lncRNA LA16c-313D11.11 modulates the development of endometrial cancer by binding to and inhibiting microRNA-205-5p function and indirectly increasing PTEN activity

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Abstract. The aim of the present study was to determine the competitive endogenous RNA (ceRNA) network associated with long-coding RNA (lncRNA) LA16c-313D11.11 in endometrial cancer (EC). Initially, the expression levels of LA16c-313D11.11 in 60 EC tissues, 20 atypical hyperplasia endometrium (EAH) tissues and 20 normal endometrium tissues was determined. MicroRNA (miRNA/miR)-205-5p mimics and LA16c-313D11.11 mimics were transfected into HEC-1A and Ishikawa cells. The expression levels of miR-205-5p, LA16c-313D11.11 and their target proteins were assessed using reverse transcription-quantitative PCR or western blot analysis. Flow cytometry, Cell Counting kit-8 assays, Transwell migration assays and wound healing assays were performed to assess the effects of LA16c-313D11.11 and miR-205-5p on the migration and proliferation of tumor cells *in vitro*. The expression levels of LA16c-313D11.11 and phosphatase and tensin homolog deleted on chromosome ten (PTEN) in human EAH and EC tissues were significantly decreased, whereas the expression levels of miR-205-5p in EAH and EC tissues were significantly increased, compared with the normal endometrium tissues. The expression of LA16c-313D11.11 in human EC tissues negatively correlated with the expression of miR-205-5p. Additionally, the overexpression of LA16c-313D11.11 significantly reduced the invasion, migration and viability of HEC-1A and Ishikawa cells *in vitro*. LA16c-313D11.11 was shown to regulate the expression of PTEN, and the invasion, migration and viability of HEC-1A and Ishikawa cells, through its microRNA response element to compete for microRNA-205-5p. LA16c-313D11.11 was also shown to modulate the PI3K/AKT signaling pathway. Therefore, LA16c-313D11.11 acts as an effective ceRNA associated with a microRNA-205-5p-PTEN axis. LA16c-313D11.11 may inhibit the development and progression of EC by acting as a sponge of miR-205-5p, thus indirectly increasing the expression of PTEN.

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Abbreviations: EC, endometrial cancer; EAH, atypical hyperplasia endometrium; NE, normal endometrium; PTEN, phosphatase and tensin homolog deleted on chromosome ten; ceRNA, competing endogenous RNA

Key words: long non-coding RNA LA16c-313D11.11, PTEN, microRNA-205-5p, endometrial cancer, competing endogenous RNA

Introduction

Endometrial cancer (EC) is one of the most common types of malignant tumors worldwide, with an age standardized incidence rate of 8.4 cases per 100,000 individuals. Each year, an estimated 382,069 new cases of EC are diagnosed (1). Compared with low-resource countries, higher-income countries have a higher morbidity rate among patients with EC, although the former have higher mortality rates. The estimated cumulative risk of endometrial cancer is 1.6% in high-income areas and 0.7% in low-income countries up to the age of 75 years (2). High-grade ECs have a high rate of recurrence, despite the fact that prognosis is generally good for the initial

cancer. The prognosis of patients with recurrent EC however, is poor. During the treatment of cancer, it is important to balance treatment efficacy with the toxicity of the therapy used (3). There are 6 major molecular changes in type I endometrioid carcinoma: Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit α (PIK3CA) gene mutations (observed in 26-39% of cases); phosphatase and tensin homolog deleted on chromosome ten (PTEN) gene mutations (observed in 30-60% of cases); microsatellite instability (observed in 25-30% of cases); k-ras gene mutations (observed in 10-30% of cases); AT-rich interaction domain 1A (ARID1A; observed in 20% of cases); and catenin beta 1 (CTNNB1) and the accumulation of nuclear protein mutations (observed in 25-38% of cases). By contrast, the majority of type II non-endometrioid carcinomas have Her-2/neu amplification, p53 mutations and multiple chromosomal loss of heterozygosity. Non-endometrioid carcinomas may also originate from endometrioid carcinomas, which are unstable due to p53 mutations, and the resultant instability drives tumor progression (4). The fundamental molecular mechanisms involving tumor suppression or oncogenic factors remain poorly understood, even though key mutational events have been characterized in EC (5). Therefore, it is of utmost importance to identify novel therapeutic targets and to develop effective cure strategies for patients with EC. To achieve this, an improved understanding of the molecular mechanisms underlying the pathogenesis of EC is required.

Numerous long non-coding RNAs (lncRNAs) have been shown to be cancer-specific (6-8), and may thus be used as novel biomarkers for the diagnosis of cancer, or as therapeutic targets for the treatment of cancer. Certain lncRNAs regulate gene expression by acting as competing endogenous RNAs (ceRNAs). This notion has been supported by numerous studies (9-13). Furthermore, lncRNAs may be more effective in downregulation of gene expression when acting as ceRNAs, without the need to interfere with translation (14). However, the roles of lncRNA-associated ceRNAs in oncogenesis are not yet fully understood, and the role of lncRNA-microRNA (miRNA/miR) networks in EC requires further investigation. PTEN has been identified as a direct target of miR-205-5p in previous studies (15,16), and the expression of miR-205-5p is significantly increased in EC. Based on Kaplan-Meier survival analysis, the upregulation of miR-205-5p is associated with a poor overall survival (17).

A previous study by the authors demonstrated that miR-205-5p targets the lncRNA LA16c-313D11.11, with one conserved target site in EC. LA16c-313D11.11 may inhibit the expression and activity of miR-205-5p in normal and cancer tissues via this post-transcriptional binding (18). In the present study, LA16c-313D11.11 was shown to modulate a miR-205-5p-PTEN axis in EC. These results will improve the understanding of the molecular mechanisms underlying the development and progression of EC.

Materials and methods

Subjects. In the present study, 60 EC tissues, 20 atypical hyperplasia endometrium (EAH) tissues and 20 normal endometrial tissues were obtained from patients who received surgery at the Obstetrics and Gynecology Hospital of Fudan University between January, 2013 and February, 2016. Normal endometrial tissues were obtained from women who had undergone hysterectomy (such as uterine fibroids or prolapse). The median ages were 55.0 years (range, 26-76 years) in the EC group, 47 years (range, 37-53 years) in the EAH group and 49 years (range, 49-61 years) in the normal group. None of the patients recruited in the present study had received chemotherapy, radiotherapy or hormone therapy prior to surgery.

Cell culture and transfection. HEC-1A and Ishikawa cells were purchased from The Cell Bank of Type Culture Collection of the Chinese Academy of Sciences. HEC-1A cells were cultured in McCoy's 5A medium (HyClone; GE Healthcare) and Ishikawa cells were cultured in Eagle's minimum essential medium (HyClone; GE Healthcare), both supplemented with 10% FBS. Cells were maintained in a humidified incubator with 5% CO_2 at 37˚C. For transfection, all vectors and mimics were transfected into Ishikawa and HEC-1A using Lipofectamine® 3000 Transfection Reagent (Invitrogen; Thermo Fisher Scientific, Inc.). Cells were cultured in plates for 24 h prior to transfection. All vectors and mimics were transfected into HEC-1A and Ishikawa cells ($5x10^5$ cells/well) with Lipofectamine® 3000 Transfection Reagent, incubated at 37˚C for 24 h, and added to the plates. The sequences were as follows: miR-205-5p mimic (5'-UCCUUCAUUCCACCG GAGUCUG-3'; 5'-GACUCCGGUGGAAUGAAGGAUU-3') and mimic NC (5'-UUCUCCGAACGUGUCACGUTT-3'; 5'-ACGUGACACGUUCGGAGAATT-3'). The lncRNA overexpression plasmid was pLenti-EF1a-EGFP-F2A-Puro-CMV-LA16C-313D11.11 and the empty vector was used as a control. The working concentration of miRNA mimics and NC were 50 nM. The concentration used for plasmids was 100 nM. The time duration between transfection subsequent experimentation was approximately 24 h. In order to analyze the dose-response association between LA16C-313D11.11, miR-205-5p and PTEN, the concentration gradient (0-5 mg/ml) of LA16C-313D11.11 was set.

Luciferase reporter assay. The wild-type 3'-untranslated region (UTR) sequence of PTEN (PTEN-WT) were cloned into the luciferase reporter vector (Obio Technology). A mutant PTEN 3'-UTR vector (PTEN-MuT) was also constructed, which contained a mutation in the predicted PTEN-binding sequence. The PTEN-MuT or PTEN-WT, were co-transfected with either miR-205-5p mimics or negative Control (NC) mimics into HEC-1A cells using Lipofectamine® 3000. After 48 h of transfection, luciferase activity was determined using a Luciferase Reporter Gene kit (Promega Corporation) and normalized to the Renilla luciferase activity.

Reverse transcription-quantitative PCR (RT-qPCR). Total RNA extraction was performed using TRIzol reagent (Life Technologies; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocols. For the analysis of miRNA expression, miRNAs were extracted using an miRNeasy Mini kit (Qiagen, Inc.) and real-time quantification of miRNAs, primer extension and RNA-tailing were performed as described previously (19). RT-qPCR was performed using the SYBR Premix Ex Taq™ (Thermo Fisher Scientific, Inc.) with primers specific for LA16c-313D11.11, PTEN and miRNA-205-5p. The

primers were designed using a primer designing software package (LA16c-313D11.11 forward, 5'-TGAAGGAGGTTATTGACGCA-3' and reverse, 5'-GAGGGGAAACAGTCCAGAGT-3'; miR-205-5p forward, 5'-TCCACCGGAGTCTGTCTCAT-3' and reverse, 5'-GCTGTCAACGATACGCTACG-3'; PTEN forward, 5'-ACCAACTGAAGTGGCTAAAGAG-3' and reverse, 5'-GGTCCAGAGTCCAGCATAAAA-3'). GAPDH was used as the internal reference RNA for lncRNA and mRNA expression analysis, and small nuclear RNA U6 was used as the internal control for miRNA analysis. Gene expression levels were calculated based on the comparative quantitative method (the $2^{-\Delta\Delta Cq}$ method) (20). All qPCR reactions were performed using an Applied Biosystems 7500 Real-Time PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc.).

Western blot analysis. Total protein was extracted from the cells using RIPA lysis buffer (Nanjing KeyGen Biotech Co., Ltd.) supplemented with 1 nM phenylmethylsulphonyl fluoride. Total protein was collected, and the concentration was determined using an enhanced bicinchoninic acid Protein assay kit (Nanjing KeyGen Biotech Co., Ltd.). A total of 30 μg of protein was loaded per lane onto a 10% SDS-gel, resolved using SDS-PAGE and transferred to PVDF membranes (EMD Millipore). The membranes were blocked in 5% non-fat milk in Tris-buffered saline (room temperature, 2 h), and subsequently incubated overnight at 4˚C with one of the following antibodies: Caspase-3 (1:1,000; Abcam; cat. no. ab32351), PTEN (1:1,000; Abcam; cat. no. ab32199), phosphoinositide-dependent kinase-1 (PDK1; 1:1,000; Abcam; cat. no. ab110025), AKT (1:1,000; Cell Signaling Technology, Inc.; cat. no. #2920), phospho-(p-) Akt (1:1,000; Cell Signaling Technology, Inc.; cat. no. #4060) and β-actin (1:1,000; ProteinTech Group, Inc.; cat. no. 66009-1-Ig). The membranes were subsequently incubated with secondary antibodies (1:5,000; Immunoway; cat. nos. #RS0001 and #RS0002) for 2 h at room temperature. Signals were visualized using an enhanced chemiluminescence kit and densitometric analysis was performed using ImageJ v1.8.0 (National Institutes of Health).

Cell proliferation analysis. To analyze cell proliferation, a Cell Counting Kit-8 assay was used (CCK-8; Dojindo Molecular Technologies, Inc.). Cells were plated in 96-well plates (2,000 cells/well) following transfection. Following 24, 48 and 72 h of incubation, the cells were incubated with CCK-8 solution at 37˚C for 2 h. The viability of the cells was determined by measuring the absorbance of the plates at 450 nm using a spectrophotometer (Bio-Tek Instruments).

Apoptosis assay. HEC-1A and Ishikawa cells ($1x10^6$) were plated in 6-well plates and collected 24 h following transfection. An Annexin V-FITC Apoptosis Detection kit (BD Biosciences) and flow cytometry (FACS Calibur; FlowJo10.0BD; Biosciences) was used to assess apoptosis, and the kit was used according to the manufacturer's protocol.

Transwell migration assay. Transwell inserts (BD Biosciences) were used to perform the invasion assays. Transfected Ishikawa and HEC-1A cells ($1x10^6$ cells/well) were added to the upper chamber of the insert in serum-free medium (200 μl). In the lower chamber, medium supplemented with 20% FBS (600 μl) was added to act as a chemoattractant. The filter membrane of the invasion assays was coated with diluted Matrigel (BD Biosciences). Following 24 h of incubation, the cells which had invaded through the membranes were fixed in methanol (room temperature, 30 min) and stained (room temperature, 5 min) with 0.05% crystal violet. The cells which had invaded were quantified using a microscope (Olympus Corporation).

Wound-healing assay. HEC-1A and Ishikawa cells ($1x10^6$ cells/well) were plated in a 24-well plate; when an 80% confluent cell monolayer had formed, the monolayer was scratched using a 100 μl pipette tip, and the cells were subsequently cultured in serum-free medium. Images of the wound were acquired at 0 and 24 h after scratching to assess cell migration using a light microscope (scale bar, 100 μm).

Statistical analysis. Data are presented as the means ± standard deviation of at least 3 experimental repeats. SPSS version 19.0 (IBM Corp.) and GraphPad Prism version 7.0 (GraphPad Software, Inc.) were used for statistical analysis. The results of RT-qPCR and western blot analysis were compared using ANOVA followed by the post hoc Student-Newman-Keuls or Tukey's tests. A two-tailed Students' t-test was used to compare differences between 2 groups. Qualitative data are expressed as rate and were analyzed using a χ^2 test. Spearman's rank correlation analysis was used for correlation analysis, and R=0 was used as the relevant standard. $P<0.05$ was considered to indicate a statistically significant difference.

Results

LA16c-313D11.11, miRNA-205-5p and PTEN expression in the endometrium. RT-qPCR was used to assess the expression of LA16c-313D11.11, PTEN and miRNA-205-5p in 60 EC, 20 EAH and 20 normal endometrium tissues. The relative expression levels of LA16c-313D11.11 and PTEN in the EC tissues were significantly decreased compared with those in the EAH and normal endometrial tissues (LA16c-313D11.11: EC vs. EAH, $P<0.0001$; EC vs. normal, $P<0.0001$; PTEN: EC vs. EAH, $P<0.05$; EC vs. normal, $P<0.0001$; Fig. 1A and B). However, the relative expression level of miRNA-205-5p in the EC tissues was significantly higher compared with that in the EAH and normal endometrial tissues (EC vs. EAH, $P<0.05$; EC vs. normal, $P<0.0001$; Fig. 1C).

Expression and clinical significance of LA16c-313D11.11 in human EC tissues. As the relative expression level of LA16c-313D11.11 in the human EC tissues was significantly lower compared with that in the normal endometrial tissues, the clinicopathological significance of LA16c-313D11.11 was assessed. The mean expression level of LA16c-313D11.11 was used to stratify patients in to the high and low expression groups. The low expression of LA16c-313D11.11 was associated with lymph node metastasis and an advanced FIGO stage ($P<0.05$; Table I). These results suggest that the decreased expression of LA16c-313D11.11 is associated with an invasive phenotype and an increased metastatic potential.

Table I. Association of LA16c-313D11.11 expression with the clinicopathological characteristics of patients with EC.

Variables	Cases (n)	LA16c-313D11.11 expression		P-value
		Low, n (%)	High, n (%)	
Age (years)				0.639
<50	16	8 (13.3)	8 (13.3)	
≥50	44	19 (31.7)	25 (41.7)	
Histological subtype				0.493
Endometrioid	45	21 (35)	24 (40)	
Serous	10	5 (8.3)	5 (8.3)	
Clear cell	5	1 (1.7)	4 (6.7)	
Menstruation				0.582
Premenopausal	20	10 (16.7)	10 (16.7)	
Menopausal	40	17 (28.3)	23 (38.3)	
FIGO stage				0.047[a]
I-II	47	18 (30)	29 (48.3)	
III-IV	13	9 (15)	4 (6.7)	
Histological grade				0.962
G1	30	14 (23.3)	16 (26.7)	
G2	8	4 (6.7)	4 (6.7)	
G3	7	3 (5)	4 (6.7)	
Myometrial invasion				0.955
<1/2	42	19 (31.7)	23 (38.3)	
≥1/2	18	8 (13.3)	10 (16.7)	
Lymph node metastasis				0.047[a]
Present	13	9 (15)	4 (6.7)	
Absent	47	18 (30)	29 (48.3)	

[*]P<0.05, indicates a significant difference. EC, endometrial cancer.

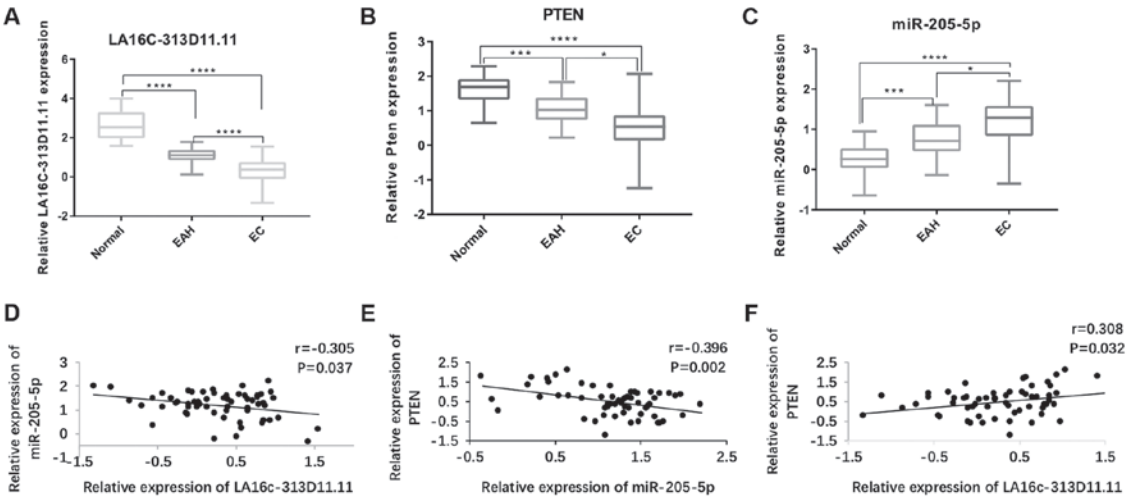

Figure 1. LA16c-313D11.11, PTEN and microRNA-205-5p are aberrantly expressed in EC, EAH and NE. (A-C) Expression levels of LA16c-313D11.11, PTEN and microRNA-205-5p in EC, EAH and NE tissues examined by RT-qPCR. (D-F) Correlation between LA16c-313D11.11, microRNA-205-5p and PTEN in EC tissues. [*]P<0.05, [***]P<0.001, [****]P<0.0001. PTEN, phosphatase and tensin homolog deleted on chromosome ten; EC, endometrial cancer; EAH, atypical hyperplasia endometrium; NE, normal endometrium.

Correlation between LA16c-313D11.11, miRNA-205-5p and PTEN expression in the endometrium. LA16c-313D11.11 expression negatively correlated with miRNA-205-5p expression in the EC tissues, suggesting a possible interaction between

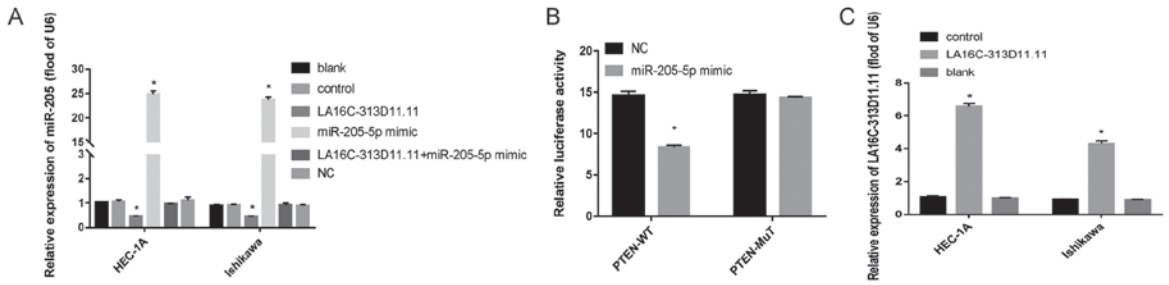

Figure 2. (A) Relative expression levels of microRNA-205-5p in HEC-1A and Ishikawa cells, transfected with control, LA16c-313D11.11, microRNA-205-5p mimic, and LA16c-313D11.11 + microRNA-205-5p mimic. *P<0.05 vs. control. (B) Relative luciferase activity in HEC-1A cells transfected with microRNA-205-5p mimic and co-transfected with PTEN-WT or PTEN-MuT. *P<0.05 vs. control. (C) Relative expression levels of LA16c-313D11.11 in HEC-1A and Ishikawa cells, transfected with control, LA16c-313D11.11 and NC were measured by RT-qPCR and normalized to U6. *P<0.05. NC, negative control; PTEN, phosphatase and tensin homolog deleted on chromosome ten.

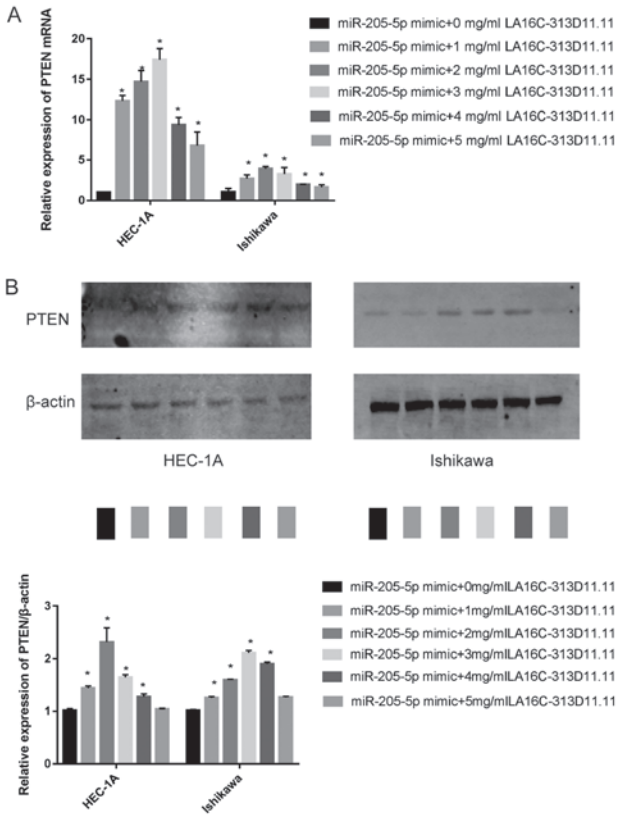

Figure 3. Regulation of PTEN by overexpression of LA16c-313D11.11 and microRNA-205-5p in HEC-1A and Ishikawa cells. (A) mRNA expression of PTEN examined by RT-qPCR; (B) Protein expression of PTEN examined by western blot analysis. *P<0.05 vs. control. PTEN, phosphatase and tensin homolog deleted on chromosome ten.

LA16c-313D11.11 and microRNA-205-5p in EC (Fig. 1D). Furthermore, PTEN expression positively corelated with LA16c-313D11.11 expression, but negatively correlated with miRNA-205-5p expression in the EC tissues (Fig. 1E and F).

Interaction between LA16c-313D11.11, miRNA-205-5p and PTEN in EC cells. LA16c-313D11.11, miRNA-205-5p and LA16c-313D11.11 + miRNA-205-5p were overexpressed in the HEC-1A and Ishikawa cells. LA16c-313D11.11 was successfully overexpressed in the HEC-1A and Ishikawa cells (Fig. 2C). The relative expression level of miRNA-205-5p was significantly decreased in the LA16c-313D11.11-overexpressing cells compared with the control (P<0.05; Fig. 2A). However, there was no statistically significant difference compared with the control when LA16c-313D11.11 and miRNA-205-5p were co-overexpressed in the HEC-1A and Ishikawa cells (Fig. 2A). Thus, it was demonstrated that miRNA-205-5p was competitively inhibited by LA16c-313D11.11 in the HEC-1A and Ishikawa cells. PTEN was found to be a direct target of miRNA-205-5p by luciferase assay (Fig. 2B). Additionally, the overexpression of LA16c-313D11.11 promoted the expression of PTEN in a concentration-dependent manner when the LA16c-313D11.11 concentration was up to the 2 mg/ml (in HEC-1A cells) or 3 mg/ml (in Ishikawa cells) (Fig. 3). At concentrations higher than these mentioned, the expression of PTEN gradually decreased. Thus, an LA16c-313D11.11-miRNA-205-5p-PTEN axis was successfully identified in the endometrial cancer cells.

LA16c-313D11.11 modulates the expression of PTEN, the endogenous target of miRNA-205-5p, and thus indirectly regulates the PI3K/Akt signaling pathway in EC cells. To investigate the function of LA16c-313D11.11 in endometrial cancer, the expression levels of PTEN and the PI3K/Akt signaling pathway were analyzed in the HEC-1A and Ishikawa cells. The expression levels of PTEN were upregulated, while those of p-AKT/AKT, PDK1 and caspase-3 levels were downregulated when LA16c-313D11.11 was overexpressed. The overexpression of miRNA-205-5p resulted in the opposite effects. However, there was no significant difference when both LA16c-313D11.11 and miR-205-5p were both overexpressed (Fig. 4). Taken together, these results demonstrate that LA16c-313D11.11 may interfere with the miRNA-205-5p mediated inhibition of PTEN in EC.

LA16c-313D11.11 decreases the viability, migration and invasion of EC cells through the inhibition of miRNA-205-5p. The regulatory effects of the LA16c-313D11.11-miRNA-205-5p-PTEN axis on the viability, migration and invasion of endometrial cancer cells were further determined. CCK-8 assay was performed to detected the proliferation of transfected Ishikawa and HEC-1A cells at the indicated periods of time. The results revealed that LA16c-313D11.11 overexpression significantly inhibited the proliferation of EC cells compared with the control, whereas miR-205-5p overexpression significantly increased the proliferation of EC cells. There was no

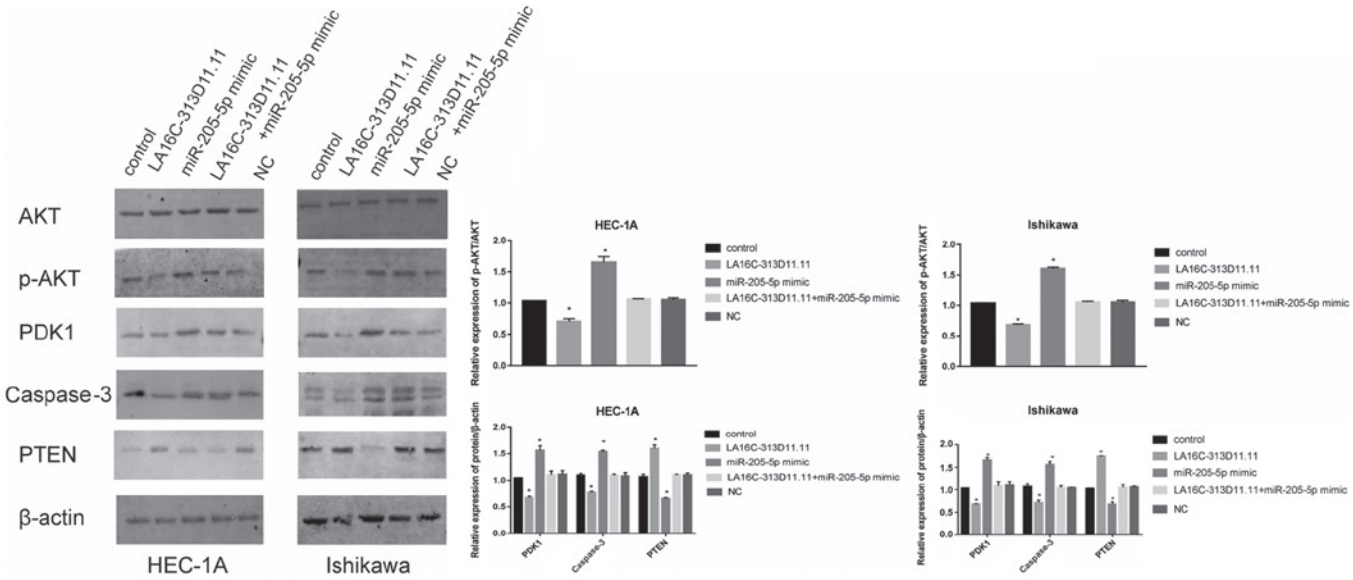

Figure 4. Effect of LA16c-313D11.11 on PTEN and PI3K/Akt signaling pathway in HEC-1A and Ishikawa cells. The protein levels of AKT, p-AKT, PDK1, caspase-3 and PTEN were evaluated by western blot analysis. *P<0.05 vs. control. NC, negative control; PTEN, phosphatase and tensin homolog deleted on chromosome ten.

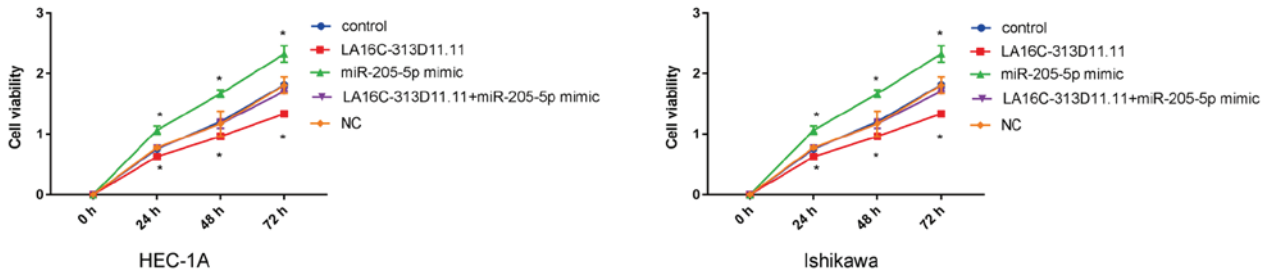

Figure 5. Role of LA16c-313D11.11 and microRNA-205-5p in cell proliferation. CCK-8 cell viability assays were performed. *P<0.05 vs. control. NC, negative control.

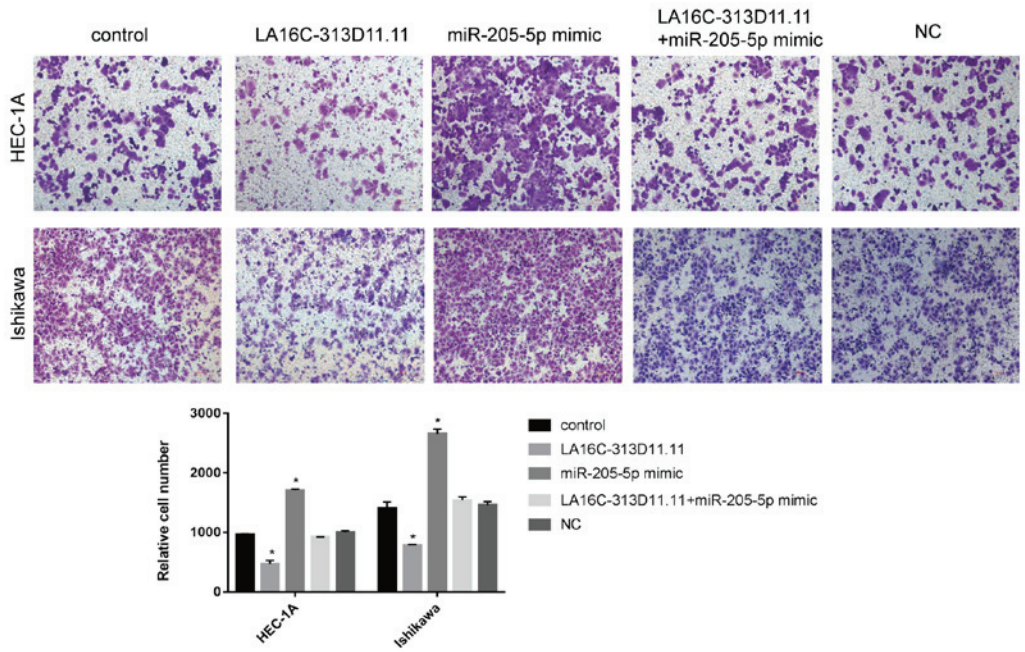

Figure 6. Functions of LA16c-313D11.11 and microRNA-205-5p in cell invasion. HEC-1A and Ishikawa cells were transfected with control, LA16c-313D11.11, microRNA-205-5p mimic, LA16c-313D11.11 + microRNA-205-5p mimic and NC groups. After 24 h, Transwell invasion assays were conducted to calculate the number of invasive cells. Magnification, x100. *P<0.05 vs. control. NC, negative control.

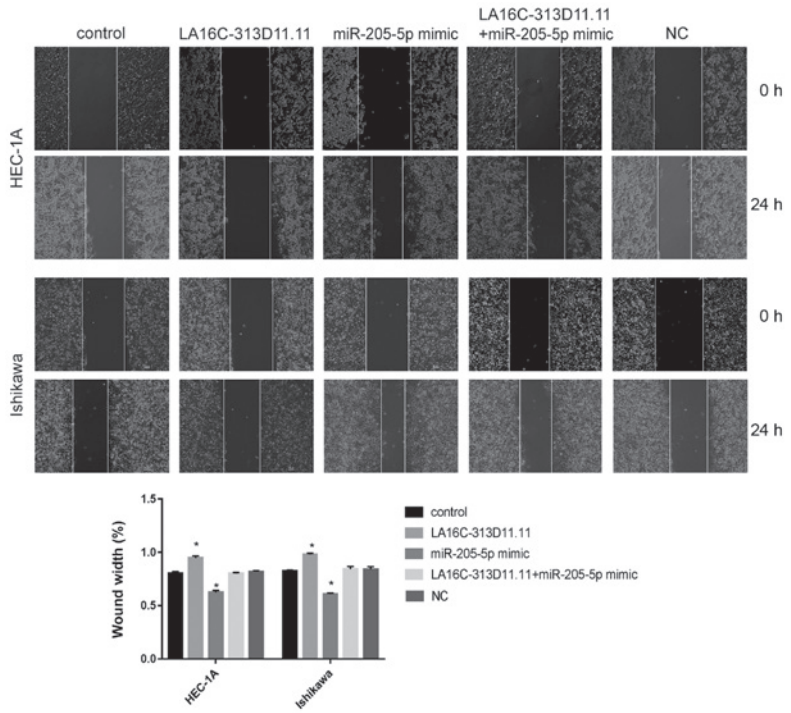

Figure 7. Functions of LA16c-313D11.11 and microRNA-205-5p in cell migration. Wound-healing assays were performed to detect the cell migratory ability of the cells in each group. Magnification, x100. *P<0.05 vs. control. NC, negative control.

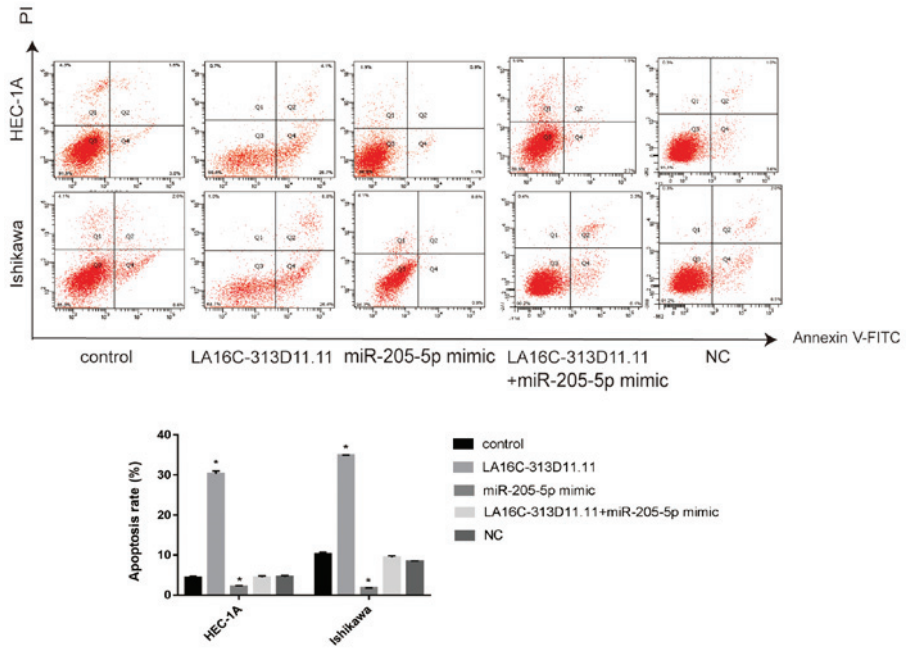

Figure 8. Functions of LA16c-313D11.11 and microRNA-205-5p in cell apoptosis. Flow cytometric assays were conducted to establish apoptosis after the cells were transfected with control, LA16c-313D11.11, microRNA-205-5p mimic, LA16c-313D11.11 + microRNA-205-5p mimic and NC groups. *P<0.05 vs. control. NC, negative control.

statistically significant difference in viability compared with the control when LA16c-313D11.11 and miRNA-205-5p were co-overexpressed in the HEC-1A and Ishikawa cell lines (Fig. 5). Transwell invasion assays were conducted to calculate the number of invasive cells. Wound-healing assays were used to evaluate the mobility of EC cells. The overexpression of LA16c-313D11.11 significantly decreased the invasion and migration of the HEC-1A and Ishikawa cells compared with the control. However, the overexpression of miRNA-205-5p significantly increased the invasion and migration of the HEC-1A and Ishikawa cells compared with the control. There was no statistically significant difference in cells overexpressing both LA16c-313D11.11 and miR-205-5p compared with the control (Figs. 6 and 7). These results suggest that LA16c-313D11.11 inhibits the effects of miRNA-205-5p on the viability, invasion and migration of EC cells.

LA16c-313D11.11 induces the apoptosis of EC cells by inhibiting the binding of miRNA-205-5p to PTEN. As LA16c-313D11.11 decreased the viability, invasion and migration of endometrial cancer cells, the effects of LA16c-313D11.11 overexpression on the apoptosis of endometrial cancer cell lines were also determined. The results revealed that LA16c-313D11.11 overexpression significantly induced the apoptosis of EC cells compared with the control, whereas miR-205-5p overexpression significantly inhibited the apoptosis of EC cells. There was no statistically significant difference in apoptosis compared with the control when LA16c-313D11.11 and miRNA-205-5p were co-overexpressed in HEC-1A and Ishikawa cell lines (Fig. 8). These results suggest that the dysregulation of LA16c-313D11.11 may contribute to the development of endometrial cancer and exert a tumor-suppressive effect.

Discussion

The non-coding regions of the genome are considered more complex compared with the coding regions in higher eukaryotes (21-23). miRNAs have been demonstrated to be involved in numerous vital biological processes, including development, hematopoiesis, organ formation, apoptosis, cell proliferation and even tumorigenesis (24-26). Similarly, lncRNAs are receiving increasing interest and are now considered a novel layer of regulation, adding to the complexity of mammalian gene regulatory networks (27,28). Recently, the ceRNA hypothesis has suggested that the regions of non-coding RNAs may act as molecular sponges of microRNAs, thereby inhibiting the target miRNAs' function, and thus increasing the activity of the target of the miRNA, indirectly (14,29-31). In the present study, it was demonstrated that lncRNA LA16c-313D11.11 served as an endogenous sponge of miRNA-205-5p, and abrogated its endogenous inhibition on PTEN, thus affecting proliferation and migration in endometrial cancer.

A previous study by the authors demonstrated that LA16c-313D11.11 was associated with the nosogenesis of EC and was shown to be a non-coding RNA. LA16c-313D11.11 is an effective ceRNA which is associated with a miRNA-205-5p-PTEN network (18). To further elucidate the molecular mechanisms through which LA16c-313D11.11 may be involved in endometrial cancer, in the present study, 60 primary endometrial cancer tissues, 20 EAH tissues and 20 normal endometrium tissues were obtained for analysis. Additionally, LA16c-313D11.11 mimic and miRNA-205-5p mimic were transfected into HEC-1A and Ishikawa cells.

The expression of miRNA-205-5p was found to be upregulated in human EC tissues, and was negatively associated with the PTEN levels in human EC tissues, suggesting a possible association between miRNA-205-5p and PTEN in the pathogenesis of EC. It was also demonstrated that the expression levels of miRNA-205-5p were negatively associated with the overall survival time of patients with EC (17). Therefore, it was concluded that miRNA-205-5p may serve as an effective marker for the diagnosis of EC, and may be used to predict the clinical prognosis of patients with EC. The expression of miRNA-205-5p in EC tissues was significantly higher compared with EAH and normal endometrial tissues. Furthermore, miRNA-205-5p expression levels were inversely associated with the viability, migration and invasion of EC cells *in vitro*, highlighting its potential role in the progression of EC.

LA16c-313D11.11 and miRNA-205-5p were shown to interact in EC cells. The relative expression level of LA16c-313D11.11 in EC tissues was significantly decreased compared with EAH and normal endometrial tissues. The clinicopathological significance of LA16c-313D11.11 indicated that the downregulation of LA16c-313D11.11 expression was significantly associated with lymph node metastasis and an advanced FIGO stage. The results demonstrated that low levels of LA16c-313D11.11 expression were associated with phenotypically invasive tumors, and in particular, with an increased metastasis. LA16c-313D11.11 expression negatively correlated with miRNA-205-5p expression in human EC tissues, and LA16c-313D11.11 regulated the viability, invasion and migration of EC cells by competing with miRNA-205-5p, which endogenously inhibits PTEN function, and PTEN physiologically inhibits the PI3K/Akt signaling pathway. Therefore, LA16c-313D11.11 indirectly inhibited the PI3K/Akt signaling pathway.

By acting as ceRNAs, lncRNAs exert their effects by acting as endogenous inhibitors of miRNAs, thus affecting the binding of miRNAs to their targets (14). The results of the present study suggest that LA16c-313D11.11 acts as a ceRNA by sequestering miRNA-205-5p, thus inhibiting the progression of EC. The identification of this novel mechanism improves our understanding of the underlying pathophysiology of EC, and may assist in the development of novel therapeutics by highlighting potential therapeutic targets. However, the present study has several limitations. To address these limitations, in future studies, the sample size will be increased to verify the association between LA16c-313D11.11 and the prognosis of patients with EC. Additionally, whether LA16c-313D11.11 regulates any other potential mechanisms will be examined, and the role of the miRNA-205-5p-PTEN axis *in vivo* will be investigated. Furthermore, the mechanisms of the three markers will be examined further using additional *in vitro* experiments.

In conclusion, LA16c-313D11.11 acts a ceRNA in a miRNA-205-5p-PTEN axis. To the best of our knowledge, the present study is the first to demonstrated that LA16c-313D11.11 regulates the development of EC by inhibiting the binding of miRNA-205-5p to its target, highlighting a novel lncRNA-miRNA network in EC. The present study enhances the current knowledge of the molecular mechanisms underlying the development and progression of EC and may assist in the development of novel therapeutics for the treatment of patients with EC, or may improve the diagnosis of patients with EC.

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Availability of data and materials

All data generated or analyzed during the present study are included in this published article or are available from the corresponding author on reasonable request.

Authors' contributions

WX, JD, QW and KH designed the study. WX and SZ performed the experiments. XH, PZ, HY, XG and PL were involved in the interpretation of the data. WX, SZ, QW and KH analyzed the data and wrote the manuscript. WX, SZ, XH and HY revised the manuscript. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The study' ethical approval was granted by the Obstetrics and Gynecology Hospital of Fudan University, Shanghai, China. Written informed consent was provided by the subjects for the collection of samples and follow-up analysis. The study was done based on the guidelines and principles stipulated in the declaration of Helsinki.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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