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LncRNA UCA1 regulates cervical cancer survival and EMT occurrence by targeting miR-155

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Abstract. – OBJECTIVE: Cervical cancer rate is increasing recently. LncRNA UCA1 plays a role in gynecological tumors, but its expression and mechanism in cervical cancer have not yet been elucidated.

PATIENTS AND METHODS: The tumor tissues and adjacent tissues of cervical cancer patients were collected to measure LncRNA UCA1 and miR-155 level by Real-time PCR. The Luciferase report analyzed the relationship between LncRNA UCA1 and miR-155. HeLa cells were separated into NC group, UCA1 siRNA group, UCA1 siRNA + miR-155 inhibitor group followed by analysis of cell proliferation, invasion and migration and EMT-related genes E-cadherin and Vimentin expression by Real time PCR.

RESULTS: UCA1 level was elevated and miR-155 was reduced in cervical cancer tissues with significant differences compared to adjacent tissues (p <0.05). UCA1 was negatively correlated with miR-155 level (p <0.05). Patients with high UCA1 level showed short survival time (p <0.05). Down-regulation of UCA1 can significantly inhibit cell proliferation, migration and invasion. It can also increase E-cadherin expression and decrease Vimentin expression (p <0.05). MiR-155 is a target miRNA of UCA1. MiR-155 inhibitor can significantly reverse UCA1 siRNA's effect (p <0.05).

CONCLUSIONS: UCA1 expression in cervical cancer is increased and related to patient survival and miR-155 expression is reduced. LncRNA UCA1 regulates EMT occurrence in cervical cancer cells by targeting miR-155.

Key Words:
LncRNA UCA1, MiR-155, EMT, Cervical cancer, Survival, Proliferation.

Introduction

The incidence of cervical cancer has always been high, especially in developing countries and economically underdeveloped areas, and it has become particularly prominent[1,2]. The incidence of cervical cancer is only behind breast cancer, lung cancer, and colorectal cancer. Due to lack of universal screening for cervical cancer, the morbidity and mortality remain high in China[3,4]. Due to inappropriate sexual behavior, HPV virus infection, etc., can cause cervical cancer. At present, the age of patients with cervical cancer becomes younger[5,6]. As the symptoms of palace cancer patients are often late, the lack of early treatment interventions leads to poor prognosis, poor quality of life, and brings a heavy mental and economic burden to patients and their families[7,8].

In recent years, long non-coding RNAs (lncRNAs) also known as lncRNA is a type of non-coding RNAs[9] and non-coding transcripts accounted for the vast majority of the human genome[10,11]. Since lncRNA itself does not participate in encoding proteins, it is initially considered to have no effect and is defined as transcription "noise". However, as the research progresses, it was found that lncRNA was involved in gene expression regulation[12,13]. In the genome, it is thought that lncRNA mainly exerts the regulation of protein coding genes[14]. But the latest research shows that lncRNA also involves in tumor pathogenesis, especially in gynecological tumors[15,16]. LncRNA UCA1 participates in many physiological and pathological fields. In oncology research, lncRNA UCA1 as a tumor cell growth and proliferation regulator can promote neovascularization, tumor invasion and metastasis and play a role in gynecological tumors[17,18]. Therefore, this study intends to assess lncRNA UCA1's level in cervical cancer and analyze the mechanism in cervical cancer.

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Patients and Methods

Selection of Research Objects

The clinical data of 20 patients who were diagnosed with cervical cancer based on at least one of the following criteria: typical histological characteristics or colposcopy biopsies were selected and admitted to our hospital for gynecological treatment by pathological examination from May 2011 to December 2011. All patients were surgically treated, aged 45-76 years, with a mean age of 52.2 ± 7.5 years old. Inclusion criteria: all patients had cervical cancer for the first time, received surgical treatment for the first time, and did not receive chemotherapy, radiotherapy or other treatments before surgery. All selected subjects signed an informed consent. Exclusion criteria: recurrent cervical cancer; previous surgical treatment; previous radiation or chemotherapy; combined with other diseases such as infectious diseases, malignant tumors, severe diabetes and other organ failure diseases, systemic immune diseases, and malignant diseases Tumor complications. Tumor tissue and adjacent tissues were collected during operation and frozen in liquid nitrogen. This study was approved by the Medical Ethics Committee of the First Affiliated Hospital of Jinzhou Medical University and all patients signed the informed consent.

Main Instruments and Reagents

RNA extraction kit and reverse transcription kit were purchased from RD Company (Minneapolis, MN, USA). Other commonly used reagents were purchased from Shanghai Shengong 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) culture fluid, fetal bovine serum (FBS), and green chain monoclonal antibody were purchased from Hyclone (San Angelo, TX, USA). Dimethyl sulfoxide (DMSO) and MTT powder were from Gibco Company (Grand Island, NY, USA); trypsin-EDTA digestion liquid was purchased from Sigma Company (St. Louis, MO, USA). The transwell cell was purchased from Corning (Corning, NY, USA). LncRNA UCA1 siRNA and miR-155 inhibitor were from Shanghai Gima Gene Corporation (Shanghai, China). The real time PCR instrument was purchased from ABI (Waltham, MA, USA). The PCR System 2400 DNA amplification instrument was purchased from PE Gene (Foster City, CA, USA). The SpectraMax iD5 microplate reader was purchased from MD Company (San Jose, CA, USA).

HeLa Cell Culture and Grouping

HeLa cell lines were stored in liquid nitrogen, subcultured after resuscitation, and 3-8 passage logarithmic growth phase cells were selected for testing. The cultured HeLa cells were randomly divided into 3 groups, the NC group (cell culture was performed normally); si-UCA1 group which was transfected with si-UCA1; si-UCA1 + miR-155 inhibitor group, which was transfected with UCA1 siRNA and miR-155 inhibitor.

Transfection of UCA1 siRNA and MiR-155 Inhibitor into HeLa Cells by Liposome Method

UCA1 siRNA and miR-155 inhibitor were transfected into HeLa cells. The UCA1 siRNA sequence is 5'-GCGUGGUGUAUUGAGGGCAUCA-3 '; 5'-UCUGACUGAUUGGAAGGU-3'. The Si-NC sequence was 5'-GGUGCGUUGAAGGUAUCA-3'; 5'-UCUGCUGAGUAUGU-3'. The miR-145 inhibitor sequence was 5'-GGCGAUAGUUGGAAUCA-3'; 5'-UGAGAGGUGCCUUUAUGU-3'. In a 6-well plate, the cell density was fused to 70-80%; UCA1 siRNA and miR-155 inhibitor liposomes were added to 200 μl serum-free medium separately or simultaneously and thoroughly mixed. Mix the mixed lipo2000 with the corresponding dilutions and then added to cultured cells. After 6 h, the serum culture medium was replaced, and cells were cultured for 48 h for experimental research.

Real-time PCR Detection of UCA1 and MiR-155 and E-cadherin and Vimentin Expression in HeLa Cells

RNA from cervical cancer tissues and adjacent tissues and HeLa tumor cells of each group were extracted on ice followed by DNA reverse transcription synthesis according to the kit instructions. The primers were designed by PrimerPremier6.0 based on each gene sequence and synthesized by Shanghai Yingjun Biotechnology Co., Ltd. (Table I). Real-time PCR reaction conditions: 55 °C 1 min, 92 °C 30 S, 58-60 °C 45 S, and 72 °C 35 S for 35 cycles. Data was collected using the PCR reactor software and GAPDH was used as a reference. According to the fluorescence quantification, the starting cycle number (CT) of all samples and standards

Table I. Primer sequences.

Gene	Forward 5′-3′	Reverse 5′-3′
GAPDH	AGTAGTCACCTGTTGCTGG	TAATACGGAGACCTGTCTGGT
UCA1	ACTGGGACTG CTTCCAA	CTGCTGAGCAA CCTGGGA
E-cadherin	GACAGTCTTGATGCG	GAG AGCAA CTGGAGGGCA
Vimentin	GACTGTGCTG TGTC	TGTGTTGGAGGCAAC
miR-155	ACCTGTGTGCTTGAGC	CGGTTCTGTGCACTGGGAG

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-ΔCt method.

MTT Method to Detect the Effect of Cell Proliferation in Each Group

HeLa cells were randomly divided into 3 groups as mentioned above. Each group was treated for 48 hours followed by addition of 20 μl of sterile MTT and 3 replicate wells were set in each treatment group. After 4 hours of continuous culture, the supernatant was completely removed, 150 μl/well of DMSO was added, and the shaker was shaken 10 min until the purple crystals were fully dissolved, followed by measuring the absorbance (A) value to calculate the proliferation rate.

Scratch Experiment

After the cells became full, a 10 μL micropipette tip was used to streak along the center axis of the well, and cells were washed off with PBS followed by addition of culture medium and observation of cell migration distance under a microscope at 0 h and 48 h. 48-hour migration rate = (0 h – 48 h scratch width) / 0 h scratch width.

Transwell Chamber Experiment

The cells of each group were digested, washed twice with PBS, centrifuged at 1×10^5 cells each, and the supernatant was removed. The cells in 100 μl serum-free medium were seeded in the Matrigel invasion chamber containing 600 μl of 10% fetal bovine serum medium and cultured in cell incubator for 28 h followed by removal of culture solution, washing the chamber twice with PBS, wiping cells above the chamber. Then, cells were fixed with ice-cold 4% paraformaldehyde for 30 min and stained with Giemsa staining solution for 15 min followed by counting cell number under a microscope. The experiment was repeated three times.

Luciferase Report Experiment

The cells were grouped as follows: pmirGLO, pmirGLO / UCA1-UTR, pmirGLO / UCA1-UTR + miRNA-NC, pmirGLO / UCA1-UTR + miRNA-155mimic, pmirGLO / UCA1-mUTR, pmirGLO / UCA1-mUTR + miRNA-NC, pmirGLO / UCA1-mUTR + miRNA-155 mimic. Prior to transfection, cells were seeded in 24-well plates, 0.5 ml 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. Mix the reporter gene lysate thoroughly and add 100 μl of each reporter gene lysate directly after absorbing the cell culture medium. After fully lysing, centrifuge at 12,000 rpm for 5 min and take the supernatant. For determination, the firefly Luciferase detection reagent and Renilla Luciferase detection buffer were melted to reach room temperature. Renilla Luciferase detection substrate (100X) was placed in an ice bath or ice box for later use. For the determination of each sample, 50 ul of sample was added to 100 μl of firefly Luciferase detection reagent and mixed to determine RLU (relative light unit). Reporter cell lysate was used as a blank control. After completing the above steps for measuring firefly luciferase, 100 μl of Renilla solution was added and mixed to measure RLU (relative light unit) with a Modulus™ microplate multifunctional photometer. In the case of using Renilla Luciferase as a control, the RLU value measured by firefly luciferase was divided by the RLU value measured by Renilla Luciferase. According to the obtained ratio, the degree of activation of the target reporter gene between different samples was compared.

Statistical Analysis

SPSS 16.0 (Chicago, IL, USA) software was applied for analyzing data which were displayed as mean ± standard deviation (SD), and assessed by one-way analysis of variance. $p < 0.05$ indicates a significance.

Results

LncRNA UCA1 Expression in Cervical Cancer

The results showed that compared to adjacent tissues, lncRNA NEAT1 was significantly upregulated in cancer tissues ($p < 0.05$) (Figure 1).

Correlation of LncRNA UCA1 with Survival

The OS curve indicates that elevated LncRNA UCA1 level shortens patients' survival. Conversely, the decreased expression of LncRNA UCA1 prolongs the survival ($p < 0.05$) (Figure 2).

Effect of Down-regulating LncRNA UCA1 on UCA1 Expression

Transfection of LncRNA UCA1 could significantly down-regulate UCA1 expression ($p < 0.05$) (Figure 3).

Effect of Down-regulating LncRNA UCA1 on Cervical Cancer Cell Proliferation

LncRNA UCA1 siRNA can down-regulate its expression and significantly inhibit cell proliferation ($p < 0.05$) (Figure 4).

Effect of Down-regulating LncRNA UCA1 on Cervical Cancer Cell Migration

The results showed that transfection of LncRNAUCA1 siRNA in cervical cancer HeLa cells can down-regulate its expression and significantly inhibit cell migration ($p < 0.05$) (Figure 5).

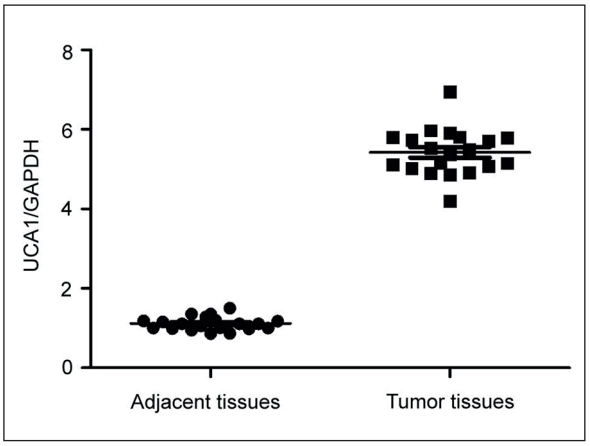

Figure 1. Expression of LncRNA UCA1 in cervical cancer. Compared with the adjacent tissues, *$p < 0.05$.

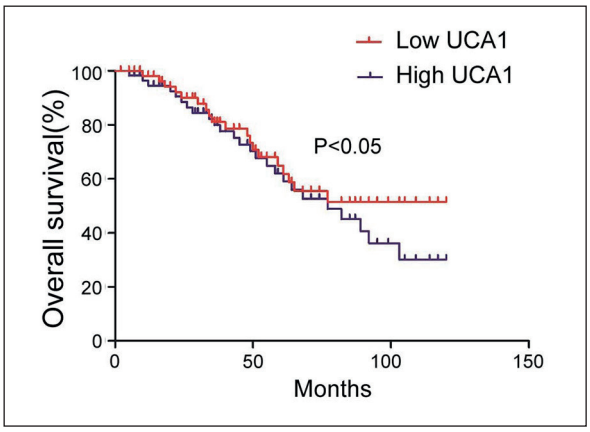

Figure 2. LncRNA UCA1 expression and OS curve.

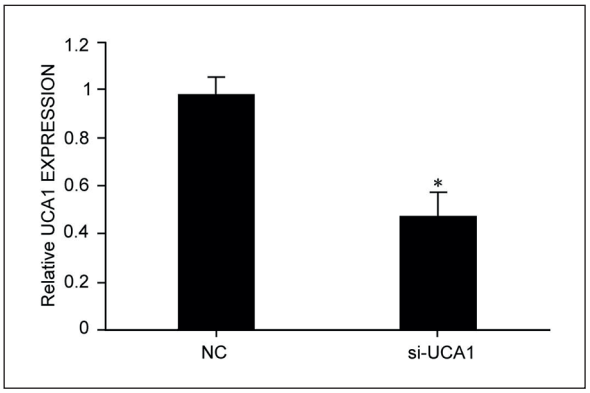

Figure 3. Effect of down-regulating LncRNA UCA1 on UCA1 expression in cervical cancer cells. Compared with NC group, *$p < 0.05$.

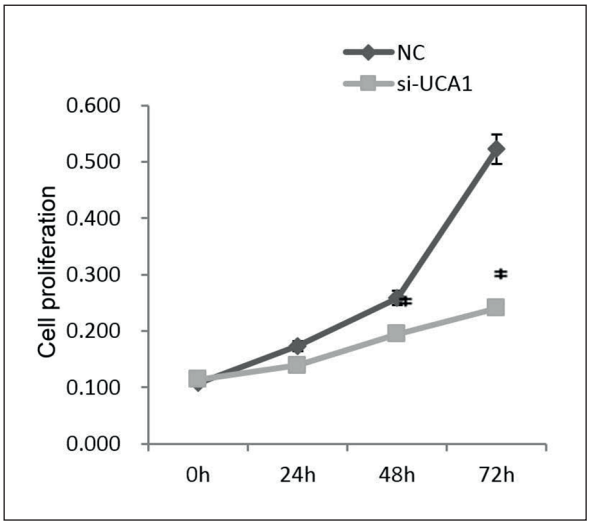

Figure 4. Effect of down-regulation of LncRNA UCA1 on cervical cancer cell proliferation. Compared with NC group, *$p < 0.05$.

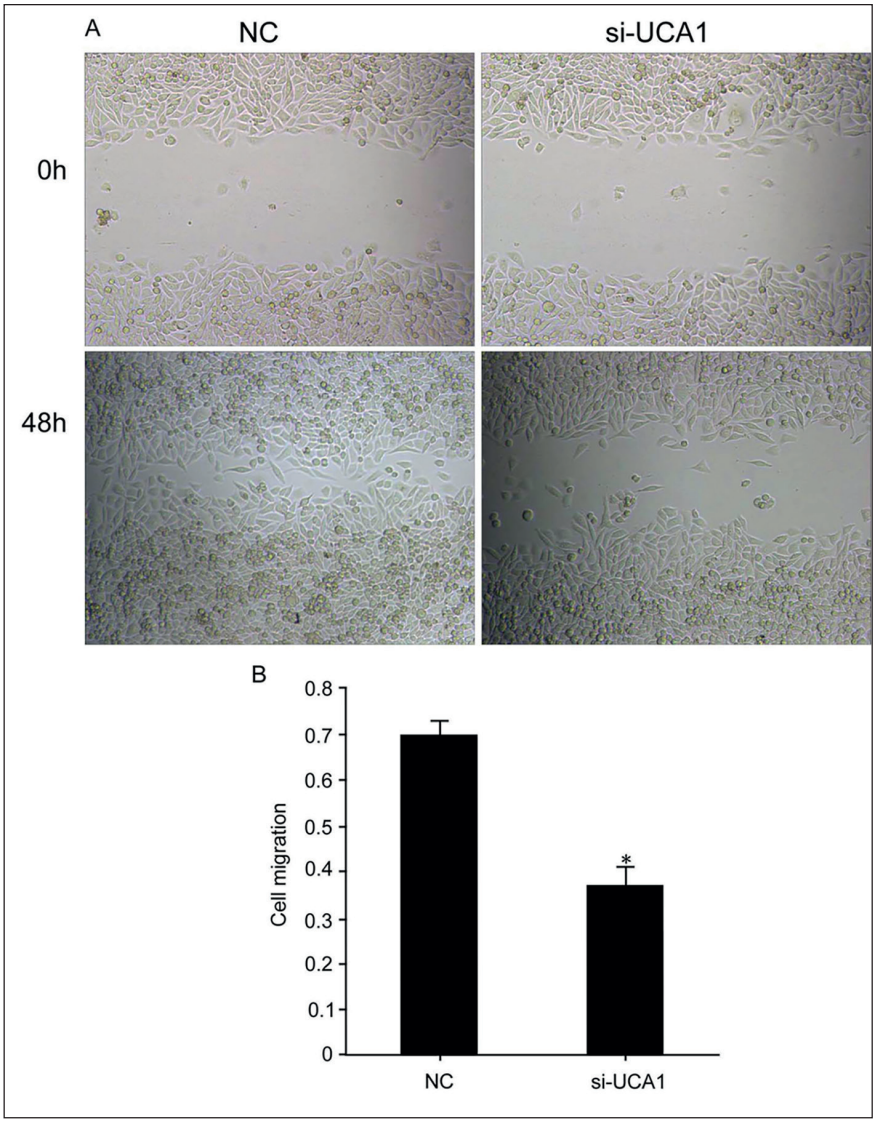

Figure 5. Effect of down-regulation of LncRNA UCA1 on cervical cancer cell migration. **A**, cell scratch test analysis of the effect of LncRNA UCA1 on cervical cancer cell migration (X50); **B**, statistical analysis of the effect of LncRNA UCA1 on cervical cancer cell migration, compared with the NC group, $*p < 0.05$.

Effect of LncRNA UCA1 on Cell Invasion

LncRNA UCA1 siRNA can down-regulate its expression and significantly decrease cell invasion ($p < 0.05$) (Figure 6).

Targeted miRNA Analysis of LncRNA UCA1

The Luciferase report was used to analyze the targeted miRNAs of LncRNA UCA1 in cervical cancer and showed miR-155 to be a target gene of LncRNA UCA1. Transfection of miR-155 mimics inhibited UCA1 luciferase activity (Figure 7).

MiR-155 Level in Cervical Cancer

Compared to adjacent tissues, miR-155 in cancer tissues was significantly downregulated ($p < 0.05$) (Figure 8).

Correlation Analysis Between LncRNA UCA1 and MiR-155

LncRNA UCA1 and miR-155 were negatively correlated in cervical cancer ($p < 0.05$) (Figure 9).

Effect of Down-regulating MiR-155 on LncRNA UCA1 in Cervical Cancer Cell Proliferation

UCA1 siRNA could significantly inhibit cervical cancer cell proliferation ($p < 0.05$). Simultaneous transfection of LncRNA UCA1 siRNA and miR-155 inhibitor can reverse the effect of UCA1 knockdown by siRNA and significantly promote cell proliferation ($p < 0.05$) (Figure 10).

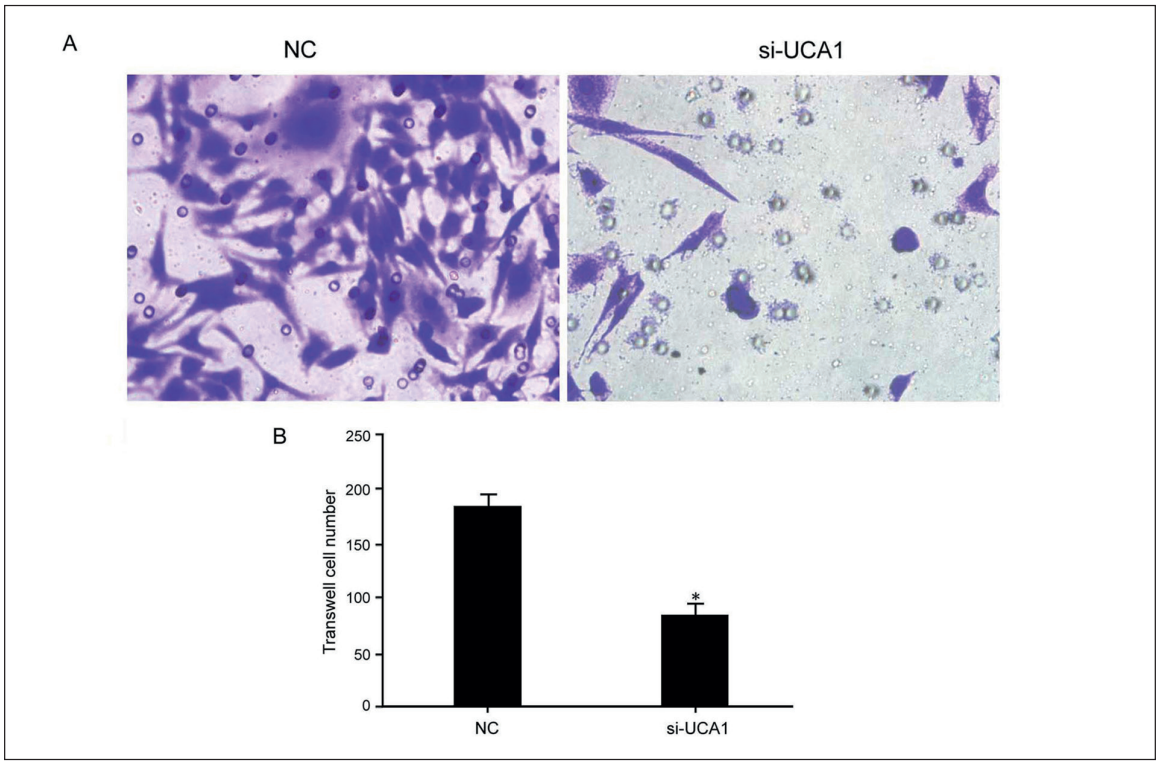

Figure 6. Effect of LncRNA UCA1 on cervical cancer cell invasion. **A**, Transwell laboratory experiment analyzes the effect of LncRNA UCA1 on cervical cancer cell invasion (×50); **B,** statistical analysis of the effect of LncRNA UCA1 on cervical cancer cell invasion, compared with NC group, *$p < 0.05$.

Effect of Down-regulating MiR-155 on LncRNA UCA1 in Cervical Cancer Cell Migration

UCA1 siRNA down-regulated its expression and significantly inhibited cell migration ($p < 0.05$). Simultaneous transfection of LncRNA UCA1 siRNA and miR-155 inhibitor can reverse the effect of UCA1 knockdown by siRNA and significantly promote cell migration ($p < 0.05$) (Figure 11).

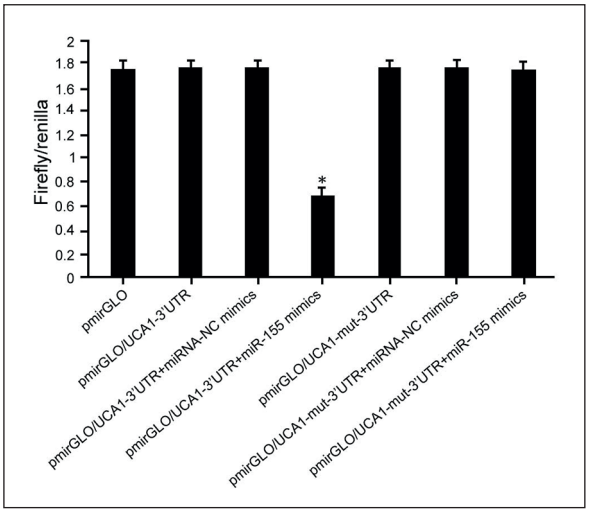

Figure 7. Targeted miRNA analysis of LncRNA UCA1 in cervical cancer. Compared with NC group, *$p < 0.05$.

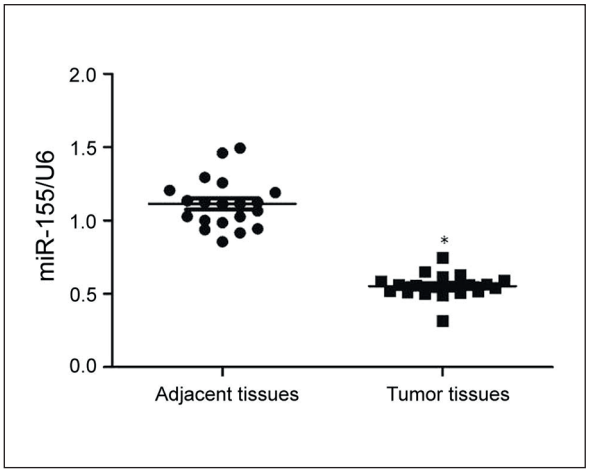

Figure 8. Expression of miR-155 in cervical cancer. Compared with adjacent tissues, $p < 0.05$.

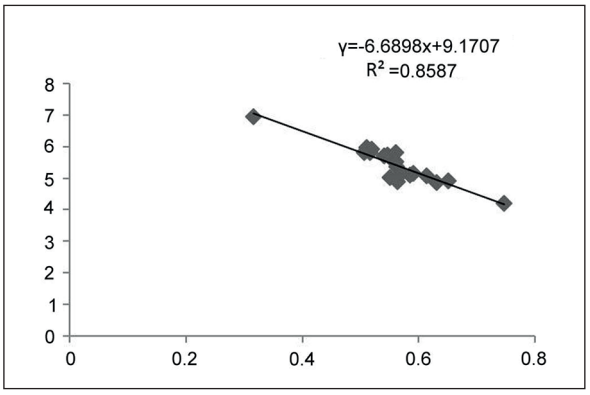

Figure 9. Correlation analysis of LncRNA UCA1 and miR-155 in cervical cancer.

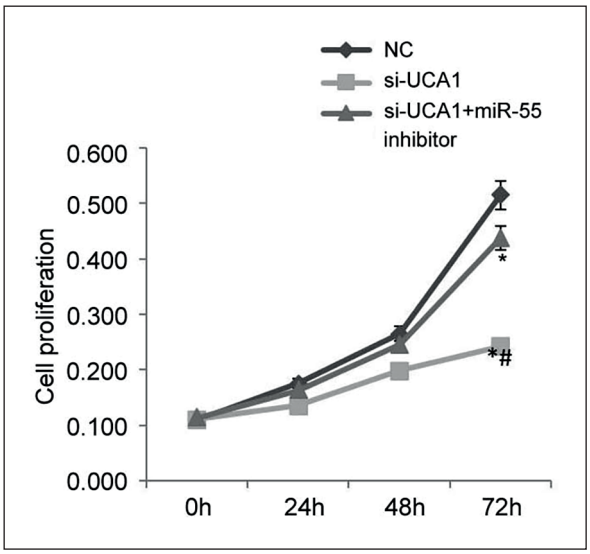

Figure 10. Effect of down-regulating miR-155 on LncRNA UCA1 in cervical cancer cell proliferation. Compared with NC group, $*p < 0.05$; compared with si-UCA1 group, $^{\#}p < 0.05$.

Effect of Down-regulating MiR-155 on Cell Invasion

UCA1 siRNA can significantly inhibit cervical cancer cell invasion after down-regulating UCA1 ($p < 0.05$); meanwhile, transfection of LncRNA UCA1 siRNA and miR-155 inhibitor

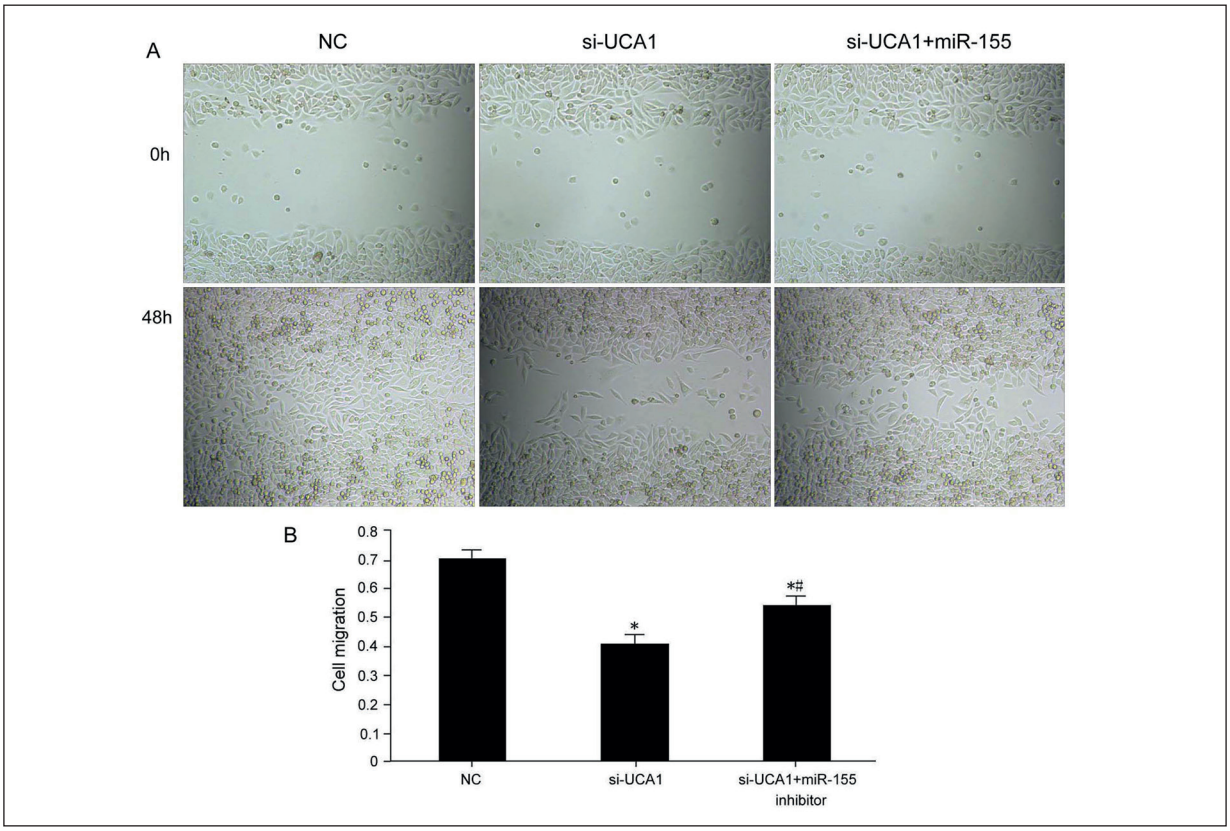

Figure 11. Effect of down-regulating miR-155 on LncRNA UCA1 in cervical cancer cell migration. **A**, Cell scratch test analyzes the effect of down-regulated miR-155 on LncRNA UCA1 in cervical cancer cell migration (X50); **B**, analysis of the effect of down-regulated miR-155 on LncRNA UCA1 in cervical cancer cell migration, compared with the NC group, $* p < 0.05$, compared with si-UCA1 group, $^{\#}p < 0.05$.

can reverse the effect of UCA1 knockdown and significantly promote cell invasion ($p < 0.05$) (Figure 12).

Effect of Down-regulating MiR-155 on E-cadherin Expression of LncRNA UCA1 in Cervical Cancer Cell Line EMT

E-cadherin was significantly upregulated after UCA1 siRNA transfection ($p < 0.05$). Transfection of LncRNA UCA1 siRNA and miR-155 inhibitor at the same time can reverse the effect of UCA1 knockdown and lead to a significant decrease in E-cadherin expression ($p < 0.05$) (Figure 13).

Effect of Down-regulating MiR-155 on Vimentin Level in Cervical Cancer

The results showed that UCA1 siRNA down-regulated its expression and significantly decreased Vimentin level ($p < 0.05$). Simultaneous transfection of LncRNA UCA1 siRNA and miR-155 inhibitor can reverse the effect of UCA1 knockdown and lead to a significant increase in Vimentin expression ($p < 0.05$) (Figure 14).

Discussion

The occurrence and development of cervical cancer involve several factors and the pathogenesis is complicated. Human papillomavirus infection is an important factor in its pathogenesis. Although the conventional treatment includes surgery, combined with chemotherapy and radiation therapy, and with the improvement of diagnosis and treatment technology, the treatment methods have become more diverse, but the ideal treatment effect has not been achieved so far leading to a poor prognosis[19,20]. Therefore, finding biomarkers to assist cervical cancer screening may reveal new targets for cervical cancer and provide potential screening targets. LncRNA participates in physiological activities through various methods such as chromatin modification, genomic imprinting, intranuclear transport, gene silencing of chromosomes, and transcriptional activation[21]. LncRNA can regulate the normal and pathological states of cells, including growth, proliferation, cell cycle, and apoptosis[22]. Abnormal lncRNA expression was found during tumor formation and cancer progression, and some ln-

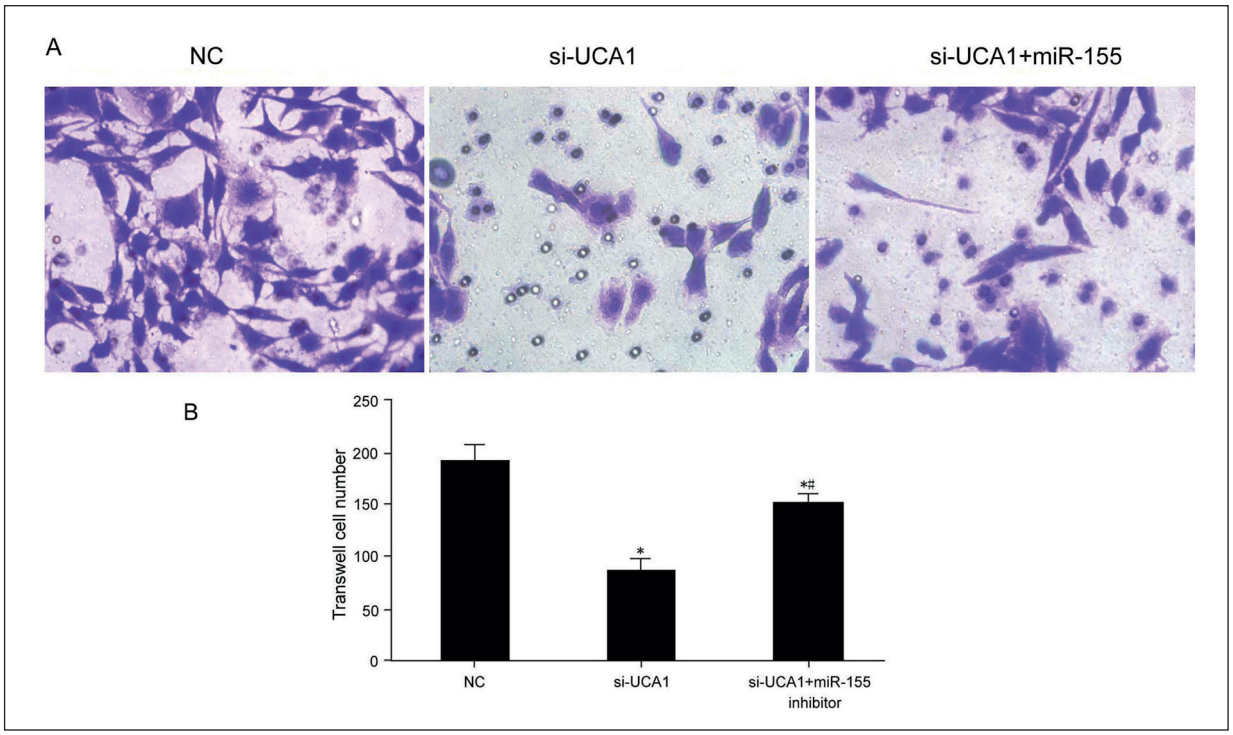

Figure 12. Effect of down-regulating miR-155 on LncRNA UCA1 invasion of cervical cancer cells. **A**, Transwell laboratory experiment analyzes the effect of down-regulated miR-155 on LncRNA UCA1 invasion of cervical cancer cells (X50); **B**, statistical analysis of the effect of down-regulated miR-155 on LncRNA UCA1 invasion of cervical cancer cells, compared with NC group, $^{*}p < 0.05$, compared with si-UCA1 group, $^{\#}p < 0.05$.

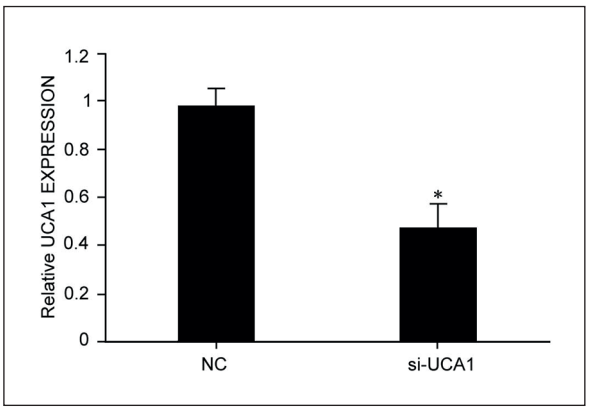

Figure 13. Effect of down-regulating miR-155 on E-cadherin expression of LncRNA UCA1 in cervical cancer cell line EMT. Compared with NC group, $*p < 0.05$, compared with si-UCA1 group, $^{\#}p < 0.05$.

cRNA expression and action mechanisms have been found in tumors[23]. LncRNA UCA1 has been confirmed to be expressed in many normal tissues, but it has been significantly increased in several tumors, further suggesting that lncRNA UCA1 may be involved in the development of tumor[24]. However, the expression and mechanism of lncRNA UCA1 in cervical cancer is unclear. Therefore, our study first confirmed that lncRNA UCA1 level in cervical cancer was significantly increased, and its increased expression was associated with shortened patient survival, suggesting that lncRNA UCA1 expression can regulate cervical cancer survival. To further analyze the related mechanism of lncRNA UCA1 in cervical cancer, this study also found that there is a targeted binding site between LncRNA UCA1 and miR-155 through bioinformatics analysis. Therefore, we further analyzed that over-expressing LncRNA UCA1 can induce down-regulation of miR-155, thereby promoting cancer cell proliferation and migration and invasion. Up-regulation of miR-155 can significantly inhibit luciferase activity of LncRNA UCA1 without affecting the activity of LncRNA UCA1MUT, indicating a targeted relationship between LncRNA UCA1 and miR-155. Moreover, reduced miR-155 is inversely related to LncRNA UCA1. LncRNA UCA1 gene knockout can significantly inhibit cervical cancer cell proliferation, migration and invasion. This study confirms that down-regulating miR-155 can reverse LncRNA UCA1 siRNA's effect. EMT involves a variety of physiological and pathological syndromes and processes, including embryonic development, fibrosis, and cancer progression. During EMT, decreased E-cadherin and increased Vimentin and N-cadherin leads to morphological changes[25,26]. EMT can regulate tumor metastasis and is a factor that determines the prognosis of cancer. During EMT, epithelial cells undergo many phenotypic changes to obtain a mesenchymal phenotype, promote tumor cell proliferation and invasion[27,28]. This study demonstrated that LncRNA UCA1 downregulation in cervical cancer cells can inhibit EMT, upregulate E-cadherin and downregulate Vimentin, and miR-155 inhibitors can reverse LncRNA UCA1's effect on EMT, suggesting that LncRNA UCA1 targets miR-155 to regulate EMT in cervical cancer.

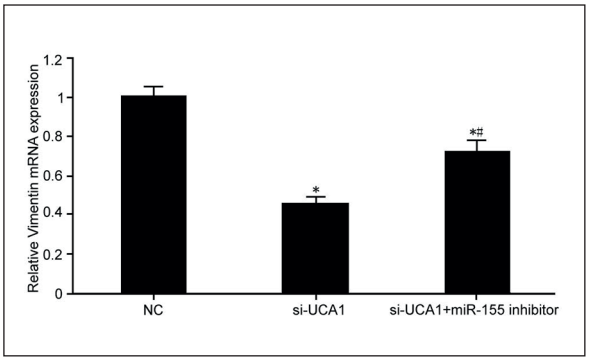

Figure 14. Effect of down-regulation of miR-155 on the expression of Vimentin in cervical cancer cells EMT by LncRNA UCA1. Compared with NC group, $*p < 0.05$, compared with si-UCA1 group, $^{\#}p < 0.05$.

Conclusions

UCA1 level in cervical cancer is related to patients' survival. MiR-155 is a target miRNA of UCA1. Lnc-RNA UCA1 regulates EMT occurrence in cervical cancer cells by targeting miR-155, thereby inhibiting tumor development.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Acknowledgements

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