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LncRNA UCA1 regulates proliferation, migration and invasion of cervical cancer cells by targeting miR-145

H. WEI[1], Y.-Q. QIU[2], Q.-S. ZENG[2], S.-F. WANG[2], C.-J. YI[2]

[1]Department of Obstetrics and Gynecology, The First Clinical Medicine College of Yangtze University, Jingzhou, Hubei, China
[2]Department of Obstetrics and Gynecology, The First Affiliated Hospital of Yangtze University, Jingzhou, Hubei, China

Hua Wei and *Yuqi Qiu* contributed equally to this work

Abstract. – OBJECTIVE: Finding a key target for the development of cervical cancer is conducive to the treatment of cervical cancer. LncRNA UCA1 plays a role in multiple tumors, such as the digestive tract and bladder. However, whether LncRNA UCA1 plays a role in cervical cancer is unclear.

PATIENTS AND METHODS: The tumor tissues (tumor group) and adjacent tissues (normal group) of cervical cancer patients were collected for analysis of expression of LncRNA UCA1 and miR-145 by Real Time-Polymerase Chain Reaction (RT-PCR). Cervical cancer HeLa cell line was cultured and divided into NC group, si-UCA1 group, and si-UCA1+ miR-145 inhibitor group. It was followed by the analysis of LucRNA UCA1 expression by Real Time-PCR, cell proliferation by MTT assay, cell migration and invasion by respective cell scratch, transwell chamber assay, and targeted relationship between UCA1 and miR-145 by Dual-Luciferase activity.

RESULTS: LncRNA UCA1 expression was significantly increased in cervical cancer and miR-145 expression was decreased compared with the normal group ($p<0.05$). There was a negative correlation between them ($p<0.05$). The downregulation of UCA1 significantly inhibited tumor cell proliferation, migration, and invasion compared with NC group ($p<0.05$). MiR-145 is the target miRNA of UCA1. The addition of miR-145 inhibitor reversed the effect of UCA1 siRNA on downregulating UCA1 expression, and the difference was statistically significant compared with the si-UCA1 group ($p<0.05$).

CONCLUSIONS: LncRNA UCA1 expression is significantly increased in cervical cancer and miR-145 expression is decreased with a negative correlation between them. Lnc-RNA UCA1 regulates cervical cancer cell proliferation, migration, and invasion by targeting miR-145.

Key Words:
Cervical cancer, LncRNA UCA1, MiR-145, Proliferation, Migration, Invasion.

Introduction

Cervical cancer is the second most common malignant tumor affecting women worldwide. However, because the early symptoms of cervical cancer are not evident, patients often have advanced cervical cancer at the time of treatment and some patients have postoperative recurrence or metastasis, so the prognosis of cervical cancer is poor[1,2]. The incidence of cervical cancer is only after breast cancer, lung cancer, and colorectal cancer. As one of the most common gynecologic tumors in women, it poses a serious threat to women's health[3,4]. Cervical cancer has a high morbidity and mortality in developing and underdeveloped areas, with approximately 570,000 new diagnoses and 310,000 deaths each year. Nearly 85% of cases occur in less developed and developing countries and become one of the malignant tumors that threaten the world, especially in developing countries[5,6]. Because the screening of cervical cancer is not popular, the prevention methods are backward, and the economic development is not balanced, the incidence of cervical cancer in developing countries is always at the forefront, and the mortality rate is higher than that of developed countries[7,8]. Epidemiological and molecular biology studies[9,10] have shown an association between cervical cancer and persistent infection with high-risk human papillomaviruses (especially HPV16 and HPV18). Despite improve-

Corresponding Author: Hua Wei, MD; e-mail: youyoucheng15@163.com

ments in cervical cancer diagnostic methods and treatment strategies, morbidity and mortality are still high, and the prognosis is poor. Therefore, it is urgent to clarify the molecular mechanism of cervical cancer, to find a key target for the treatment of cervical cancer, to promote the development of cervical cancer treatment strategies, and to facilitate the diagnosis and treatment of cervical cancer and prognosis evaluation.

Long non-coding RNAs (LncRNAs) are a group of non-coding RNAs longer than 200 nucleotides involved in various physiological and biological processes, such as cell development, survival, differentiation, and apoptosis[11]. Moreover, lncRNAs regulate the expression of genes[12,13]. In the genome, lncRNA is the most transcribed non-protein coding sequence and is thought to play a major role in the regulation of protein-coding genes[14]. However, Cheng et al[15] have shown that lncRNA also plays an important role in the occurrence and development of tumors, such as cervical cancer. So far, LncRNA UCA1 has been considered a carcinogenic lncRNA, involved in the migration, invasion, and tumor development. It was found that pvt1 is the most amplified gene in ovarian cancer patients and is closely related to low survival rate. LncRNA UCA1 has been shown to play a role in multiple tumors, such as the digestive tract and bladder[16-18], but the expression and mechanism of LncRNA UCA1 in cervical cancer has not been elucidated.

Patients and Methods

Study Object Selection

Clinical data of 109 patients who underwent pathological histological examination of cervical cancer admitted to our hospital for gynecological treatment from June 2018 to December 2019 were selected. All patients were treated with Primary Surgical Treatment (PST) with an age of 47-71 years old, average age (55.5 ± 12.5) years old. Inclusion criteria: screening out patients with direct operative squamous cell carcinoma of the International Federation of Gynecology and Obstetrics (FIGO) stage I-II; preoperative biopsy and postoperative pathological diagnosis were clear; all patients were first diagnosed as cervical cancer, the first surgery, the patient underwent hysterectomy or extensive hysterectomy + pelvic lymphadenectomy + abdominal para-aortic lymphadenectomy in our hospital; no other chemotherapy or radiotherapy before surgery; all investigated consent forms were signed for all selected subjects. Exclusion criteria: recurrent cervical cancer; previous surgical treatment; previous radiotherapy or chemotherapy; combination with other diseases, such as infectious diseases, malignant tumors, severe diabetes mellitus, and other organ-borne diseases, systemic immune diseases and malignancy tumor complications, etc.

The tumor tissues and adjacent tissues were collected during operation, and frozen in liquid nitrogen. Patients and their families participated voluntarily in the study and signed informed consent. The study was approved by the Medical Ethics Committee of the hospital.

Main Instruments and Reagents

The RNA extraction kit and the reverse transcription kit were purchased from RD Corporation (Minneapolis, MN, USA). Other commonly used reagents were purchased from Shanghai Shenggong Biological Co., Ltd (Shanghai, China). Real Time-PCR reagents were purchased from Thermo Fisher Scientific (Waltham, MA, USA). Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), and cyan chain double antibody were purchased from HyClone (San Angelo, TX, USA). Dimethyl sulfoxide, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) powder was purchased from Gibco (Grand Island, NY, USA); trypsin-ethylenediaminetetraacetic acid (EDTA) digest was purchased from Sigma-Aldrich (St. Louis, MO, USA). The transwell chamber was purchased from Corning (Corning, NY, USA). LncRNA UCA1 siRNA and miR-145 inhibitor were designed and synthesized by Shanghai Gemma Gene Company (Shanghai, China). The Real Time-PCR instrument was purchased from ABI (Waltham, MA, USA). The PCR System 2400 DNA Amplifier was purchased from PE Gene (Foster City, CA, USA). The SpectraMax iD5 microplate reader was purchased from MD (Burlington, MA, USA). The AccuriTM C6 flow cytometer was purchased from BD Corporation (San Jose, CA, USA).

HeLa Cell Culture and Grouping

The HeLa cell line was preserved by liquid nitrogen, sub-cultured after resuscitation, and cells of 3-8 generation logarithmic growth phase were selected for testing. The cultured HeLa cells were randomly divided into 3 groups, including NC group (normal cell culture), si-UCA1 (si-

UCA1 transfected into cultured HeLa cells), and si-UCA1+ miR-145 inhibitor group (HeLa cells were transfected with UCA1 siRNA and miR-145 inhibitor).

Transfection of UCA1 siRNA and MiR-145 Inhibitor in HeLa Cells

UCA1 siRNA and miR-145 inhibitor were transfected into HeLa cells, respectively. The UCA1 siRNA sequence was 5'-GCGUGUGAGGUGUAUGGCAUCA-3'; 5'-UCUGGAUUGGAACUAGGU-3'. The Si-NC sequence was 5'-GGUGCGAGGUUUGAAUCA-3'; 5'-UCUUGAGGCUAUGU-3'. The miR-145 inhibitor sequence was 5'-GGUUGCGAUAGGAAUCA-3'; 5'-UGAGAGGUGCCUUUAUGU-3'. The cell density was fused to 70-80% in a 6-well plate; the UCA1 siRNA and miR-145 inhibitor liposomes were separately or simultaneously added to 200 µl of serum-free medium, mixed well, and incubated at room temperature for 15 min. The mixed Lipofectamine 2000 was separately mixed with the corresponding dilution and incubated for 30 min at room temperature. The serum of the cells was removed, phosphate-buffered saline (PBS) was gently rinsed, 1.6 ml of serum-free medium was added, and each system was added to each system, and cultured in a 5% CO_2 incubator at 37°C for 6 hours. The serum culture solution was replaced and cultured for 48 hours for experimental research.

Real Time-PCR Detection of UCA1 and MiR-145 Expression in HeLa Cells

RNA was isolated from cervical cancer and adjacent tissues and each group of HeLa cells on ice by TRIzol reagent, and reverse transcription synthesis was performed according to the kit instructions. The primers were designed by Primer Premier 6.0 according to each gene sequence and synthesized by Shanghai Yingjun Biotechnology Co., Ltd. (Shanghai, China) (Table I). Real-Time-PCR reaction conditions: 55°C 1 min, 92°C 30 s, 58-60°C 45 s, 72°C 35 s, a total of 35 cycles. Data were collected using the PCR reactor software and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as a reference. According to the fluorescence quantification, the starting cycle number (CT) of all samples and standards was calculated. Based on the standard CT value, a standard curve was drawn, and then, the semi-quantitative analysis was carried out by the $2^{-\Delta Ct}$ method.

MTT Assay Analysis of the Proliferation of Cells in Each Group

The HeLa cells in good growth state were transferred to the second generation and inoculated into a 96-well culture plate, and the HeLa cells in the logarithmic growth phase were collected and seeded in a 96-well culture plate with a cell culture solution containing 10% fetal bovine serum DMEM at a dose of 5×10^3. The treatment methods and grouping were the same as mentioned above. After culturing for 24 hours, the supernatant was discarded, 20 µl of sterile MTT was added to the well to be tested, and 3 replicate wells were set at each time point. After 4 hours of continuous culture, the supernatant was completely removed, and DMSO 150 µl/well was added and shaken 10 min. After the purple crystals were fully dissolved, the absorbance (A) value was measured at a wavelength of 492 nm by a microplate reader, and the proliferation rate of each group was calculated.

Cell Scratch Test

When cells overgrew, 10 µL micro-dosing tips was used to draw a line along the central axis of the well. The crossed cells were washed away with PBS, and then, the medium was added. The 24-well plates were taken at 0 h and 48 h, respectively, and microscopic photographs were taken to observe the migration distance of the cells, and the cell migration rates at different time points were calculated, and a histogram was drawn. 48-hour migration rate = (scratch width of 0 h - scratch width of 48 h)/scratch width of 0 h.

Transwell Invasion Experiment

The cells from different treatment group were digested, washed twice with PBS, and

Table I. Primer sequences.

Gene	Forward 5'-3'	Reverse 5'-3'
GAPDH	AGTAGTCACCTGTTGCTGG	TAATACGGAGACCTGTCTGGT
UCA1	ACTGGGACTG CTTCCAA	CTGCTGAGCAA CCTGGGA
miR-145	ACCTG CTGTGTTGAGC	CGGGTG CTTCTACTGGGAG

centrifuged to remove the supernatant. The cells were resuspended in 100 ul of serum-free medium, inoculated into Matrigel invasion chamber, and 600 µl of medium containing 10% fetal bovine serum was added to the lower chamber; the cells were cultured for 28 hours in a cell culture incubator. We discarded the old culture solution, washed the upper and lower parts of the chamber twice with PBS, wiped the cells on the chamber with cotton swab, fixed the ice pre-cooled 4% paraformaldehyde for 30 min, stained with Giemsa dye solution (Sigma-Aldrich, St. Louis, MA, USA) for 15 min followed by observation of the invaded cells under a microscope.

Luciferase Reporting Experiment Verifying the Relationship Between MiR-145 and UCA1

The cells were grouped as follows: pmirGLO, pmirGLO/UCA1-UTR, pmirGLO/UCA1-UTR+miRNA-NC, pmirGLO/UCA1-UTR+miRNA-145mimic, pmirGLO/UCA1-mUTR, pmirGLO/UCA1-mUTR+miRNA-NC, pmirGLO/UCA1-mUTR+miRNA-145 mimic. The cells were seeded in 24-well plates one day before transfection with addition of 0.5 ml of antibiotic-free medium per well, and the cells reached 60-80% per well on the second day of transfection. Lipofectamine 3000 was used for transfection with the above plasmid and mixed with cells and incubated at 37°C for 48 h. The Dual-Luciferase reporter gene detection of lysed cells was done as follows: we mixed the reporter cell lysate thoroughly, and after draining the cell culture solution, directly added 100 µl per well of the reporter cell lysate; after fully lysing, they centrifuged at 12,000 rpm for 5 min followed by measurement. The Renilla Luciferase Assay Substrate (100x) was placed in an ice bath or icebox for later use. For each sample measurement, we took 50 µl of the sample, added 100 µl of firefly Luciferase detection reagent, and mixed well to determine the RLU (relative light unit). The reporter cell lysate was used as a blank control. After completing the above-mentioned measurement of the firefly Luciferase step, 100 ul of Renilla Luciferase test solution was added, homogenized, and the RLU (relative light unit) was determined by a ModulusTM microplate type multifunctional photometer (Promega, Madison, WI, USA). In the case of Renilla Luciferase as an internal reference, the RLU value obtained by firefly Luciferase measurement was divided by the RLU value determined by Renilla Luciferase. The degree of activation of the reporter gene of interest between different samples was compared based on the ratio.

Statistical Analysis

Data were analyzed by Statistical Product and Service Solution 16.0 statistical software (SPSS Inc., Chicago, IL, USA). Measurement data were expressed as mean ± standard deviation (SD), and comparison of multiple groups of samples was performed using one-way ANOVA with Bonferroni post-hoc analysis. The correlation between LncRNA UCA1 and miR-145 was analyzed using Person correlation analysis. $p < 0.05$ was considered statistically significant.

Results

LncRNA UCA1 Expression in Cervical Cancer

The expression of lnc RNA UCA1 in cervical cancer was detected by Real Time-PCR. The results showed that compared with the normal group, lncRNA UCA1 expression was significantly increased in cervical cancer ($p < 0.05$) (Figure 1).

Expression of MiR-145 in Cervical Cancer

The expression of miR-145 in cervical cancer was detected by Real Time-PCR. The results showed that miR-145 was significantly decreased in cervical cancer compared with the normal group ($p < 0.05$) (Figure 2).

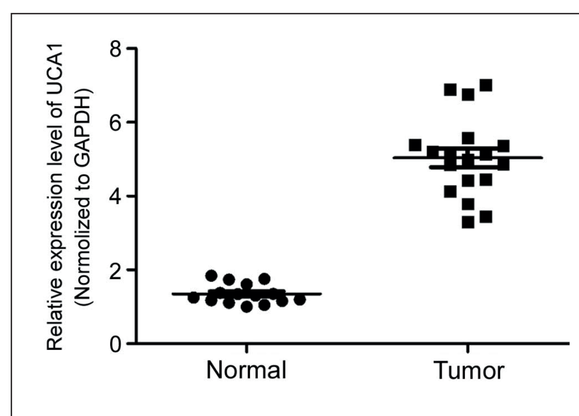

Figure 1. Expression of LncRNA UCA1 in cervical cancer. Compared with the normal group, *$p < 0.05$.

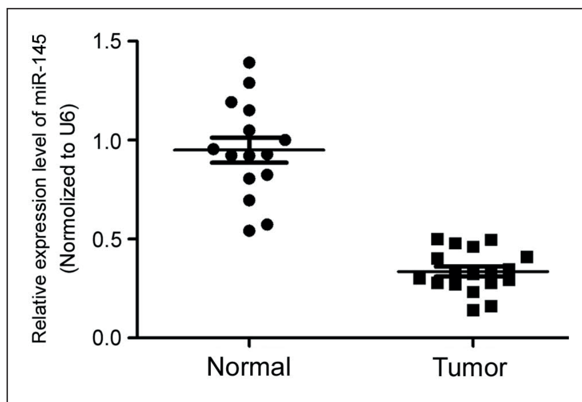

Figure 2. Expression of miR-145 in cervical cancer. Compared with the normal group, $*p < 0.05$.

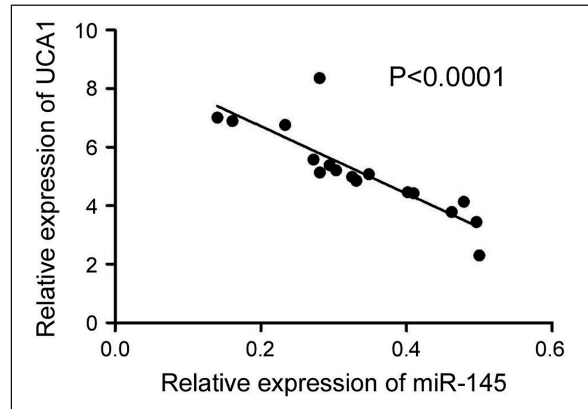

Figure 3. Correlation analysis between LncRNA UCA1 and miR-145 in cervical cancer.

Correlation Analysis Between LncRNA UCA1 and MiR-145 in Cervical Cancer

Further analysis of the correlation between LncRNA UCA1 and miR-145 in cervical cancer showed that the expression of LncRNA UCA1 and miR-145 was negatively correlated in cervical cancer ($p < 0.05$) (Figure 3).

Effect of LncRNA UCA1 on Proliferation of Cervical Cancer Cells

LncRNA UCA1 siRNA was transfected into cervical cancer HeLa cells *in vitro* for 24 h, 48 h, and 72 h. The results showed that transfection of LncRNA UCA1 siRNA in cervical cancer HeLa cells could downregulate its expression and inhibit cell proliferation. Compared with NC group, the difference was statistically significant ($p < 0.05$) (Figure 4).

Effects of LncRNA UCA1 on Migration of Cervical Cancer Cells

LncRNA UCA1 siRNA was transfected into cervical cancer HeLa cells *in vitro*. It was found that transfection of LncRNA UCA1 siRNA in cervical cancer HeLa cells could downregulate its expression and inhibit cell migration. Compared with NC group, the difference was statistically significant ($p < 0.05$) (Figure 5).

Effect of LncRNA UCA1 on Invasion of Cervical Cancer Cells

LncRNA UCA1 siRNA was transfected into cervical cancer HeLa cells *in vitro*. The results showed that transfection of LncRNA UCA1 siRNA in cervical cancer HeLa cells could downregulate its expression and inhibit cell invasion. Compared with NC group, the difference was statistically significant ($p < 0.05$) (Figure 6).

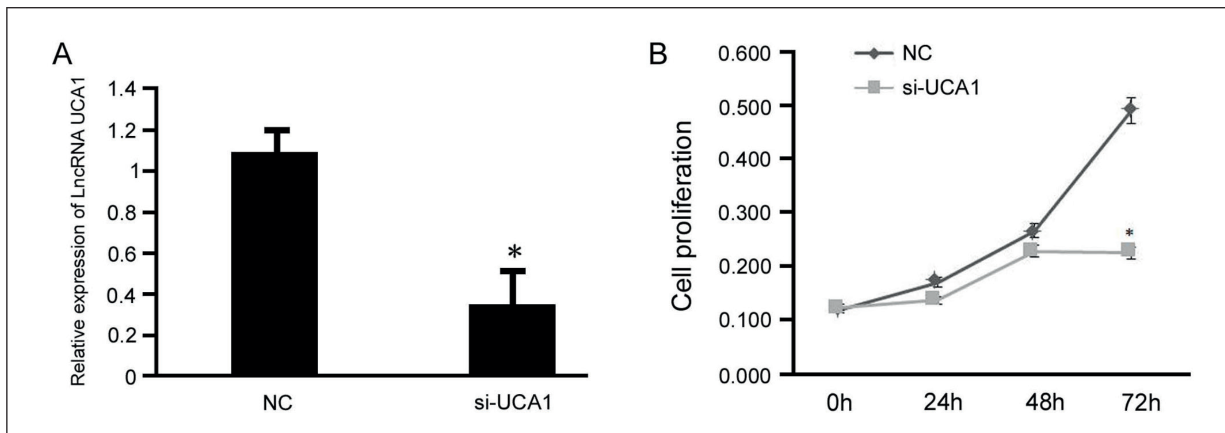

Figure 4. Effect of LncRNA UCA1 on proliferation of cervical cancer cells. **A,** Real time PCR analysis of LncRNA UCA1 expression in cervical cancer cells. **B,** MTT assay analysis of LncRNA UCA1 on cervical cancer cell proliferation, compared with NC group, $*p < 0.05$.

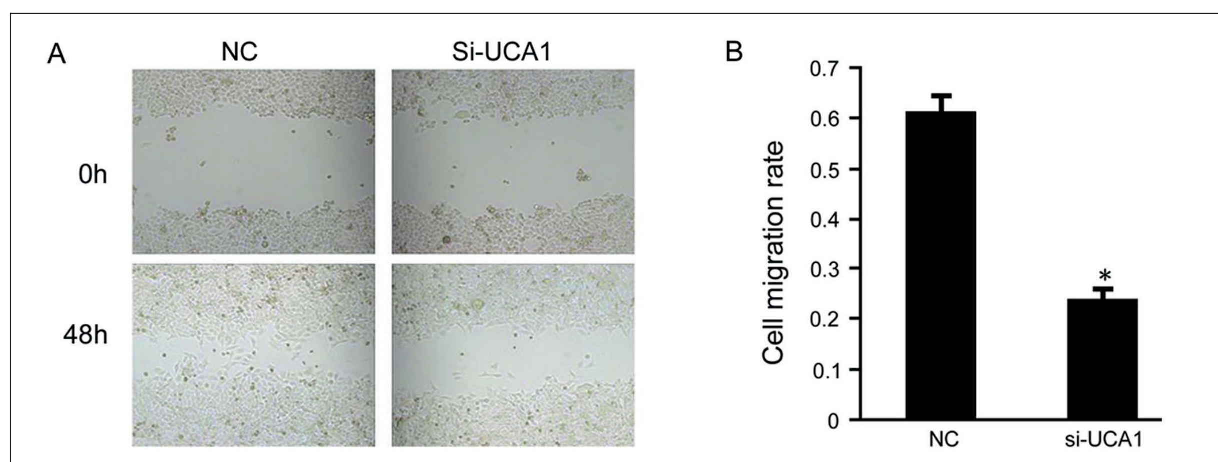

Figure 5. Effect of LncRNA UCA1 on migration of cervical cancer cells. **A,** cell scratch assay analyzes the effect of LncRNA UCA1 on cervical cancer cell migration (× 40). **B,** Statistical analysis of the effect of BLncRNA UCA1 on cervical cancer cell migration, compared with NC group, $*p < 0.05$.

Targeted miRNA Analysis of LncRNA UCA1 in Cervical Cancer

Dual-Luciferase reporter assay was performed to analyze the targeting miRNA of LncRNA UCA1 in cervical cancer, by establishing UCA1 wild type (WT) and variant (Mut), respectively and showed that miR-145 was the LncRNA UCA1 target gene as shown by decreased UCA1 Luciferase activity after transfection of miR-145 mimics (Figure 7).

Down-Regulation of MiR-145 on the Proliferation of LncRNA UCA1 in Cervical Cancer Cells

LncRNA UCA1 siRNA and miR-145 inhibitor were transfected into cervical cancer cells, respectively. The results showed that UCA1 siRNA downregulated the expression of UCA1 in cervical cancer cells, which inhibited cell proliferation. Compared with the NC group, the difference was statistically significant ($p < 0.05$). Simultaneous transfection of LncRNA UCA1 siRNA and miR-145 inhibitor reversed the effect of UCA1 siRNA, leading to the proliferation of cervical cancer cells. Compared with the si-UCA1 group, the difference was statistically significant ($p < 0.05$) (Figure 8).

Downregulation of MiR-145 on the Migration of LncRNA UCA1 in Cervical Cancer Cells

LncRNA UCA1 siRNA and miR-145 inhibitor were transfected into cervical cancer cells, respectively. The results showed that UCA1 siRNA downregulated the expression of UCA1 in cervical cancer cells, which inhibited cell migration.

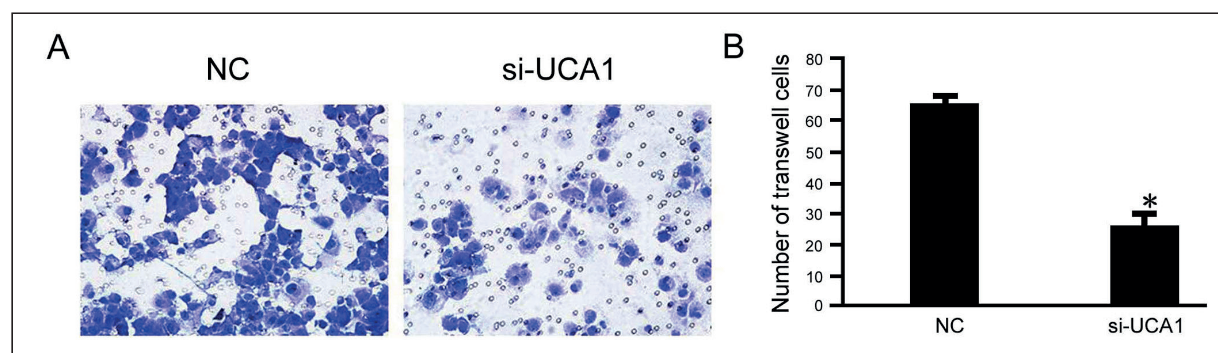

Figure 6. Effect of LncRNA UCA1 on invasion of cervical cancer cells. **A,** Transwell chamber assay analyzes the effect of LncRNA UCA1 on cervical cancer cell invasion (× 100). **B,** Statistical analysis of the effect of LncRNA UCA1 on invasion of cervical cancer cells, compared with NC group, $*p < 0.05$.

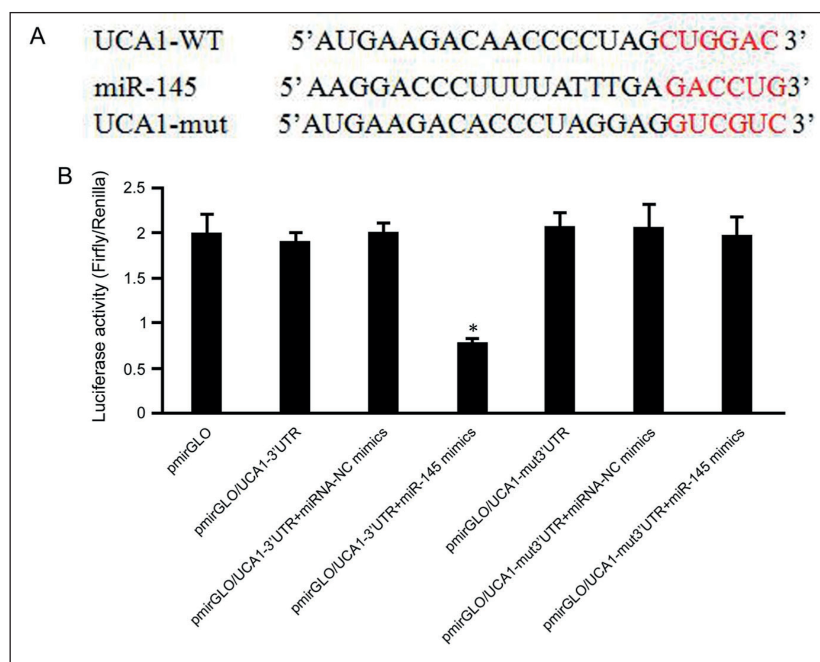

Figure 7. Targeted miRNA analysis of LncRNA UCA1 in cervical cancer. **A,** Targeted miRNA predictive analysis of LncRNA UCA1 in cervical cancer. **B,** statistical analysis of luciferase report compared with pmirGLO group, $*p < 0.05$.

Compared with the NC group, the difference was statistically significant ($p < 0.05$). Simultaneous transfection of LncRNA UCA1 siRNA and miR-145 inhibitor reversed the effect of UCA1 siRNA, leading to the promotion of cervical cancer cell migration. Compared with the si-UCA1 group, the difference was statistically significant ($p < 0.05$) (Figure 9).

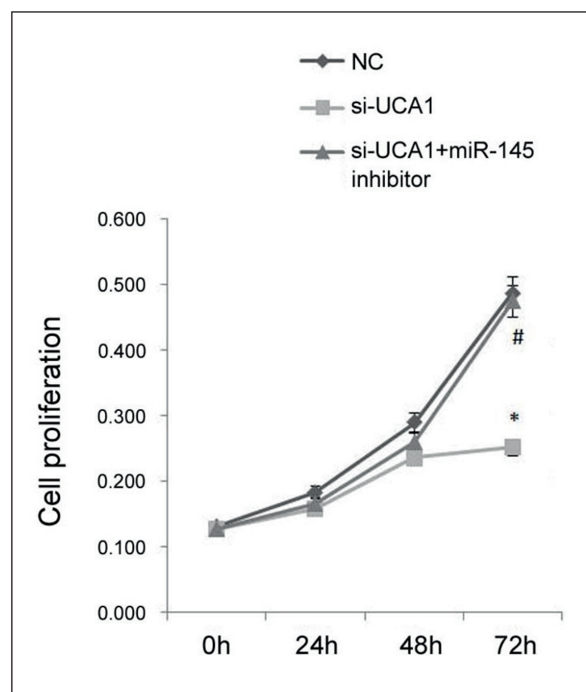

Figure 8. Downregulation of miR-145 on the proliferation of cervical cancer cells by LncRNA UCA1. Compared with the NC group, $*p < 0.05$; compared with the si-UCA1 group, $^{\#}p < 0.05$.

Downregulation of MiR-145 on the Invasion of LncRNA UCA1 in Cervical Cancer Cells

LncRNA UCA1 siRNA and miR-145 inhibitor were transfected into cervical cancer cells, respectively. The results showed that UCA1 siRNA downregulated the expression of UCA1 in cervical cancer cells, which inhibited cell invasion. Compared with the NC group, the difference was statistically significant ($p < 0.05$). Simultaneous transfection of LncRNA UCA1 siRNA and miR-145 inhibitor reversed the effect of UCA1 siRNA, leading to the promotion of cervical cancer cell invasion. Compared with the si-UCA1 group, the difference was statistically significant ($p < 0.05$) (Figure 10).

Discussion

Zhang et al[19] suggest that abnormal expression of LncRNA plays an important role in a variety of tumorigenesis, invasion, and metastasis, including cervical cancer. LncRNA UCA1 is one of the newly discovered LncRNAs and has an oncogenic effect in many cancers. However, the role of LncRNA UCA1 in the progression of cervical cancer and the underlying mechanism

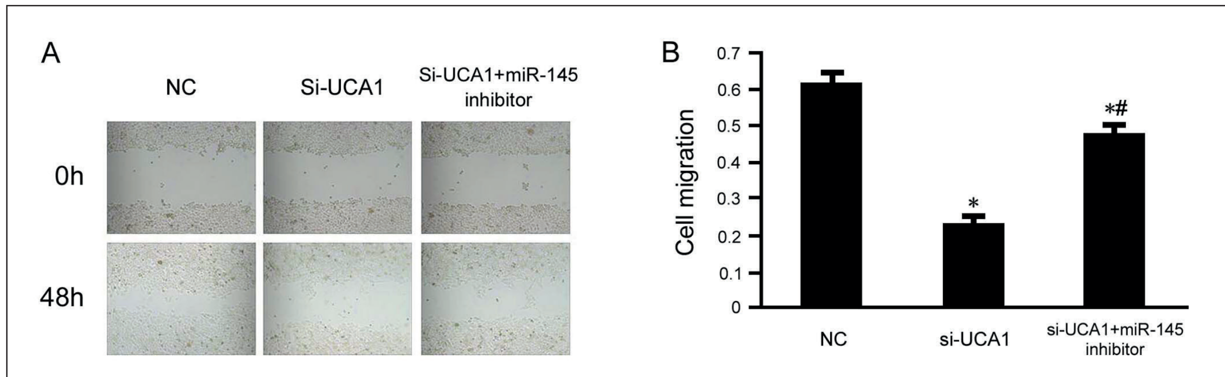

Figure 9. Downregulation of miR-145 on the migration of LncRNA UCA1 in cervical cancer cells. **A,** Cell scratch assay analyzes the effect of miR-145 on the migration of LncRNA UCA1 in cervical cancer cells (× 40). **B,** Downregulation of the effect of miR-145 on the migration of LncRNA UCA1 in cervical cancer cells, with NC group Comparison, *$p < 0.05$, compared with the si-UCA1 group, $^{\#}p < 0.05$.

has not yet been elucidated. LncRNA UCA1 is upregulated in many cancers as a carcinogen and can promote the proliferation and invasion of gastrointestinal tumors, including colon cancer and gastric cancer by regulating miRNA[16-18]. Our results also showed that the expression of LncRNA UCA1 was significantly upregulated in the cervical cancer group and the knockdown of LncRNA UCA1 gene significantly inhibited the proliferation, migration, and invasion of cervical

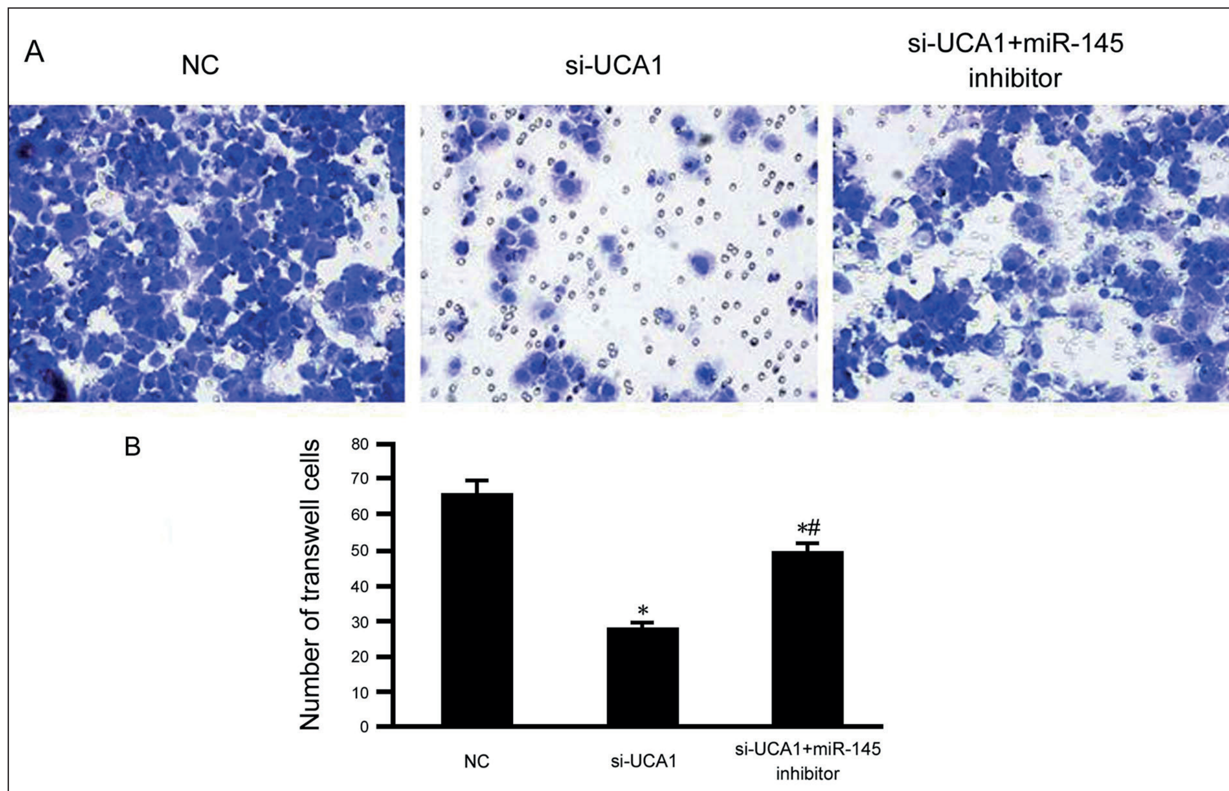

Figure 10. Downregulation of miR-145 on the invasion of LncRNA UCA1 in cervical cancer cells. **A,** Transwell chamber assay downregulates the effect of miR-145 on the invasion of LncRNA UCA1 in cervical cancer cells (× 100). **B,** Downregulation of the effect of miR-145 on the invasion of LncRNA UCA1 in cervical cancer cells, compared with NC group, *$p < 0.05$, compared with the si-UCA1 group, $^{\#}p < 0.05$.

cancer cells. These findings suggest that LncRNA UCA1 may play an oncogenic role in the development of cervical cancer, which is consistent with previous reports.

MicroRNAs (MiRNAs) are a group of non-coding RNAs between 17-25 nucleotides in length that affect the pathogenesis of many human diseases, including cancer. MiRNAs are usually dysregulated in human cancers and can be used as oncogenes or tumor suppressor genes[20]. The downregulation of miR-145 expression in cancer tissues is inversely correlated with the prognosis of cancer, suggesting the anti-cancer effect of miR-145 in tumor formation[21-23]. The occurrence and metastasis of miR-145 in cervical cancer has yet to be determined. In this study, bioinformatics analysis also revealed that there is a targeted binding relationship between LncRNA UCA1 and miR-145. Therefore, we hypothesized that upregulation of LncRNA UCA1 can promote cervical cancer cells in cervical cancer by targeting miR-145. Our results indicate that the expression of miR-145 is significantly downregulated in cervical cancer tissues and HeLa cells. LncRNA UCA1 and miR-145 were negatively correlated in cervical cancer, and upregulation of miR-145 significantly inhibited the Luciferase activity of LncRNA UCA1 but did not affect the Luciferase activity of LncRNA UCA1 MUT, indicating a targeted relationship between LncRNA UCA1 and miR-145. LncRNA UCA1 promotes metastasis and proliferation of cervical cancer by inhibiting miR-145. This study demonstrates that the downregulation of miR-145 reverses the effect of LncRNA UCA1 downregulation on cervical cancer cell proliferation, invasion, and migration, suggesting the role of interaction of LncRNA UCA1 and miR-145 in the progression of cervical cancer cells.

Conclusions

The expression of LncRNA UCA1 is significantly increased in cervical cancer and the expression of miR-145 is decreased with a negative correlation between them. Lnc-RNA UCA1 regulates cervical cancer cell proliferation, migration, and invasion by targeting miR-145.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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