Long non-coding RNA (IncRNA) HOXB-AS3 promotes cell proliferation and inhibits apoptosis by regulating ADAM9 expression through targeting miR-498-5p in endometrial carcinoma

Ying Xing1,*, Xianhua Sun2,*, Feng Li3,*, Xuan Jiang4,*, Afang Jiang5, Xiaofan Li6, Ruiting Lv7 and Liwei Shao8

Abstract
Objective: Long non-coding RNA (IncRNA) expression is closely related to the pathogenesis and progression of various tumors. In this study, we investigated the mechanisms of IncRNA HOXB cluster antisense RNA 3 (HOXB-AS3), miRNA(miR)-498-5p, and disintegrin and metalloproteinase domain-containing protein 9 (ADAM9) in endometrial carcinoma (EC) cells.

Methods: The expression levels of IncRNA HOXB-AS3 in EC tissues and cells were detected using RT-qPCR assays. The effects of HOXB-AS3 knockdown on EC cell proliferation and apoptosis were measured using CCK-8 assays, colony formation assays, and flow cytometry. In addition, putative
miR-498-5p binding sites were identified in HOXB-AS3 and ADAM9. The targeted relationships were further verified using dual-luciferase reporter and RNA pull-down assays.

**Results:** HOXB-AS3 expression was upregulated in EC tissues and cells. EC cell proliferation and viability decreased significantly in HOXB-AS3 knockdown groups. A putative miR-498-5p binding site in HOXB-AS3 was verified. Inhibition of miR-498-5p rescued the effects of HOXB-AS3 knockdown on cell proliferation and apoptosis. Finally, ADAM9 was verified as a direct target gene of miR-498-5p.

**Conclusions:** Our results suggest that IncRNA HOXB-AS3 is highly expressed in EC tissues and cells. Downregulation of HOXB-AS3 inhibits cell proliferation and promotes apoptosis in EC cells. HOXB-AS3 can upregulate ADAM9 expression by sponging miR-498-5p.

**Keywords**
Disintegrin and metalloproteinase domain-containing protein 9, endometrial carcinoma, long non-coding RNA HOXB cluster antisense RNA 3, miR-498-5p, tumorigenesis, tumor progression

**Introduction**

As the fifth most common cancer in females, endometrial cancer (EC) originates in the endometrium layer of the uterus. Recently, the increased incidence of EC has led to concerns regarding disease management. Early screening and accurate diagnostic assessment are critical steps for successful treatment of EC.

Studies have shown the emerging role of long non-coding RNAs (lncRNAs) in carcinogenesis. LncRNAs are found in both the cell nucleus and cytoplasm, indicating its role in epigenetics and translational and post-translational gene regulation. Aberrant expression of lncRNAs in EC tissues, as well as the associated regulatory networks, has been recognized and identified, suggesting the significance of lncRNAs in EC diagnosis and prognosis. Several lncRNAs have been found to be associated with EC, including small nucleolar RNA host gene 12 (ASLNC04080), H19 imprinted maternally expressed transcript (H19), ovalbumin (OVAL), cancer susceptibility 2 (CASC2), metastasis associated lung adenocarcinoma transcript 1 (MALAT1), HOX transcript antisense RNA (HOTAIR), sister of ramosa 3 (SRA), and long intergenic non-coding RNA, regulator of reprogramming (Linc-RoR). Li et al. summarized and reviewed the roles of various lncRNAs in the initiation and progression of EC. LncRNA HOXB cluster antisense RNA 3 (HOXB-AS3) has a crucial role in multiple tumors, including hepatocellular carcinoma, acute myeloid leukemia, colon cancer, and epithelial ovarian cancer. However, its potential functions and mechanisms in EC pathogenesis and progression remain unknown.

MicroRNAs (miRNAs) are small non-coding RNAs that are responsible for post-transcriptional control of gene expression. Dysregulation of miRNAs is closely related to cancer initiation and progression. The role of miRNAs in EC has been identified and investigated in many studies. Cohn et al. provided a comprehensive analysis of the miRNA profile for surgically-staged EC, suggesting miRNAs could serve as predictive and therapeutic biomarkers for EC. Among multiple miRNAs, miR-498-5p has been identified in various types of cancer, such as ovarian cancer and cervical cancer, and functions in either an oncogenic or tumor suppressive manner. More importantly, Xue et al. investigated the role of miR-498-5p in bronchial epithelial cell inflammation
responses. However, the function of miR-498-5p in the progression of EC remains unclear.

The disintegrin and metalloproteinase domain (ADAM) family is a group of cell surface proteins that are responsible for cell adhesion and migration. Studies have shown that the ADAM family also modulates cell invasion and proliferation in various cancers. ADAM9 is a membrane-anchored metalloprotease that has been shown to be upregulated in different human carcinomas, such as prostate and gastric cancers.

Therefore, this research aims to elucidate the potential mechanisms and interactions among HOXB-AS3, miR-498-5p, and ADAM9 in EC, which may provide significant benefits for EC diagnosis and treatment.

Materials and methods

Patient tissue samples

Sixty patients diagnosed with EC were selected for this study. No radiotherapy or chemotherapy were given to the patients before surgery. The tumor tissue samples and adjacent normal tissue samples were obtained during surgical resection of EC. All samples were stored at −80°C until use. All experiments were approved by the ethics committee at Southern Medical University (Approval number SMU-HL93-2017; 7 March 2017). Written informed consent was obtained from all patients that participated in this study.

Cell culture and cell transfections

Three EC cell lines, including HEC-1-A (HTB-112), HEC-1-B (HTB-113), and Ishikawa (HTB-113), and human embryonic stem cells (ESC) (SCRC-2002) were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). The cells were cultured in 5% CO2 at 37°C. The cell culture medium used for each cell line follows the recommended formulation and required additives on the ATCC website (https://www.atcc.org/products/all/HTB-113.aspx).

Cell transfections were performed when cultures reached 80% confluence using Lipofectamine 2000 Transfection Reagent (Invitrogen, Carlsbad, CA, USA). Specific small interfering RNA (siRNA) oligonucleotides targeting HOXB-AS3 (si-HOXB-AS3; 5'-UUCUUGUCUGGAGAUGGAGCCACUA-3'), ADAM9 (5'-CGCUCUUGGAGAUAACUAGUU-3'), miR-498-5p (5'-UUUCAAGGCCAGGGGGCGUUUUUC-3'), and negative control siRNA (si-NC) were transfected into EC cells. The si-HOXB-AS3, si-ADAM9 (5'-CUCCUUGGAGAUAAACUAGUU-3'), anti-miR-498-5p (5'-UUUCAAGGCCAGGGGGCGUUUUUC-3'), miR-498-5p mimic (5'-TTTCAAGCCAGGGGGCGGT TTTC-3'), and negative control (5'-TGTAGTTGTACTCAGCTTGUGCT T-3') were synthesized and purchased from GenePharma (Shanghai, China). In our study, miR-498-5p was used in all experiments. Quantitative reverse transcription polymerase chain reaction (RT-qPCR) assays were used to confirm HOXB-AS3 knockdown 48 hours after transfection. The sequence of si-HOXB-AS3 was designed as UGCUUGUCUGGAGAUGGAGCCACUA.

RNA isolation and quantitative reverse transcription polymerase chain reaction assays

Total RNA was extracted from EC tissues and cell samples using TRIzol RNA Isolation Reagents (Thermo Fisher Scientific, Rockford, IL, USA), then reverse transcribed into complementary DNA (cDNA) using PrimeScript RT Reagent Kit with gDNA Eraser (Takara, Kyoto, Japan). Then, RT-qPCR assays were carried out using an ABI 7500 Fast Real-Time PCR system (Applied
Biosystems, Foster City, CA, USA) according to the manufacturer’s suggested protocol. SYBR® Green JumpStart™ Taq ReadyMix™ (S1816, Sigma-Aldrich, St. Louis, MO, USA) was used in all RT-qPCRs. The 2$^{-\Delta\Delta Ct}$ method was used to quantify the relative expression levels of genes. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an endogenous control. Primer sequences for HOXB-AS3: Forward, 5’-TGCTTGTCTGGAGATGGAGC-3’; Reverse, 5’-GAATAAGAGCGATGAGGCGCT-3’. Primer sequences for GAPDH: Forward, 5’-TCTTACTCCTTGGAGGAGCCAT-3’. Dual-luciferase reporter assay
To confirm the putative miR-498-5p binding sites in HOXB-AS3 or ADAM9, relative luciferase activity was measured using the dual-luciferase reporter assay system (Promega, Madison, WI, USA). pGL4 luciferase reporter vectors were constructed for wild type (WT) HOXB-AS3 fragments (HOXB-AS3 WT) with miR-498-5p binding sites and HOXB-AS3 mutant (HOXB-AS3 MUT) without miR-498-5p binding sites. Mutants were generated using a GeneEditor in vitro Site-Directed Mutagenesis System (Promega). Ishikawa cells were co-transfected with HOXB-AS3 WT or HOXB-AS3 MUT and NC miRNA mimic or miR-498-5p mimic using Lipofectamine 2000 Transfection Reagent (Invitrogen). Similarly, WT or mutant ADAM9 3’ untranslated region (3’ UTR) with or without miR-498-5p binding sites were also cloned into pGL4 luciferase reporter vectors to form reporter vector ADAM9 WT and ADAM9 MUT, respectively. The relative luciferase activity was measured according to the manufacturer’s suggested protocol.

RNA pull-down assay
Ishikawa cells were transfected with biotinylated miR-498-5p (miR-498-5p probe) using Lipofectamine 2000 Transfection Reagent (Invitrogen). Forty-eight hours after transfection, cells were collected, lysed, and incubated with streptavidin-coupled beads for the pull-down assay using an RNA-protein pull-down kit (Millipore, Burlington, MA, USA). HOXB-AS3 expression levels were further quantified using RT-qPCR assays.

Cell viability and apoptosis assays
A Cell Counting Kit-8 (CCK-8) assay (Dodinjo, Kumamoto, Japan) was used to detect cell viability. The cell viability was evaluated 24 hours after seeding the transfected cell suspensions at a density of 3500 cells/well in a 96-well plate. The absorbance was measured at 450 nm.

Colony formation assays were also used to detect cell proliferation. After transfection, the cells were plated at density of 1000 cells/well in a six-well plate and allowed to grow for 15 days. Cell colonies were fixed using methanol, stained using 0.1% crystal purple (Sigma, St. Louis, MO, USA), then counted under a light microscope.

The apoptotic cells were quantified using flow cytometry. Cell suspensions were washed with PBS and fixed in 4% formaldehyde overnight. An Annexin V-FITC Apoptosis Detection Kit (Sigma) was used to label the cellular DNA.

Western blotting and immunohistochemistry (IHC) assay
Cells were scraped and lysed in radioimmunoprecipitation assay (RIPA) buffer (R0278, Sigma) on ice. The total protein was measured using a BCA Protein Assay Kit (Pierce, Thermo Fisher Scientific). Then, 50 μg of total protein was loaded on
an SDS-PAGE gel for electrophoresis, and then transferred to PVDF membranes. The membranes were blocked using 5% non-fat milk at room temperature for two hours, then incubated with specific primary antibodies at 4°C overnight. The membranes were washed with Tris-buffered saline containing Tween-20 (TBST) three times, then incubated with secondary antibody at room temperature for 2 hours. The protein bands were detected by chemiluminescence (ECL) based on manufacturer’s instruction (Santa Cruz Biotechnology, Dallas, TX, USA). All antibodies were purchased from Abcam (Cambridge, MA, USA). The anti-ADAM9 antibody (ab226459) is a rabbit polyclonal. A 1:10,000 dilution was used in all experiments.

Immunohistohemistry (IHC) assays for ADAM9 was performed on 4-μm sections of formalin-fixed paraffin-embedded (FFPE) tissue using VENTANA BenchMark Special Stains (Roche, Indianapolis, IN, USA) based on the manufacturer’s instructions. A rabbit polyclonal antibody against ADAM9 (ab186833, Abcam, Cambridge, MA, USA) was used in our study.

Statistical analysis

All experiments were conducted in triplicate and repeated three times. All data were measured as mean ± standard deviation (SD). All statistical analyses were performed using SPSS 20.0 software (IBM Corp., Armonk, NY, USA). A paired t-test or one-way analysis of variance (ANOVA) was applied to determine the differences between groups. Kaplan–Meier analysis was performed using GraphPad Prism 8.0 (GraphPad Software Inc., San Diego, CA, USA). A P-value less than 0.05 was considered to be statistically significant.

Results

Overexpression of HOXB-AS3 in EC tissues and cell lines

First, The Cancer Genome Atlas (TCGA) database was used to compare the expression of HOXB-AS3 in EC tissues and corresponding healthy tissues. HOXB-AS3 expression data in TCGA and normal samples were obtained from the Gene Expression Profiling Interactive Analysis (GEPIA) database (http://gepia2.cancer-pku.cn/#analysis). As shown in Figure 1a, the expression levels of HOXB-AS3 in EC tissues are significantly higher than those in healthy tissues. Second, HOXB-AS3 expression was examined in 60 pairs of EC tissues and adjacent normal tissues using RT-qPCR assays. The results showed that the expression of HOXB-AS3 was significantly upregulated in tumor tissues (P < 0.001, Figure 1b). Subsequently, the expression levels of HOXB-AS3 in EC cell lines, including HEC1A, HEC-1-B and Ishikawa, and human ESCs were also examined. The results suggested that HOXB-AS3 has much higher expression in the three EC cell lines, especially in HEC1A and Ishikawa cells (P < 0.05, Figure 1c). In addition, we investigated a potential correlation between HOXB-AS3 expression and the survival rate of patients with EC. Kaplan–Meier analysis was used to evaluate the survival rate of patients with EC in low (n = 30) and high (n = 30) HOXB-AS3 expression groups. Those patients with higher HOXB-AS3 expression had shorter survival times than those with lower expression (P < 0.001, Figure 1d).

HOXB-AS3 suppresses EC cell proliferation and promotes apoptosis

To verify the function of HOXB-AS3 in vitro, cell proliferation rates following HOXB-AS3 knockdown were examined in
EC cell lines. First, two EC cell lines (HEC1A and Ishikawa) with higher HOXB-AS3 expression were used for loss-of-function studies. Successful knockdown of HOXB-AS3 expression was seen using qRT-PCR assays in the si-HOXB-AS3 group compared with the control group (si-NC) (Figure 2a). Next, the EC cell proliferation rates were verified using CCK-8 assays. The optical density (OD) values at 450 nm were significantly lower in the si-HOXB-AS3 group compared with the si-NC group \( (P < 0.01, \text{Figure } 2b) \), which suggested that cell viability was reduced after knocking down HOXB-AS3. Furthermore, EC cell survival was tested using colony formation assays in both cell lines. Plate cloning experiments showed that HOXB-AS3 knockdown reduced the colony forming ability in both EC cell lines (Figure 2c). In addition, apoptosis was evaluated by flow cytometry. The results showed that both EC cell lines had significantly higher apoptosis levels in the HOXB-AS3 knockdown groups compared with the controls (Figure 2d).

**miR-498-5p has a binding site in HOXB-AS3 in EC cells**

A miR-498-5p binding site in the HOXB-AS3 sequence was predicted using
To further verify this binding site, dual-luciferase reporter analysis was carried out on Ishikawa cells. The luciferase activity was significantly reduced in cells co-transfected with HOXB-AS3 WT and miR-498-5p mimic compared with that in cells co-transfected with HOXB-AS3 WT and miR-NC mimic. No significant change was seen in the cells transfected with HOXB-AS3 MUT (\(P < 0.01\), Figure 3a). Next, RNA pull-down assays were used to confirm that miR-498-5p could bind to HOXB-AS3. HOXB-AS3 was successfully pulled down with a miR-498-5p probe (\(P < 0.01\), Figure 3b). Additionally, RT-qPCR assays were used to measure miR-498-5p expression in Ishikawa cells. The results showed that the expression of miR-498-5p was significantly upregulated in the HOXB-AS3 knockdown group (\(P < 0.01\), Figure 3c). We also examined the expression levels of miR-498-5p in three EC cell lines and EC tissues. miR-498-5p expression was much lower in the three EC cell lines than in ESCs, especially in HEC1A and Ishikawa cells (\(P < 0.05\), Figure 3d). Also, miR-498-5p was downregulated in EC tissues (n = 30) compared with adjacent healthy tissues (n = 30) (\(P < 0.05\), Figure 3e). Furthermore, miR-498-5p expression was negatively correlated with HOXB-AS3 expression in EC tissues (\(R^2 = 0.2645\), \(P < 0.0001\)) (Figure 3f).

**HOXB-AS3 promotes cell proliferation and inhibits apoptosis of EC cells by sponging miR-498-5p**

Next, we further explored the mechanisms of HOXB-AS3 in EC cell proliferation via LncBase (http://carolina.imis.athena-innovation.gr/diana_tools/web/). To further verify this binding site, dual-luciferase reporter analysis was carried out on Ishikawa cells. The luciferase activity was significantly reduced in cells co-transfected with HOXB-AS3 WT and miR-498-5p mimic compared with that in cells co-transfected with HOXB-AS3 WT and miR-NC mimic. No significant change was seen in the cells transfected with HOXB-AS3 MUT (\(P < 0.01\), Figure 3a). Next, RNA pull-down assays were used to confirm that miR-498-5p could bind to HOXB-AS3. HOXB-AS3 was successfully pulled down with a miR-498-5p probe (\(P < 0.01\), Figure 3b). Additionally, RT-qPCR assays were used to measure miR-498-5p expression in Ishikawa cells. The results showed that the expression of miR-498-5p was significantly upregulated in the HOXB-AS3 knockdown group (\(P < 0.01\), Figure 3c). We also examined the expression levels of miR-498-5p in three EC cell lines and EC tissues. miR-498-5p expression was much lower in the three EC cell lines than in ESCs, especially in HEC1A and Ishikawa cells (\(P < 0.05\), Figure 3d). Also, miR-498-5p was downregulated in EC tissues (n = 30) compared with adjacent healthy tissues (n = 30) (\(P < 0.05\), Figure 3e). Furthermore, miR-498-5p expression was negatively correlated with HOXB-AS3 expression in EC tissues (\(R^2 = 0.2645\), \(P < 0.0001\)) (Figure 3f).

**HOXB-AS3 promotes cell proliferation and inhibits apoptosis of EC cells by sponging miR-498-5p**

Next, we further explored the mechanisms of HOXB-AS3 in EC cell proliferation via
cell proliferation assays. First, we confirmed that miR-498-5p expression can be downregulated after transfection with anti-miR-498-5p in Ishikawa cells \((P < 0.01, \text{Figure 4a})\). Second, a series of experiments was carried out to confirm that HOXB-AS3 silencing could be restored via co-introduction of anti-miR-498-5p. The CCK-8 assays showed that the OD values at 450 nm were relatively higher in cells co-transfected with si-HOXB-AS3 and anti-miR-498-5p compared with cells transfected with si-HOXB-AS3 alone \((P < 0.01, \text{Figure 4b})\). The colony formation assays showed that colony forming ability was partially regained in cells co-transfected with si-HOXB-AS3 and anti-miR-498-5p \((P < 0.01, \text{Figure 4c})\). The flow cytometry results showed that apoptosis was relieved after cells were co-transfected with si-HOXB-AS3 and anti-miR-498-5p \((P < 0.01, \text{Figure 4d})\).

**HOXB-AS3 promotes ADAM9 expression by sponging miR-498-5p**

A putative miR-498-5p binding site in the ADAM9 3’ UTR was predicted using bioinformatics software starBase v2.0. To verify this binding site, dual-luciferase reporter analysis was used to show that the miR-498-5p mimic significantly inhibited the luciferase activity of ADAM9 WT but not ADAM9 MUT in Ishikawa cells.
Moreover, ADAM9 protein expression was significantly reduced following miR-498-5p overexpression (Figure 5b). Silencing HOXB-AS3 reduced the expression of ADAM9, but such reduction was restored under the effect of a miR-498-5p inhibitor (Figure 5c). Furthermore, we evaluated the expression levels and function of ADAM9 in EC cells. ADAM9 had higher expression in the three EC cell lines than in ESCs ($P < 0.05$, Figure 5d). ADAM9 was also upregulated in EC tissues ($n = 30$) compared with adjacent healthy tissues ($n = 30$) ($P < 0.05$, Figure 5e and f). Kaplan–Meier analysis suggested that the patients with EC and higher ($n = 30$) ADAM9 expression had shorter survival times than those with lower ($n = 30$) expression ($P < 0.05$, Figure 5g). Moreover, ADAM9 mRNA expression was negatively correlated with miR-498-5p expression ($R^2 = 0.2436$, $P < 0.0001$) (Figure 5h), but positively correlated with HOXB-AS3 in EC tissues ($R^2 = 0.3185$, $P < 0.0001$) (Figure 5i). In addition, the impact of ADAM9 on EC cell growth and apoptosis was detected by loss-of-function assays. After knockdown of ADAM9 in Ishikawa cells, ADAM9 expression decreased (Figure 5j), EC cell viability also decreased (Figure 5k), and EC cell apoptosis was significantly higher (Figure 5l).

**Discussion**

Numerous IncRNAs and miRNAs have been identified and reported to be dysregulated in the carcinogenesis and tumor progression processes. A newly identified IncRNA, HOXB-AS3, has been reported...
Figure 5. ADAM9 is targeted by miR-498-5p in endometrial carcinoma (EC) cell lines. (a) A miR-498-5p binding site is present in the ADAM9 3’ untranslated region (3’ UTR), and miR-498-5p overexpression inhibits the luciferase activity of ADAM9 WT. (b) Western blot analysis quantification showing ADAM9 protein levels after cell transfection with miR-498-5p mimic; miR-498-5p overexpression inhibits ADAM9 protein expression. (c) Western blot analysis quantification showing ADAM9 protein levels after cell transfection with si-HOXB-AS3 and anti-miR-498-5p; miR-498-5p inhibitor treatment restores ADAM9 protein expression. (d and e) Upregulation of ADAM9 in three EC cell lines EC tissues, as measured by RT-qPCR assays. (f) ADAM9 expression in EC tissues, as determined by immunohistochemistry assays. (g) Overexpression of ADAM9 is associated with lower survival in patients with EC. (h) Negative correlation between ADAM9 and miR-498-5p in EC tissues ($R^2 = 0.2436, P < 0.0001$). (i) Positive correlation between ADAM9 and HOXB-AS3 in EC tissues ($R^2 = 0.3185, P < 0.0001$). (j) Downregulation of ADAM9 measured by qRT-PCR assays in Ishikawa cells after ADAM9 knockdown. (k) The viability of ADAM9 knockdown cells, as detected by CCK-8 assays. (l) Apoptosis of ADAM9 knockdown cells quantified by flow cytometry. **$P < 0.05$; ***$P < 0.001$. 
in several different cancers. Zhuang et al.\textsuperscript{33} discovered that HOXB-AS3 is overexpressed in epithelial ovarian cancer (EOC) tissues and cells, and also showed that HOXB-AS3 acts as an oncogene through the Wnt/\(\beta\)-catenin signaling pathway. The authors also demonstrated that silencing HOXB-AS3 can inhibit EOC cell proliferation and invasion. This study suggested that there may be a potential correlation between HOXB-AS3 and the progression of EC. In our study, we observed that HOXB-AS3 and ADAM9 were significantly overexpressed in both EC tissues and cell lines (Figure 1b, c, and Figure 5d–f). The loss-of-function study also showed that both HOXB-AS3 knockdown and ADAM9 knockdown could reduce EC cell proliferation and promote apoptosis (Figure 2a–d, and Figure 5j–l).

MiRNAs are important post-transcriptional regulators of gene expression. Interestingly, IncRNAs can serve as miRNA sponges to suppress their expression.\textsuperscript{34} Several previous studies have uncovered a role for miR-498-5p in the progression of various tumors, where it acts as a tumor suppressor in ovarian cancer cells and esophageal squamous cell carcinoma cells.\textsuperscript{26,35–38} Shen et al.\textsuperscript{38} proved that miR-498-5p is directly targeted by lncRNA AFAP1 antisense RNA 1 (AFAP1-AS1) in esophageal squamous cell carcinoma cells and showed that AFAP1-AS1 downregulates miR-498-5p expression by competitively binding to this miRNA. To further understand the mechanism of HOXB-AS3 in EC cells, we identified a miR-498-5p binding site in HOXB-AS3. We showed that miR-498-5p was downregulated in EC cells and tissues (Figure 3d, e), and its expression was negatively correlated with HOXB-AS3 levels (Figure 3f). This binding site was predicted by LncBase and validated using dual-luciferase reporter and RNA pull-down assays (Figure 3a, b). Moreover, the inhibitory effect of HOXB-AS3 knockdown on cell proliferation and viability can be restored via sponging miR-498-5p (Figure 4c–d).

Finally, we identified ADAM9 as a target gene of miR-498-5p. As transmembrane proteins, the ADAM family of proteins are key modulators for cell matrix interactions and have both cell adhesion and protease activities.\textsuperscript{39,40} Mazzocca et al.\textsuperscript{29} suggested that ADAM9 may play an essential role for modulating tumor-stromal interactions. Yin et al.\textsuperscript{41} discovered that ADAM9 expression is upregulated in gastric cancer tissue and cells, and lncRNA LINC00689 can promote ADAM9 expression by sponging miR-526b-3p. In our study, we found that overexpression of miR-498-5p inhibits protein expression levels of ADAM9 (Figure 5b). Additionally, silencing HOXB-AS3 also reduced the protein levels of ADAM9, and this inhibitory impact of HOXB-AS3 knockdown on ADAM9 expression can be restored by using a miR-498-5p inhibitor (Figure 5c).

**Conclusion**

Overall, this study shows that lncRNA HOXB-AS3 is overexpressed in EC tissues and cell lines. Interference with HOXB-AS3 expression can inhibit EC cell proliferation and promote apoptosis. Furthermore, silencing HOXB-AS3 could upregulate miR-498-5p expression but downregulate ADAM9 expression, indicating that HOXB-AS3 can act as a sponge of miR-498-5p to regulate ADAM9. Therefore, HOXB-AS3 could be used as a potential biomarker for EC diagnosis and prognosis.

**Declaration of conflicting interest**

The authors declare no relevant or material financial interests that relate to the research described in this paper.
Funding
This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

ORCID iD
Ruiting Lv https://orcid.org/0000-0001-5427-7556

References
1. Morice P, Leary A, Creutzberg C, et al. Endometrial cancer. *Lancet* 2016; 387: 1094–1108.
2. Burke WM, Orr J, Leitao M, et al. Endometrial cancer: a review and current management strategies: part II. *Gynecol Oncol* 2014; 134: 393–402.
3. Hung T and Chang HY. Long noncoding RNA in genome regulation: prospects and mechanisms. *RNA Biol* 2010; 7: 582–585.
4. Wang KC, Yang YW, Liu B, et al. A long noncoding RNA maintains active chromatin to coordinate homeotic gene expression. *Nature* 2011; 472: 120–124.
5. Jia H, Osak M, Bogu GK, et al. Genome-wide computational identification and manual annotation of human long non-coding RNA genes. *RNA* 2010; 16: 1478–1487.
6. Hajjar M, Khoshnevisan A and Shin YK. Molecular function and regulation of long non-coding RNAs: paradigms with potential roles in cancer. *Tumour Biol* 2014; 35: 10645–10663.
7. Zhai W, Li X, Wu S, et al. Microarray expression profile of IncRNAs and the upregulated ASLNC04080 IncRNA in human endometrial carcinoma. *Int J Oncol* 2015; 46: 2125–2137.
8. Doucrasy S, Coll J, Barrois M, et al. Expression of the human fetal bac h19 gene in invasive cancers. *Int J Oncol* 1993; 2: 753–758.
9. Akrami R, Jacobsen A, Hoell J, et al. Comprehensive analysis of long non-coding RNAs in ovarian cancer reveals global patterns and targeted DNA amplification. *PLoS One* 2013; 8: e80306.
10. Baldinu P, Cossu A, Manca A, et al. CASC2a gene is down-regulated in endometrial cancer. *Anticancer Res* 2007; 27: 235–243.
11. Ying J, Li H, Seng TJ, et al. Functional epigenetics identifies a protocadherin PCDH10 as a candidate tumor suppressor for nasopharyngeal, esophageal and multiple other carcinomas with frequent methylation. *Oncogene* 2006; 25: 1070–1080.
12. You QY, Tao H and Ling B. Long noncoding RNA HOX transcript antisense intergenic RNA (HOTAIR) as a foe and novel potential therapeutic target for endometrial carcinoma. *Int J Gynecol Cancer* 2014; 24: 1536.
13. Lanz RB, Chua SS, Barron N, et al. Steroid receptor RNA activator stimulates proliferation as well as apoptosis in vivo. *Mol Cell Biol* 2003; 23: 7163–7176.
14. Zhou X, Gao Q, Wang J, et al. Linc-RNA-RoR acts as a “sponge” against mediation of the differentiation of endometrial cancer stem cells by microRNA-145. *Gynecol Oncol* 2014; 133: 333–339.
15. Li BL and Wan XP. The role of IncRNAs in the development of endometrial carcinoma. *Oncl Lett* 2018; 16: 3424–3429. DOI: 10.3892/ol.2018.9065.
16. Zhang X, Chen H, Zhou B, et al. IncRNA HOXB-AS3 promotes hepatoma by inhibiting p53 expression. *Eur Rev Med Pharmacol Sci* 2018; 22: 6784–6792.
17. Papaioannou D, Petri A, Dovey OM, et al. The long non-coding RNA HOXB-AS3 regulates ribosomal RNA transcription in NPM1-mutated acute myeloid leukemia. *Nat Commun* 2019; 10: 5531.
18. Huang HH, Chen FY, Chou WC, et al. Long non-coding RNA HOXB-AS3 promotes myeloid cell proliferation and its higher expression is an adverse prognostic marker in patients with acute myeloid leukemia and myelodysplastic syndrome. *BMC Cancer* 2019; 19: 617.
19. Huang JZ, Chen M, Chen D, et al. A peptide encoded by a putative IncRNA HOXB-AS3 suppresses colon cancer growth. *Mol Cell* 2017; 68: 171–184.e176.
20. Zhuang XH, Liu Y and Li JL. Overexpression of long noncoding RNA HOXB-AS3 indicates an unfavorable prognosis and promotes tumorigenesis in
epithelial ovarian cancer via Wnt/β-catenin signaling pathway. *Biosci Rep* 2019; 39: BSR20190906.

21. Xu S, Jia G, Zhang H, et al. LncRNA HOXB-AS3 promotes growth, invasion and migration of epithelial ovarian cancer by altering glycolysis. *Life Sci* 2020; 264: 118636.

22. Croce CM. Causes and consequences of microRNA dysregulation in cancer. *Nat Rev Genet* 2009; 10: 704–714.

23. Cohn DE, Fabbri M, Valeri N, et al. Comprehensive miRNA profiling of surgically staged endometrial cancer. *Am J Obstet Gynecol* 2010; 202: 656.e651–656.e658.

24. Matamala N, Vargas MT, González-Cámpora R, et al. MicroRNA deregulation in triple negative breast cancer reveals a role of miR-498 in regulating BRCA1 expression. *Oncotarget* 2016; 7: 20068–20079.

25. Islam F, Gopalan V, Law S, et al. MiR-498 in esophageal squamous cell carcinoma: clinicopathological impacts and functional interactions. *Hum Pathol* 2017; 62: 141–151.

26. Liu R, Liu F, Li L, et al. MiR-498 regulated FOXO3 expression and inhibited the proliferation of human ovarian cancer cells. *Biomed Pharmacother* 2015; 72: 52–57.

27. Rong X, Gao W, Yang X, et al. Downregulation of hsa_circ_0007534 restricts the proliferation and invasion of cervical cancer through regulating miR-498/BMI-1 signaling. *Life Sci* 2019; 235: 116785. DOI: https://doi.org/10.1016/j.lfs.2019.116785.

28. Xue H, Yu F, Zhang X, et al. circ_0000638 inhibits neodymium oxide-induced bronchi-al epithelial cell inflammation through the miR-498–5p/NF-κB axis. *Ecotoxicol Environ Saf* 2020; 195: 110455.

29. Mazzocca A, Coppari R, De Franco R, et al. A secreted form of ADAM9 promotes carcinoma invasion through tumor-stromal interactions. *Cancer Res* 2005; 65: 4728–4738.

30. Peduto L, Reuter VE, Shaffer DR, et al. Critical function for ADAM9 in mouse prostate cancer. *Cancer Res* 2005; 65: 9312–9319.

31. Carl-McGrath S, Lendeckel U, Ebert M, et al. The disintegrin-metalloproteinases ADAM9, ADAM12, and ADAM15 are upregulated in gastric cancer. *Int J Oncol* 2005; 26: 17–24.

32. Wang J, Zhang X, Chen W, et al. Regulatory roles of long noncoding RNAs implicated in cancer hallmarks. *Int J Cancer* 2020; 146: 906–916.

33. Zhuang XH, Liu Y and Li JL. Overexpression of long noncoding RNA HOXB-AS3 indicates an unfavorable prognosis and promotes tumorigenesis in epithelial ovarian cancer via Wnt/β-catenin signaling pathway. *Biosci Rep* 2019; 39: BSR20190906. DOI: 10.1042/bsr20190906.

34. Hansen TB, Jensen TI, Clausen BH, et al. Natural RNA circles function as efficient microRNA sponges. *Nature* 2013; 495: 384–388.

35. Gopalan V, Smith RA and Lam AKY. Downregulation of microRNA-498 in colorectal cancers and its cellular effects. *Exp Cell Res* 2015; 330: 423–428.

36. Kasiappan R, Shen Z, Tse AKW, et al. 1,25-Dihydroxyvitamin D3 suppresses telomerase expression and human cancer growth through microRNA-498. *J Biol Chem* 2012; 287: 41297–41309.

37. Wang M, Zhang Q, Wang J, et al. MicroRNA-498 is downregulated in non-small cell lung cancer and correlates with tumor progression. *J Cancer Res Ther* 2015; 11: C107–C111.

38. Shen W, Yu L, Cong A, et al. Silencing lncRNA AFAP1-AS1 Inhibits the Progression of Esophageal Squamous Cell Carcinoma Cells via Regulating the miR-498/VEGFA Axis. *Cancer Manag Res* 2020; 12: 6397–6409.

39. Seals DF and Courtneidge SA. The ADAMs family of metalloproteases: multidomain proteins with multiple functions. *Genes Dev* 2003; 17: 7–30.

40. Black RA and White JM. ADAMs: focus on the protease domain. *Curr Opin Cell Biol* 1998; 10: 654–659.

41. Yin G, Tian P, BuHe A, et al. LncRNA LINC00689 Promotes the Progression of Gastric Cancer Through Upregulation of ADAM9 by Sponging miR-526b-3p. *Cancer Manag Res* 2020; 12: 4227–4239.