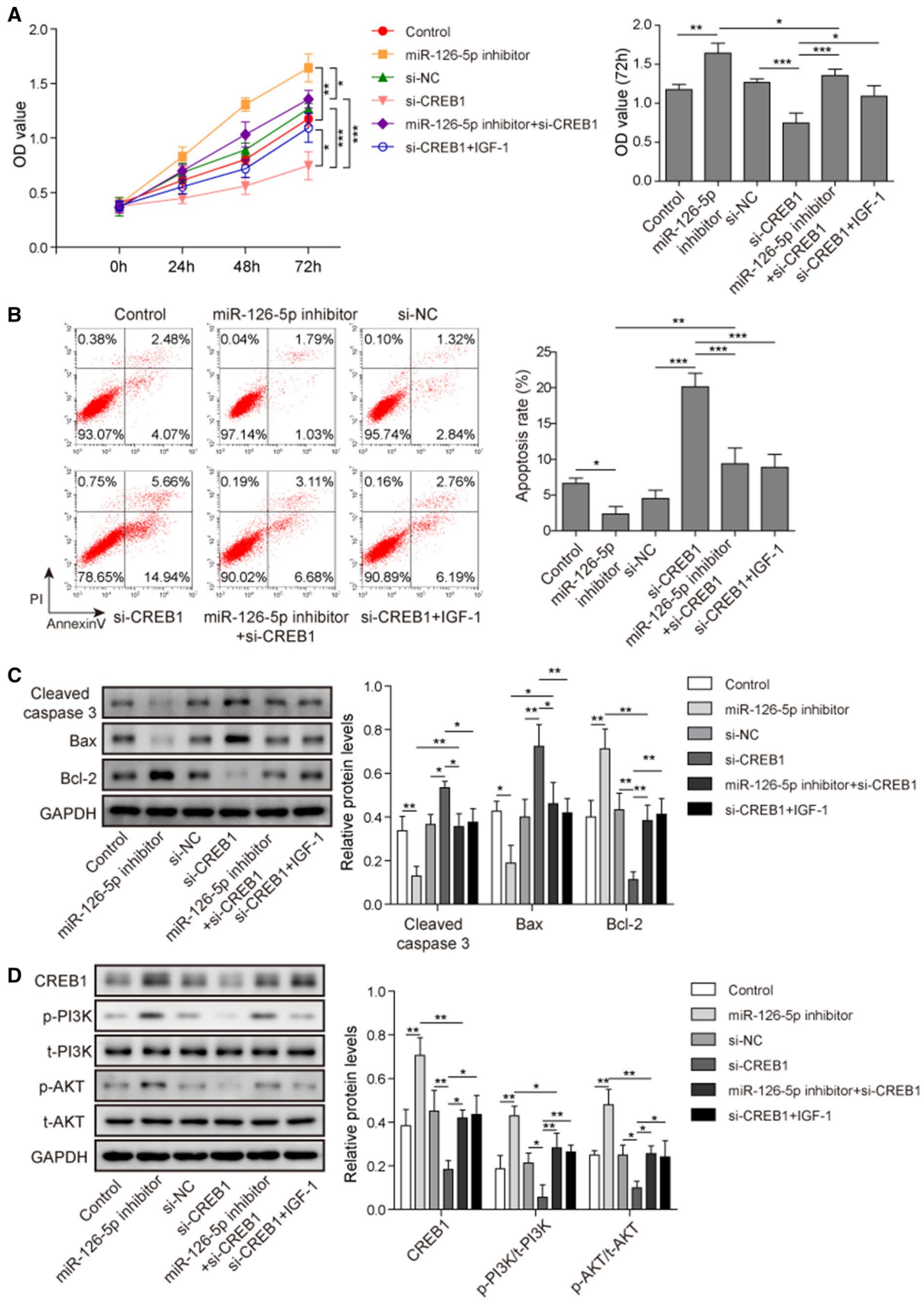


Fig. 5 MiR-126-5p facilitates apoptosis of HESCs through CREB1-mediated PI3K/AKT pathway. HESCs were transfected with miR-126-5p inhibitor, si-CREB1, or treated with IGF-1 (100 ng/mL). **a** MTT assay for evaluating the viability of HESCs from the indicated group. **b** The percentage of apoptotic HESCs that were subjected to various treatments was detected by AnnexinV/PI staining using flow cytometry. **c** and **d** The levels of target proteins in HESCs were determined by western blotting. All results were shown as mean \pm standard deviation ($n=3$). * $P<0.05$, ** $P<0.01$, *** $P<0.001$ versus designated group

In conclusion, our results extend previous findings on the effect of MALAT1 on endometriosis and indicated that loss of MALAT1 promoted apoptosis and suppressed growth of HESCs, probably through miR-126-5p-CREB1 axis via inactivation of PI3K-AKT pathway. Although these findings need to be further verified and investigated in vivo, these data uncover the regulation of MALAT1-miR-126-5p-CREB1 axis during the progression of endometriosis and provide evidence for developing promising therapy against endometriosis.

Author contributions YF contributed to the study conception and design. Material preparation, data collection and analysis were performed by YF and B-ZT. The first draft of the manuscript was written by YF and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Compliance with ethical standards

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

Ethics approval The experiment was carried out in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards, and approved by the Ethics Committee of the Second Affiliated Hospital of Nanchang University.

Informed consent All patients signed written informed consent. The informed consent was obtained from study participants.

References

- Vercellini P, Vigano P, Somigliana E, Fedele L (2014) Endometriosis: pathogenesis and treatment. *Nat Rev Endocrinol* 10:261–275. <https://doi.org/10.1038/nrendo.2013.255>
- Swiersz LM (2002) Role of endometriosis in cancer and tumor development. *Ann N Y Acad Sci* 955:281–292. <https://doi.org/10.1111/j.1749-6632.2002.tb02788.x>
- Macer ML, Taylor HS (2012) Endometriosis and infertility: a review of the pathogenesis and treatment of endometriosis-associated infertility. *Obstet Gynecol Clin N Am* 39:535–549. <https://doi.org/10.1016/j.ogc.2012.10.002>
- Matsuzaki S, Pouly JL, Canis M (2017) Effects of U0126 and MK2206 on cell growth and re-growth of endometriotic stromal cells grown on substrates of varying stiffness. *Sci Rep* 7:42939. <https://doi.org/10.1038/srep42939>
- Santulli P, Marcellin L, Chouzenoux S, Boulard V, Just PA, Nicco C, Chereau C, Tosti C, Chapron C, Batteux F (2016) Role of the protein kinase BRAF in the pathogenesis of endometriosis. *Expert Opin Ther Targets* 20:1017–1029. <https://doi.org/10.1080/14728222.2016.1180367>
- Liu S, Qiu J, Tang X, Cui H, Zhang Q, Yang Q (2019) LncRNA-H19 regulates cell proliferation and invasion of ectopic endometrium by targeting ITGB3 via modulating miR-124-3p. *Exp Cell Res* 381:215–222. <https://doi.org/10.1016/j.yexcr.2019.05.010>
- Liu J, Wang Y, Chen P, Ma Y, Wang S, Tian Y, Wang A, Wang D (2019) AC002454.1 and CDK6 synergistically promote endometrial cell migration and invasion in endometriosis. *Reproduction*. <https://doi.org/10.1530/REP-19-0005>
- Yu J, Chen LH, Zhang B, Zheng QM (2019) The modulation of endometriosis by lncRNA MALAT1 via NF-kappaB/iNOS. *Eur Rev Med Pharmacol Sci* 23:4073–4080. https://doi.org/10.26355/eurrev_201905_17908
- Liu H, Zhang Z, Xiong W, Zhang L, Du Y, Liu Y, Xiong X (2019) Long non-coding RNA MALAT1 mediates hypoxia-induced pro-survival autophagy of endometrial stromal cells in endometriosis. *J Cell Mol Med* 23:439–452. <https://doi.org/10.1111/jcmm.13947>
- Zhou CF, Liu MJ, Wang W, Wu S, Huang YX, Chen GB, Liu LM, Peng DX, Wang XF, Cai XZ, Li XX, Feng WQ, Ma Y (2019) miR-205-5p inhibits human endometriosis progression by targeting ANGPT2 in endometrial stromal cells. *Stem Cell Res Ther* 10:287. <https://doi.org/10.1186/s13287-019-1388-5>
- Zhu H, Cao XX, Liu J, Hua H (2019) MicroRNA-488 inhibits endometrial glandular epithelial cell proliferation, migration, and invasion in endometriosis mice via Wnt by inhibiting FZD7. *J Cell Mol Med* 23:2419–2430. <https://doi.org/10.1111/jcmm.14078>
- Meng X, Liu J, Wang H, Chen P, Wang D (2019) MicroRNA-126-5p downregulates BCAR3 expression to promote cell migration and invasion in endometriosis. *Mol Cell Endocrinol* 494:110486. <https://doi.org/10.1016/j.mce.2019.110486>
- Shabestari RM, Safa M, Alikarami F, Banan M, Kazemi A (2017) CREB knockdown inhibits growth and induces apoptosis in human pre-B acute lymphoblastic leukemia cells through inhibition of pro-survival signals. *Biomed Pharmacother* 87:274–279. <https://doi.org/10.1016/j.biopha.2016.12.070>
- Li Y, Chen D, Jin L, Liu J, Su Z, Qi Z, Shi M, Jiang Z, Ni L, Yang S, Gui Y, Mao X, Chen Y, Lai Y (2016) Oncogenic cAMP responsive element binding protein 1 is overexpressed upon loss of tumor suppressive miR-10b-5p and miR-363-3p in renal cancer. *Oncol Rep* 35:1967–1978. <https://doi.org/10.3892/or.2016.4579>
- Lv X, Wang D, Ma Y, Long Z (2018) Analysis of the oncogene BRAF mutation and the correlation of the expression of wild-type BRAF and CREB1 in endometriosis. *Int J Mol Med* 41:1349–1356. <https://doi.org/10.3892/ijmm.2017.3342>
- Liu H, Zhang Z, Xiong W, Zhang L, Xiong Y, Li N, He H, Du Y, Liu Y (2017) Hypoxia-inducible factor-1alpha promotes endometrial stromal cells migration and invasion by upregulating autophagy in endometriosis. *Reproduction* 153:809–820. <https://doi.org/10.1530/REP-16-0643>
- Kim HS, Kim TH, Chung HH, Song YS (2014) Risk and prognosis of ovarian cancer in women with endometriosis: a meta-analysis. *Br J Cancer* 110:1878–1890. <https://doi.org/10.1038/bjc.2014.29>
- Kao AP, Wang KH, Chang CC, Lee JN, Long CY, Chen HS, Tsai CF, Hsieh TH, Tsai EM (2011) Comparative study of human eutopic and ectopic endometrial mesenchymal stem cells and the development of an in vivo endometriotic invasion model.

- Fertil Steril 95(1308–15):e1. <https://doi.org/10.1016/j.fertnstert.2010.09.064>
19. Coutinho LM, Vieira EL, Dela Cruz C, Casalechi M, Teixeira AL, Del Puerto HL, Reis FM (2016) Apoptosis modulation by activin A and follistatin in human endometrial stromal cells. *Gynecol Endocrinol* 32:161–165. <https://doi.org/10.3109/09513590.2015.1103222>
 20. Zhu MB, Chen LP, Hu M, Shi Z, Liu YN (2019) Effects of lncRNA BANC1 on endometriosis through ERK/MAPK pathway. *Eur Rev Med Pharmacol Sci* 23:6806–6812. https://doi.org/10.26355/eurrev_201908_18719
 21. Huang H, Zhu Z, Song Y (2019) Downregulation of lncRNA uc1 as a diagnostic and prognostic biomarker for ovarian endometriosis. *Rev Assoc Med Bras* 65:336–341. <https://doi.org/10.1590/1806-9282.65.3.336>
 22. Li ZX, Zhu QN, Zhang HB, Hu Y, Wang G, Zhu YS (2018) MALAT1: a potential biomarker in cancer. *Cancer Manag Res* 10:6757–6768. <https://doi.org/10.2147/CMAR.S169406>
 23. Liang Z, Chen Y, Zhao Y, Xu C, Zhang A, Zhang Q, Wang D, He J, Hua W, Duan P (2017) miR-200c suppresses endometriosis by targeting MALAT1 in vitro and in vivo. *Stem Cell Res Ther* 8:251. <https://doi.org/10.1186/s13287-017-0706-z>
 24. Zhang H, Li W, Gu W, Yan Y, Yao X, Zheng J (2019) MALAT1 accelerates the development and progression of renal cell carcinoma by decreasing the expression of miR-203 and promoting the expression of BIRC5. *Cell Prolif*. <https://doi.org/10.1111/cpr.12640>
 25. Fang H, Li HF, He MH, Yan JY, Yang M, Zhang FX, Wang RR, Wang QY, Zhang JP (2019) Long non-coding RNA MALAT1 sponges microRNA-429 to regulate apoptosis of hippocampal neurons in hypoxic-ischemic brain damage by regulating WNT1. *Brain Res Bull* 152:1–10. <https://doi.org/10.1016/j.brainresbull.2019.06.004>
 26. Sun Z, Ou C, Liu J, Chen C, Zhou Q, Yang S, Li G, Wang G, Song J, Li Z, Zhang Z, Yuan W, Li X (2019) YAP1-induced MALAT1 promotes epithelial-mesenchymal transition and angiogenesis by sponging miR-126-5p in colorectal cancer. *Oncogene* 38:2627–2644. <https://doi.org/10.1038/s41388-018-0628-y>
 27. Lima Queiroz A, Zhang B, Comstock DE, Hao Y, Eriksson M, Hydbring P, Vakifahmetoglu-Norberg H, Norberg E (2018) miR-126-5p targets malate dehydrogenase 1 in non-small cell lung carcinomas. *Biochem Biophys Res Commun* 499:314–320. <https://doi.org/10.1016/j.bbrc.2018.03.154>
 28. Villain G, Poissonnier L, Noueihed B, Bonfils G, Rivera JC, Chemtob S, Soncin F, Mattot V (2018) miR-126-5p promotes retinal endothelial cell survival through SetD5 regulation in neurons. *Development*. <https://doi.org/10.1242/dev.156232>
 29. Rao M, Zhu Y, Cong X, Li Q (2017) Knockdown of CREB1 inhibits tumor growth of human gastric cancer in vitro and in vivo. *Oncol Rep* 37:3361–3368. <https://doi.org/10.3892/or.2017.5636>
 30. Yan L, You WQ, Sheng NQ, Gong JF, Hu LD, Tan GW, Chen HQ, Wang ZG (2018) A CREB1/miR-433 reciprocal feedback loop modulates proliferation and metastasis in colorectal cancer. *Aging (Albany NY)* 10:3774–3793. <https://doi.org/10.18632/aging.101671>
 31. Kong WQ, Bai R, Liu T, Cai CL, Liu M, Li X, Tang H (2012) MicroRNA-182 targets cAMP-responsive element-binding protein 1 and suppresses cell growth in human gastric adenocarcinoma. *FEBS J* 279:1252–1260. <https://doi.org/10.1111/1/j.1742-4658.2012.08519.x>
 32. Chhabra A, Fernando H, Watkins G, Mansel RE, Jiang WG (2007) Expression of transcription factor CREB1 in human breast cancer and its correlation with prognosis. *Oncol Rep* 18:953–958
 33. Zhao Y, Sun H, Feng M, Zhao J, Zhao X, Wan Q, Cai D (2018) Metformin is associated with reduced cell proliferation in human endometrial cancer by inhibiting PI3K/AKT/mTOR signaling. *Gynecol Endocrinol* 34:428–432. <https://doi.org/10.1080/09513590.2017.1409714>
 34. Lee MS, Jeong MH, Lee HW, Han HJ, Ko A, Hewitt SM, Kim JH, Chun KH, Chung JY, Lee C, Cho H, Song J (2015) PI3K/AKT activation induces PTEN ubiquitination and destabilization accelerating tumorigenesis. *Nat Commun* 6:7769. <https://doi.org/10.1038/ncomms8769>
 35. Xu X, Zhu Y, Liang Z, Li S, Wang X, Wu J, Hu Z, Meng S, Liu B, Qin J, Xie L, Zheng X (2016) c-Met and CREB1 are involved in miR-433-mediated inhibition of the epithelial-mesenchymal transition in bladder cancer by regulating Akt/GSK-3beta/Snail signaling. *Cell Death Dis* 7:e2088. <https://doi.org/10.1038/cddis.2015.274>
 36. Rosa E, Fahnestock M (2015) CREB expression mediates amyloid beta-induced basal BDNF downregulation. *Neurobiol Aging* 36:2406–2413. <https://doi.org/10.1016/j.neurobiolaging.2015.04.014>

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