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Long non-coding RNA UCA1 mediates proliferation and metastasis of laryngeal squamous cell carcinoma cells *via* regulating miR-185-5p/HOXA13 axis

Y.-D. SUN, Q. LIU, H.-X. YANG, L. TIAN, J. WANG, L. ZENG, X.-W. ZHOU

Department of Otolaryngology, Hospital of Traditional Chinese Medicine Affiliated to Southwest Medical University, Luzhou, Sichuan Province, China

Abstract. – OBJECTIVE: LncRNA urothelial cancer associated 1 (UCA1) is involved in the development of laryngeal squamous cell carcinoma (LSCC), however, its specific mechanism is not fully clear.

PATIENTS AND METHODS: Quantitative reverse transcription-PCR (RT-qPCR) was conducted to determine the expressions of lncRNA-UCA1, miR-185-5p and homeobox A13 (HOXA13) in LSCC tissues and cell lines. Cell Counting Kit-8 assay, colony formation assay, wound healing assay, Transwell and flow cytometry, DIANA-LncBase V2, as well as Starbase, Targetscan, and Dual-Luciferase reporter gene system were conducted to detect and confirm the crosstalk networks among lncRNA-UCA1, miR-185-5p, and HOXA13.

RESULTS: The levels of UCA1 and HOXA13 were significantly higher and the expression of miR-185-5p was reduced in LSCC tissues and cell lines. Moreover, miR-185-5p was predicted as a target gene for lncRNA UCA1, while HOXA13 was the target gene for miR-185-5p. UCA1 siRNA inhibited the proliferation and invasion of LSCC cells, moreover, the proliferation and invasion of LSCC cells were suppressed by miR-185-5p mimics but were enhanced by miR-185-5p inhibitor. UCA1 siRNA and overexpressed HOXA13 reversed the promotive effects of miR-185-5p inhibitor and inhibitory effects of miR-185-5p mimics on cell proliferation and metastasis, respectively.

CONCLUSIONS: The current findings reveal the important role of lncRNA UCA1/miR-185-5p/HOXA13 regulatory network in LSCC cells, and potentially provide new insights into the pathogenesis of LSCC.

Key Words:
Urothelial cancer associated 1, MiR-185-5p, Homeobox A13, LSCC, Proliferation.

Introduction

Head and neck squamous cell carcinoma (HNSCC) has become a cancer with high incidence in the past 20 years in the world[1]. Laryngeal squamous cell carcinoma (LSCC) in HNSCC is possibly associated with factors such as drinking, smoking, air pollution, sex hormones and certain occupations[2]. Worldwide, approximately 150,000 patients are diagnosed with LSCC annually, with an incidence of about 1.2% and a mortality rate of about 1.1%[3]. Although LSCC surgery, chemoradiotherapy and targeted drug therapy have been widely applied to treat LSCC, survival rate of LSCC patients is generally decreasing (from 57.1% to 51.9%)[4].

LncRNAs are RNA molecules with nucleotides longer than 200 nt and localize in the cytoplasm and/or nucleus but lack protein-encoding functions. Abnormal expressions of lncRNAs are closely related to occurrence and development of various diseases including malignant tumors[5-7]. Studies[8,9] showed that lncRNAs can control downstream target genes through signals, decoys, guide, and scaffolds. Research[10-12] found that lncRNAs also act as competitive endogenous RNAs (ceRNAs) or miRNA sponges, and their miRNA response elements (MREs) compete for binding to miRNAs to inhibit functions of miRNAs, thereby regulating the expressions of target gene mRNAs at post-transcriptional levels.

Urothelial cancer associated 1 (UCA1), which is a lncRNA molecule first discovered in bladder cancer and locates on chromosome 19p13.12, contains 3 exons and encodes 2 transcript variants. Previous study[13-15] reported that UCA1 is high-expressed in tumors such as bladder cancer, colorectal cancer and breast cancer. Moreover, UCA1 can promote the proliferation and metastasis of bladder cancer cells through signaling pathways such as PDK, Wnt and Akt[16,17]. In addition, UCA1 activates mTOR-STAT3-miR-

Corresponding Author: Xingwei Zhou, MD; e-mail: xingwei_zhouxw@163.com

143-HK2 pathway to accelerate glucose metabolism in bladder cells, while miR-1 inhibits the growth of bladder cancer cells by down-regulating UCA1 expression, indicating that there is a mutual regulation between UCA1 and miRNAs in tumor cells[18,19]. Sun et al[16] indicated that UCA1 is elevated in LSCC and participates in proliferation and metastasis of LSCC cells; however, whether UCA1 is involved in the development of LSCC and its underlying mechanism remain unknown.

Therefore, based on the previous findings, the present study investigated the expression level of UCA1 in LSCC cancer tissues and cell lines and explored the mechanism of lncRNA UCA1-miR-185-5p- homeobox A13 (HOXA13) pathway regulatory network in LSCC development.

Patients and Methods

LSCC Patient Tissues

From March 2017 to March 2019, 30 LSCC cancer tissues and adjacent normal tissues were collected from patients who attended Hospital of Traditional Chinese Medicine Affiliated to Southwest Medical University. All the patients did not receive chemotherapy, radiotherapy, targeted therapy or other surgical treatments prior to the study. The specimens were immediately saved in liquid nitrogen. All LSCC cancer tissues and adjacent normal tissues were confirmed by pathology. The signed informed consent was obtained from each patient before the surgery, and the study was approved by Hospital of Traditional Chinese Medicine Affiliated to Southwest Medical University Ethics Committee.

Cells and Culture

Human normal immortalized epidermal cell line Hacat and human LSCC cell line AMC-HN-8 were purchased from Shanghai Institute of Biochemistry and Cell Biology, Tu 177 was purchased from Shanghai Baili Biotechnology Co., Ltd., and SNU-46, SNU-899 and SNU-1076 were obtained from Korean Cell Bank. The cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) medium (Gibco, Grand Island, NY, USA) containing 10% fetal bovine serum (FBS, Gibco, Grand Island, NY, USA), 100 U/mL penicillin and 100 μg/mL streptomycin (Gibco, Grand Island, NY, USA) in an incubator at 37°C with 5% CO_2 in 95% humidity.

Transfection

UCA1 siRNA (GCCACCUACAUUAAAGCUAdTdT), miR-185-5p mimic (AGGGGCUGGCUUUCCUCUGGUC), inhibitor (GACCAGAGGAAAGCCAGCCCCU) and HOXA13 expression plasmid pcDNA3.1-HOXA13 were purchased for transfection (GenePharma, Shanghai, China). The plasmids and RNA were transfected with Tu 177 using liposome Lipofectamine™ 2000 (Invitrogen, Carlsbad, CA, USA).

Cell Counting Kit-8 (CCK-8) Assay

Tu 177 at 5×10^4 cells/mL was added into a 96-well plate and incubated for 24 h. After transfection for 24, 48 and 72 h, 10 μL CCK-8 solution (Beyotime, Beijing, China) was added to the cells. After 2 h, the absorbance at 450 nm was measured by a microplate reader (MD SpectraMax M5; Molecular Devices, Sunnyvale, CA, USA).

Cloning Formation Experiment

One hundred cells were seeded in a 6-well plate containing medium pre-warmed to 37°C, cultured at 37°C with 5% CO_2, and allowed to stand for 2 to 3 weeks. The medium was changed every two days. The cells were then fixed by 1: 3 acetic acid/ methanol for 30 min, and stained by Giemsa stain for 20 min. The pictures of cloning formation were then obtained by using a camera. Clones of the cells were counted, and colony formation rate was calculated (clonal formation rate = number of clones formed / number of cells seeded × 100%).

Flow Cytometry for the Detection of Apoptosis and Cell Cycle

Cell suspension of Tu 177 cells was prepared at the final concentration of 1×10^6 cells/mL using 500 μL of 1× Annexin V Binding Solution and then removed to a 6-well plate. 5 μL Annexin V-FITC was added to the cell suspension, 5 μL propidium iodide (PI) was added to the cells and cultured in the dark for 15 min at room temperature. Cell apoptosis was detected by flow cytometry (version 10.0, FlowJo, FACSCalibur™, BD Biosciences, San Jose, CA, USA). Necrotic cells were displayed in the upper left area (Annexin V^-, PI^+) and late apoptotic cells were displayed in the upper right area (Annexin V^+, PI^+), while the living cells were shown in the lower left area (Annexin V^-, PI^-) and the early apoptotic cells were shown in lower right area (Annexin V^+, PI^-).

Transfected Tu 177 cells were digested, collected and fixed at 4°C by pre-cooled 70% ethanol overnight. Cells were centrifuged at 1000 rpm for 5 min, and the cells were washed twice with phosphate-buffered saline (PBS) and then resuspended with PBS. 10 μL 50 μg/mL propidium iodide (PI) was added to the cells for 10 min at 4°C and the cell cycle was detected using flow cytometry (version 10.0, FlowJo, FACS Calibur™, BD Biosciences, San Jose, CA, USA).

Wound Healing Assay

Tu 177 cells were seeded in a 6 well plate at a density of 8×10^5 for culture. The cell layer was scratched using a sterile sampler tip, and the floating cells were removed by PBS. The cells were then cultured for 48 h and photographed.

Transwell

Invasion ability of Tu 177 cells was measured using a 24-well transwell (Corning, Corning, NY, USA). The cells were seeded at a density of 4×10^5 cells/well into the upper chamber containing 200 mg/mL Matrigel (BD, Biosciences, San Jose, CA, USA). Supernatant containing serum-free medium was added to the upper chamber and 20% serum medium was added to the lower chamber to form a concentration gradient of nutrients that promote cell transfer into the lower chamber. After incubation at 37°C for 48 h, the remaining cells in the upper chamber were removed using a cotton swab, and transferred cells were fixed in methanol for 10 min at room temperature and stained by 0.1% crystal violet (Beyotime, Beijing, China). The number of cells per field of view was calculated under an inverted microscope.

Quantitative Reverse Transcription-PCR

To quantitatively determine mRNA expressions of lncRNA-UCA1, miR-185-5p and Homeobox A13 (HOXA13) in the cells, RT-qPCR was performed using the following primer pairs: lncRNA-UCA1 forward primer, 5′- CTCTCCATTGGGTTCACCATTC-3′ and lncRNA-UCA1 reverse primer, 5′-GCGGCAGGTCTTAAGAGATGAG-3′. miR-185-5p forward primer, 5′-CAATGGAGAGAAAGGCAGTTCC-3′ and miR-185-5p reverse primer, 5′-AATCCATGAGAGATCCCTACCG-3′. HOXA13 forward primer, 5′-GAACGGCCAAATGTACTGCC-3′ and HOXA13 reverse primer, 5′-GTATAAGGCACGCGCTTCTTTC-3′. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (GAPDH forward primer, 5′-AGGTCGGTGTGAACGGATTTG-3′ and GAPDH reverse primer, 5′-GGGGTCGTTGATGGCAACA-3′), U6 forward primer, 5′-CTCGCTTCGGCAGCACA-3′ and U6 reverse primer, 5′- AACGCTTCACGAATTTGCGT-3′ served as an internal control. RNAs isolation and conversion to complementary DNAs (cDNAs) was performed using RevertAid™ First Strand cDNA Synthesis Kit (#K1622, Fermentas, Hanover, MD, USA). CDNA suspension (1 μg) was amplified by SYBR Green Quantitative PCR Master Mix (Applied Biosystems, Foster City, CA, USA).

Western Blot

Total protein extracts were separated on 8% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a nitrocellulose membrane, which was then blocked by 5% non-fat milk and 0.1% Tris buffered saline/Tween 20 (TBST-20) at room temperature for 2 h. The membrane was then incubated with Anti- B-cell lymphoma-2 (Bcl-2) (26 kDa, Rabbit, 1:1000, ab59348, Abcam, Cambridge, MA, USA), Anti- BCL2 associated X (Bax) (21 kDa, Rabbit, 1:1000, ab32503, Abcam, Cambridge, MA, USA), Anti- cleaved caspase 3 (C caspase-3) (17 kDa, Rabbit, 1:1000, ab2302, Abcam, Cambridge, MA, USA), Anti- HOXA13 (40 kDa, Rabbit, 1:1000, ab106503, Abcam, Cambridge, MA, USA) or Anti-GAPDH antibody (36 kDa, mouse, 1:1000, ab8245, Abcam, Cambridge, MA, USA) overnight at 4°C. Target bands were detected by horseradish peroxidase-conjugated secondary antibody (1:2000, sc-516102/ sc-2357, Santa Cruz Biotechnology, Santa Cruz, CA, USA) and visualized by enhanced chemiluminescence detection system (EZ-ECL kit, Biological Industries, Kibbutz Beit Haemek, Israel). Gray values of the strips were analyzed and counted by ImageJ (version 5.0, Bio-Rad, Hercules, CA, USA).

Bioinformatics Prediction

For identifying the target gene for lncRNA UCA1, gene prediction was performed using DIANA-LncBase V2 (https://omictools.com/diana-lncbase-tool) and StarBase (http://starbase.sysu.edu.cn/agoClipRNA.php?source=lncRNA). The target gene for miR-185-5p was predicted using targetscan7.2 (http://www.targetscan.org/vert_72/).

Dual-Luciferase Activity

Tu 177 cells were respectively transfected with 100 nM UCA1-3'-UTR plasmid (The UCA1 mutant (MT), wild-type (WT)) and with (or without) 100 nM mimic to determine the binding ability

of UCA1 to miR-185-5p. For determining the binding ability of miR-185-5p to HOXA13, Tu 177 cells were transfected with 100 nM HOXA13-3'-UTR plasmid (The HOXA13 mutant (MT) and wild-type (WT)) and with or without 100 nM mimic. Then, Luciferase activity was measured by luciferase reporter assay system (Promega, Madison, WI, USA) in Lmax II luminescence meter (Molecular Devices, Sunnyvale, CA, USA) 48 h after the transfection.

Statistical Analysis

The data were shown as the mean ± SD and analyzed by GraphPad Prism 7 (La Jolla, CA, USA). Comparisons between the groups were performed by one-way analysis of variance (ANOVA) followed by Bonferroni's post hoc test. $p<0.05$ was considered to be significantly different.

Results

LncRNA UCA1 was Upregulated in LSCC, and Silencing of UCA1 Inhibited LSCC Cell Proliferation

Expression of lncRNA UCA1 in 30 pairs of LSCC specimens and paraneoplastic tissues was determined using specific primers for UCA1. As shown in Figure 1A, the expression of lncRNA UCA1 was increased significantly in LSCC tissues in comparison with that in paraneoplastic tissues. Next, we determined the expressions of lncRNA UCA1 in 5 LSCC cell lines (AMC-HN-8, Tu 177, SNU-46, SNU-899 and SNU-1076 and Human normal immortalized epidermal cell line Hacat, as shown in Figure 1B, expression of lncRNA UCA1 was increased in the 5 LSCC cell lines compared with Hacat. Tu 177 cells

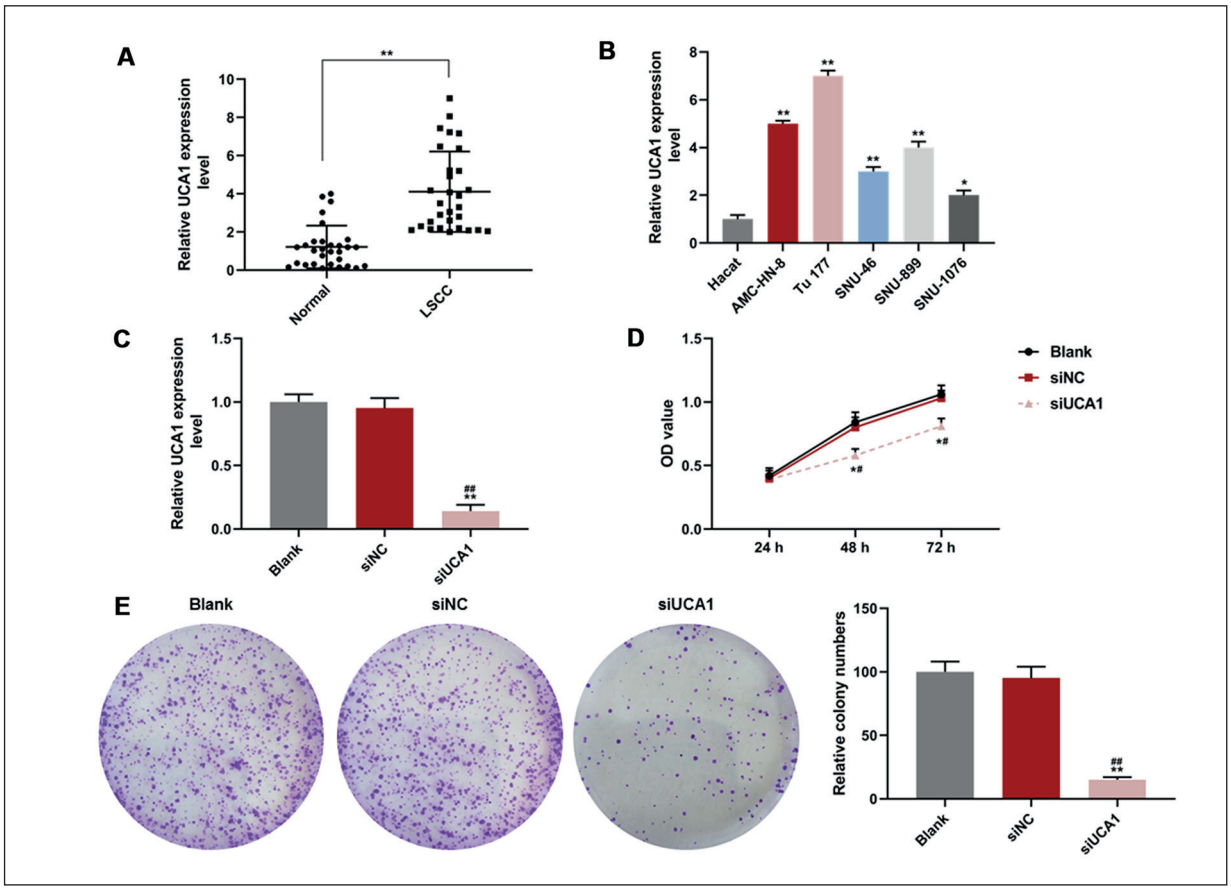

Figure 1. LncRNA UCA1 was upregulated in LSCC, and siRNA inhibited proliferation. **A**, Analyses of the expression levels of lncRNA UCA1 in paired LSCC and adjacent non-tumorous tissues (n = 30). **B**, Analyses of the expression levels of lncRNA UCA1 in 5 LSCC cell lines and human normal immortalized epidermal cell line Hacat. **C**, Stable Tu 177 cell line down-regulating lncRNA UCA1 was established. CCK-8 assay (**D**) and cloning formation experiment (**E**) were performed to detect viability and proliferation of Tu 177 cells. Magnification: ×1. *$p<0.05$, **$p<0.01$ *vs.* normal/ Hacat/ Blank, $^{\#}p<0.05$, $^{\#\#}p<0.01$ *vs.* siNC. UCA1: urothelial cancer associated 1; CCK-8: Cell Counting Kit-8.

were selected for further assays, as it had the highest LSCC expression. To further investigate the effects of lncRNA UCA1 on LSCC cell proliferation, lncRNA UCA1 knockdown cell line was established by siRNA in Tu 177 cells and confirmed by RT-qPCR (Figure 1C). LncRNA UCA1 knockdown was found to significantly inhibit viability of Tu 177 cells (Figure 1D) and colony formation (Figure 1E).

The Inhibition of lncRNA UCA1 Promoted Apoptosis and Reduced Tu 177 Cells Invasion

As lncRNA UCA1 siRNA suppressed Tu 177 cell proliferation, we further investigated the roles of UCA1 in cell apoptosis and invasion and found that down-regulation of UCA1 increased apoptosis of Tu 177 cells (Figure 2A). Flow cytometry analyses on cell cycle distribution demonstrated that UCA1 siRNA increased the proportion of cells in the G1 phase but reduced the proportion of those in the S phase compared with control cells (Figure 2B). Of note, lower UCA1 expression in Tu 177 cells reduced markedly expression of Bcl-2 and increased expressions of Bax and C caspase-3 (Figure 2C). Tu 177 cells transfected with UCA1 siRNA showed slower wound healing in comparison with that of the cells transfected with UCA1-siNC (Figure 2D). Moreover, Tu 177 cells induced by UCA1 knockdown showed a slow invasion rate (Figure 2E).

LncRNA UCA1 Bound to MiR-185-5p and Promoted Tu 177 Cell Proliferation

To understand the regulatory mechanism of lncRNA UCA1, bioinformatics analysis software predicted that miR-185-5p could be target by lncRNA UCA1 (Figure 3A), moreover, Dual-Luciferase activity showed that Luciferase activity of UCA1-WT was visibly reduced by miR-185-5p mimic, proving that lncRNA UCA1 could interact with miR-185-5p (Figure 3B). Moreover, miR-185-5p expression was noticeably increased in Tu 177 cells with lncRNA UCA1 siRNA (Figure 3C). We also found that miR-185-5p level was down-regulated in LSCC tissues (Figure 3D). Furthermore, miR-185-5p mimic and inhibitor were transfected into Tu 177 cells to increase and knock down the expression of miR-185-5p, so as to investigate whether miR-185-5p was involved in the effects of lncRNA UCA1 on proliferation. The results showed that UCA1 siRNA promoted expression of miR-185-5p and reduced the inhibitory effect of miR-185-5p inhibitor (Figure 3E). The data also revealed that cell viability (Figure 3F) and proliferation (Figure 3G, 3H) were significantly inhibited by up-regulating miR-185-5p but were promoted by down-regulating miR-185-5p, however, the effects on promoting cell functions by the inhibitor were partially reversed by lncRNA UCA1 siRNA.

The Inhibition of MiR-185-5p Partly Reversed Tumor-Suppressive Effects Induced by the Down-Regulation of lncRNA UCA1

We explored whether lncRNA modulated apoptosis, invasion and migration of Tu 177 cell line through regulating miR-185-5p. Our findings indicated that overexpression of miR-185-5p induced cell apoptosis, which was suppressed by the inhibitor, moreover, the inhibitory effect on cell apoptosis was partially reversed by lncRNA UCA1 siRNA (Figure 4A, 4B). Flow cytometry on the cell cycle distribution demonstrated that cell cycle was arrested at G1 phase by mimic and the inhibitor had the opposite results to mimic, moreover, the effect of inhibitor on cell cycle could be reversed by lncRNA UCA1 siRNA(Figure 4C, 4D). Wound-healing assay indicated that mimic noticeably reduced the migration ability of Tu 177 cells, while the inhibitor promoted the migration ability of Tu 177 cells compared with their controls, moreover, the promotion effect was partly reversed by down-regulating lncRNA UCA1 (Figure 4E, 4F). Transwell invasion assay indicated that mimic greatly reduced the invasion ability of Tu 177 cells, while the inhibitor promoted the invasion ability of Tu 177 cells compared with their controls, and the promotion effect was partly reversed by the down-regulation of lncRNA UCA1 (Figure 4G, 4H).

HOXA13 Was a Novel Target for MiR-185-5p

TargetScan software predicted that HOXA13 was a putative target gene for miR-185-5p in LSCC (Figure 5A), furthermore, Luciferase activity experiment demonstrated that miR-185-5p mimic significantly reduced the luciferase activity of HOXA13-WT expression in comparison with HOXA13-MUT, but did not affect the luciferase activity of HOXA13-MUT (Figure 5B). Furthermore, the mRNA expression level of HOXA13 was significantly up-regulated in LSCC tissue samples compared with adjacent healthy tissue

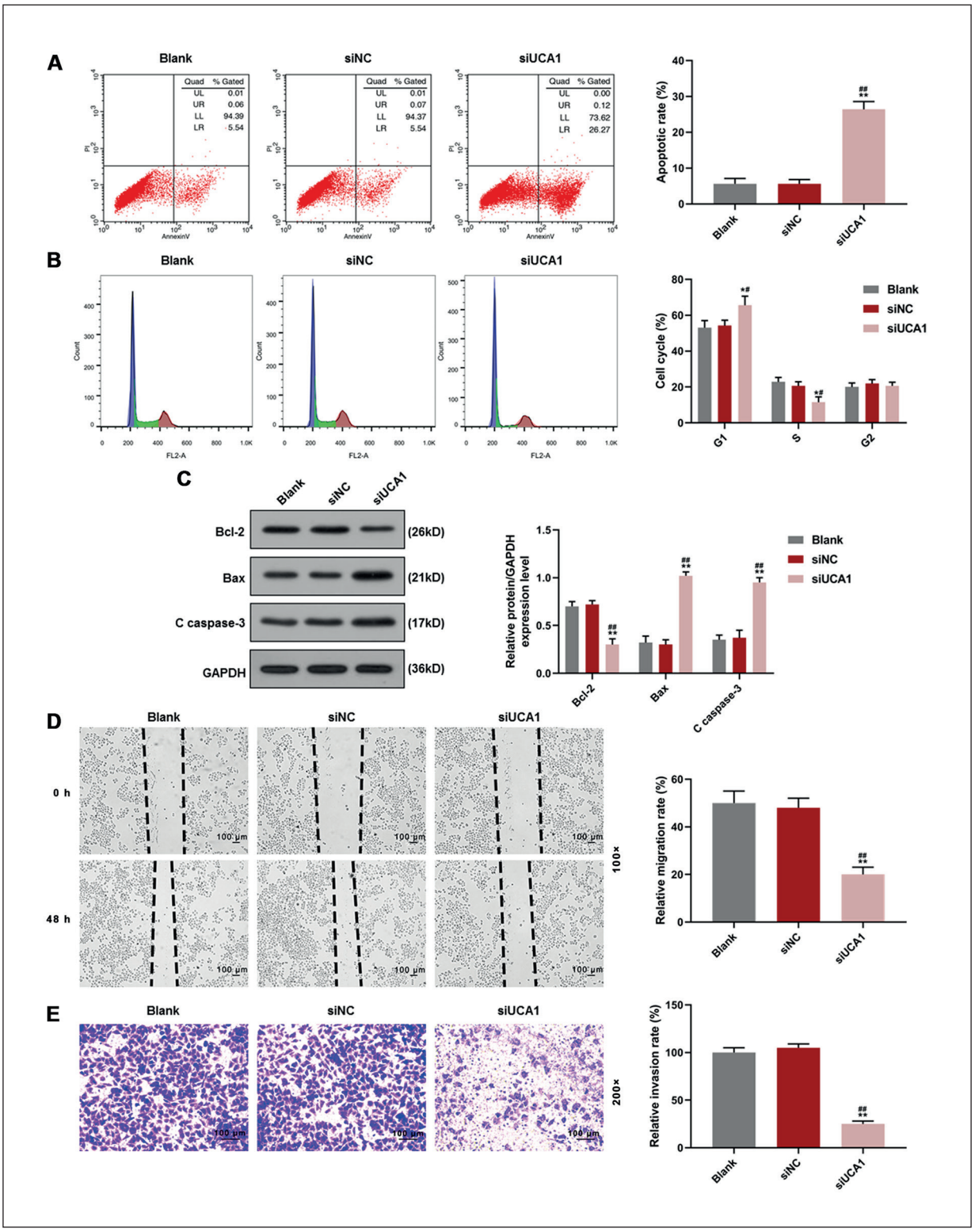

Figure 2. The inhibition of lncRNA UCA1 promoted apoptosis and reduced Tu 177 cells invasion. Flow cytometry was carried out to detect the apoptosis (**A**) and cell cycle (**B**) in Tu 177 cells. **C**, Western blot was performed to measure the levels of Bcl-2, Bax and C caspase-3 in Tu 177 cells. Wound healing and Transwell were performed to detected cell migration (**D**, ×100) and invasion (**E**, ×200), respectively. *p<0.05, **p<0.01 *vs.* Blank, $^{\#}p$<0.05, $^{\#\#}p$<0.01 *vs.* siNC. Bcl-2: B-cell lymphoma-2; Bax: BCL2 associated X; C caspase 3: cleaved caspase 3.

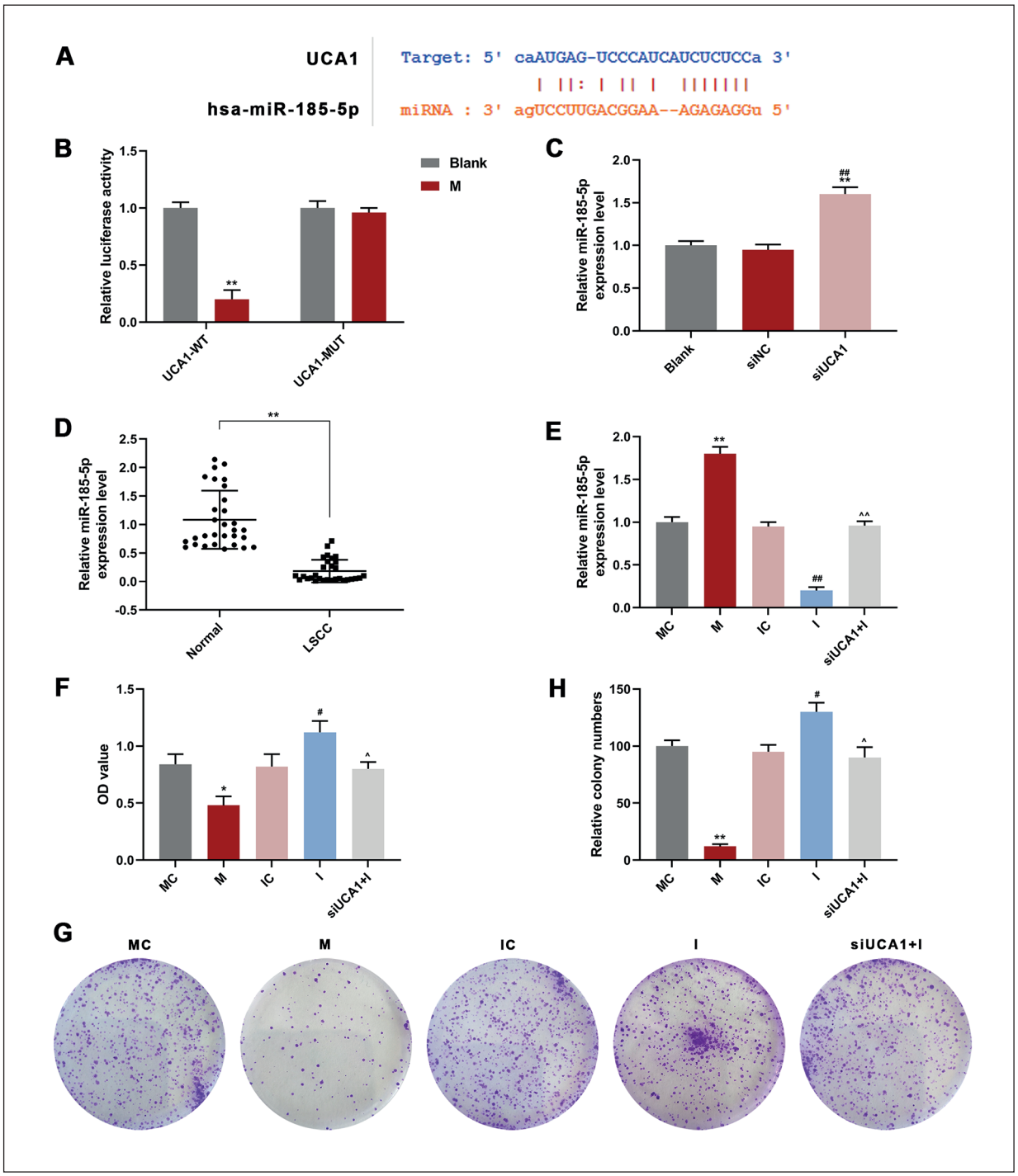

Figure 3. LncRNA UCA1 bound to miR-185-5p and promoted Tu 177 cell proliferation. **A**, DIANA-LncBase V2 and StarBase, and Dual-Luciferase activity **(B)** was performed to identify miR-185-5p binding sites in lncRNA UCA1. **C**, Following transfection with si-UCA1 or control, the mRNA expression level of miR-185-5p was determined by RT-qPCR in Tu 177 cells. **D**, The mRNA expression level of miR-185-5p was determined by RT-qPCR in LSCC tissues. (E) RT-qPCR analysis of miR-185-5p levels in Tu 177 cells transfected with miR-185-5p mimics, inhibitor or miR-NC in the presence or absence of siUCA1. **F,** CCK-8 assay analysis of cell viability in Tu 177 cells transfected with miR-185-5p mimics, inhibitor or miR-NC in the presence or absence of siUCA1. **G**, **H**, Cloning formation analysis of Tu 177 cells transfected with miR-185-5p mimics, inhibitor or miR-NC in the presence or absence of siUCA1. Magnification: ×1. *p<0.05, **p<0.01 *vs.* Blank/Normal/MC, $^{\#}p$<0.05, $^{\#\#}p$<0.01 *vs.* IC, $^{\wedge}p$<0.05, $^{\wedge\wedge}p$<0.01 *vs.* I. MC: mimic control. IC, inhibitor control. NC: negative control. M: miR-185-5p mimic. I: miR-185-5p inhibitor.

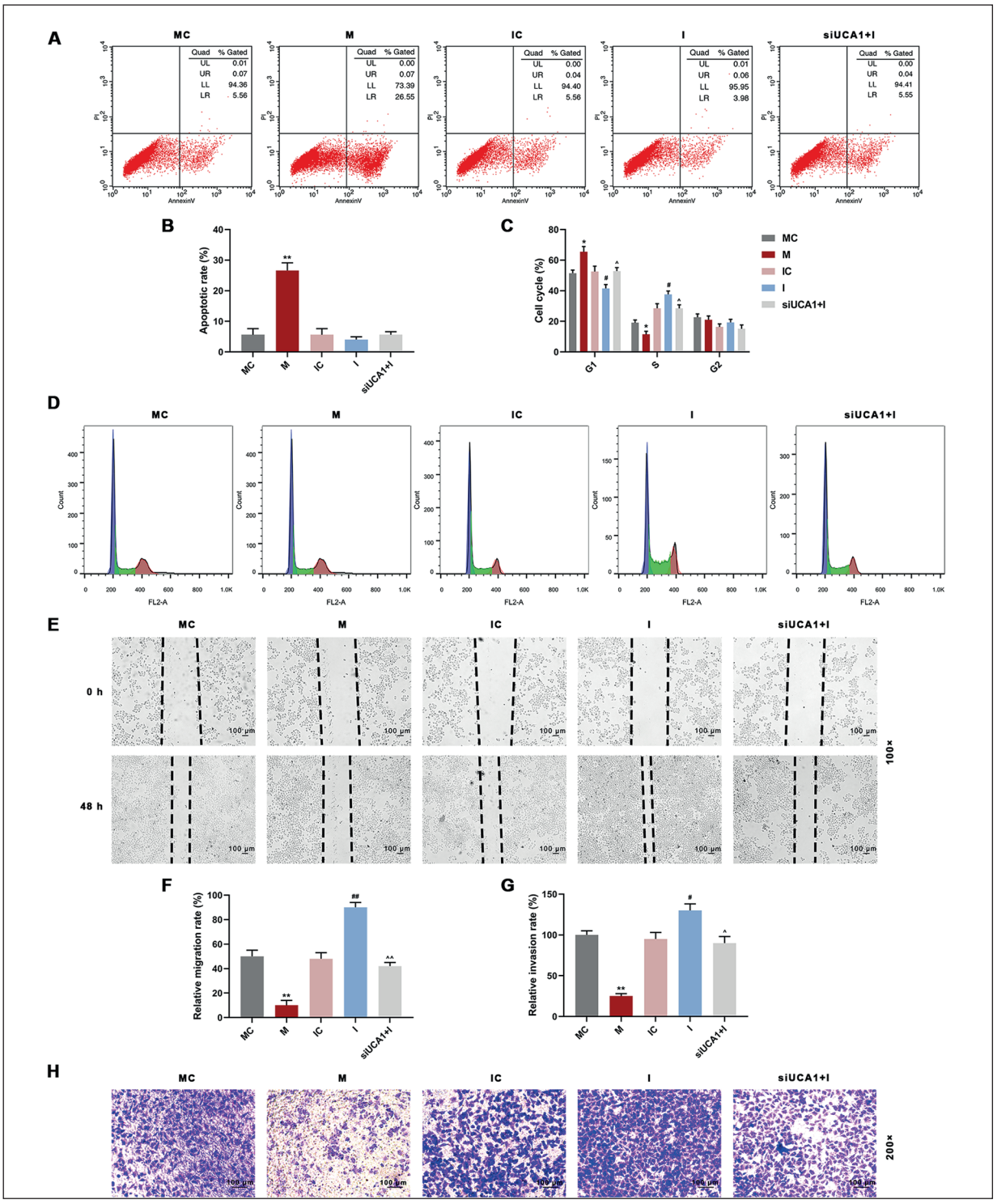

Figure 4. The inhibition of miR-185-5p partly reversed regulatory effects induced by down-regulation of LncRNA UCA1. **A**, **B**, Flow cytometry analysis of cell apoptosis in Tu 177 cells transfected with miR-185-5p mimics, inhibitor or miR-NC in the presence or absence of siUCA1. **C**, **D**, Flow cytometry analysis of cell cycle distribution in Tu 177 cells transfected with miR-185-5p mimics, inhibitor or miR-NC in presence or absence of siUCA1. **E**, **F**, Wound healing analysis of the migration capacity of Tu 177 cells transfected with miR-185-5p mimics, inhibitor or miR-NC in the presence or absence of siUCA1. Magnification: ×100. **G**, **H**, Transwell analysis of invasion ability of Tu 177 cells transfected with miR-185-5p mimics, inhibitor or miR-NC in the presence or absence of siUCA1. Magnification: ×200. **p*<0.05, ***p*<0.01 *vs.* MC, $^{\#}p$<0.05, $^{\#\#}p$<0.01 *vs.* IC, $^{\wedge}p$<0.05, $^{\wedge\wedge}p$<0.01 *vs.* I. MC: mimic control. IC, inhibitor control. M: miR-185-5p mimic. I: miR-185-5p inhibitor.

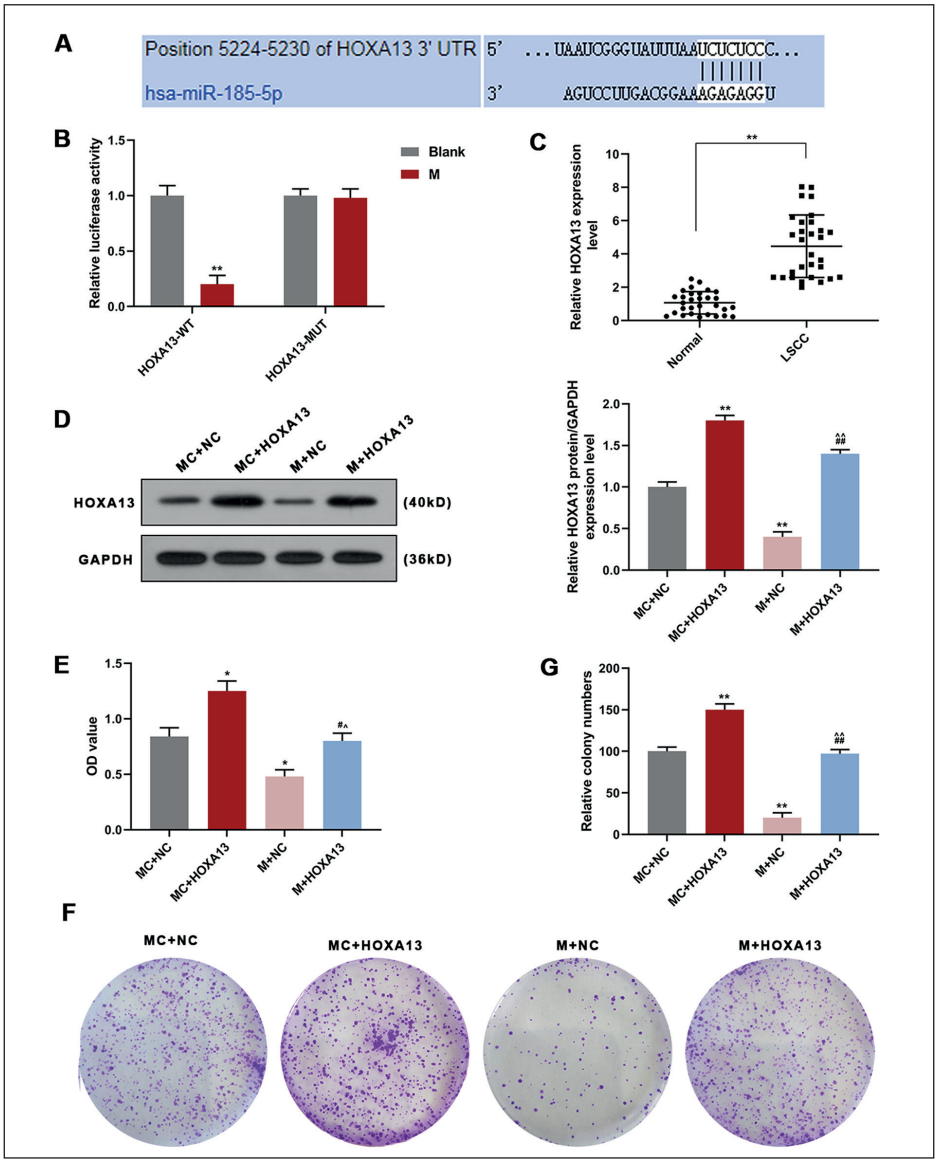

Figure 5. HOXA13 was a novel target of miR-185-5p. **A**, Targetscan and Dual-Luciferase activity (**B**) were performed to identify miR-185-5p binding sites in HOXA13. **C**, The mRNA expression level of HOXA13 was determined by RT-qPCR in LSCC tissues. **D**, The protein expression level of HOXA13 was determined by Western blot in Tu 177 cells under miR-185-5p mimic and HOXA13 overexpression conditions. **E**, CCK-8 assay analysis of Tu 177 cells transfected with miR-185-5p mimic or miR-NC in the presence or absence of HOXA13 overexpression. **F**, Cloning formation analysis of Tu 177 cells transfected with miR-185-5p mimic or miR-NC in the presence or lack HOXA13 overexpression. Magnification: ×1. **G**, The numbers of cloning formation were calculated. *p<0.05, **p<0.01 *vs.* Blank/ Normal/ MC+NC, $^{\#}p$<0.05, $^{\#\#}p$<0.01 *vs.* MC+ HOXA13, $^{\wedge}p$<0.05, $^{\wedge\wedge}p$<0.01 *vs.* M+NC. MC: mimic control. NC: negative control. M: miR-185-5p mimic. HOXA13: homeobox A13.

samples (Figure 5C). Furthermore, the effect of miR-185-5p on HOXA13 expression was determined in Tu 177 after transfection with miR-185-5p mimic or miR-NC with HOXA13- pcDNA3.1, and we found that overexpressed miR-185-5p significantly down-regulated the protein expression level of HOXA13 but was compensated by HOXA13 overexpression (Figure 5D). In addition, Tu 177 cell viability (Figure 5E) and colony formation (Figure 5F, 5G) were significantly increased after transfection with miR-185-5p + HOXA13 compared with miR-185-5p.

The Up-Regulation of HOXA13 Reversed the Antitumor Effect of MiR-185-5p on Tu 177 Cells

Rescue experiments were conducted to investigate whether the tumor-suppressive function

of miR-185-5p in Tu 177 cells was directly regulated by suppressing the HOXA13 level. As expected, overexpression of miR-185-5p promoted cell apoptosis (Figure 6A), arrested the cell cycle at G1-S transition (Figure 6B) and blocked cell migration (Figure 6C) and invasion (Figure 6D) of Tu 177 cells, and those phenomena were reversed by up-regulating HOXA13.

Discussion

The expression profiling and functional assays of lncRNAs conducted on various cancers have provided mounting evidence supporting the vital roles of lncRNAs in tumor malignancy[20]. Sun et al reports that LSCC progression is often accompanied with lncRNAs changes,

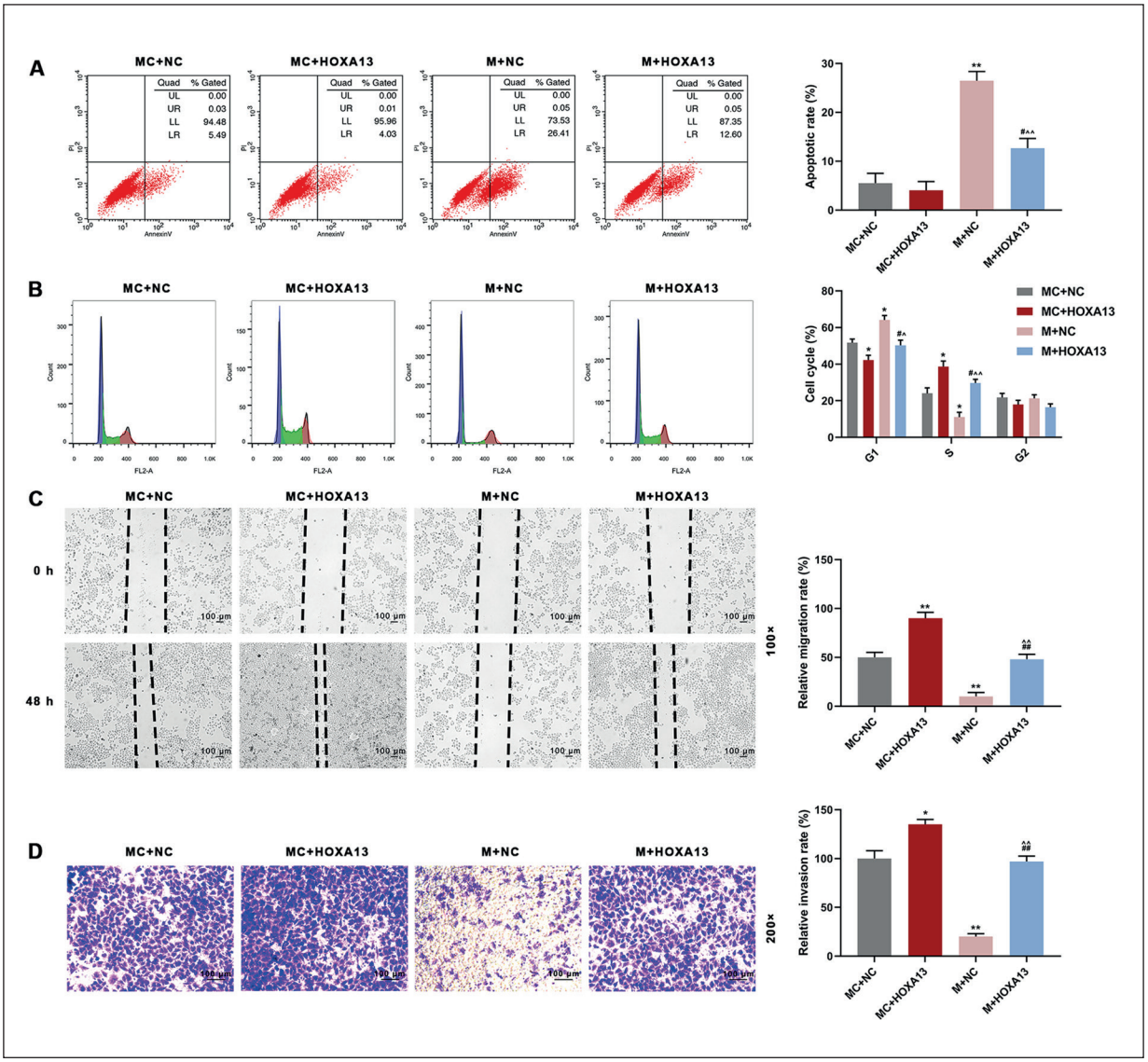

Figure 6. Up-regulation of HOXA13 reversed the tumor-suppressing effects of miR-185-5p on Tu 177 cells. **A**, Flow cytometry analysis of cell apoptosis in Tu 177 cells transfected with miR-185-5p mimics or miR-NC in the presence or absence of HOXA13 overexpression. **B**, Flow cytometry analysis of cell cycle in Tu 177 cells transfected with miR-185-5p mimics or miR-NC in the presence or absence of HOXA13 overexpression. **C**, Wound healing analysis of cell migration in Tu 177 cells transfected with miR-185-5p mimics or miR-NC in the presence or absence of HOXA13 overexpression. Magnification: ×100. **D**, Transwell analysis of cell invasion in Tu 177 cells transfected with miR-185-5p mimics or miR-NC in the presence or absence of HOXA13 overexpression. Magnification: ×200. *$p<0.05$, **$p<0.01$ *vs.* MC+NC, $^{\#}p<0.05$, $^{\#\#}p<0.01$ *vs.* MC+ HOXA13, $^{\wedge}p<0.05$, $^{\wedge\wedge}p<0.01$ *vs.* M+NC. MC: mimic control. NC: negative control.

and that lncRNA UCA1 is a key factor in the pathogenesis of LSCC[16]. However, the potential mechanism of lncRNA UCA1 in LSCC is not fully understood.

In clinical specimens of ovarian cancer, higher expression of UCA1 has been observed in the cancer tissues in comparison to paracancer tissues[21], moreover, elevated lncRNA UCA1 expression promotes cell invasion and migration through activating Wnt/β-catenin in LSCC[16]. Cisplatin resistance could occur when lncRNA UCA1 is overexpressed in ovarian cancer[22]. In ovarian cancer, PTX resistance has been observed to be induced by high UCA1 expression *via* the miR-129/ABCB1 axis[23]. Knocking down lncRNA-UCA1 inhibits the proliferation and migration of melanoma cells[24]. The present study observed that the expression of lncRNA UCA1 was significantly up-regulated in 30 LSCC tissues and 5 LSCC cell lines, and that inhibition of UCA1 expression markedly reduced LSCC cell migration and proliferation, moreover, these findings were consistent with a previous report[16].

MicroRNAs (miRNAs) participate in various progresses in cell through modulating protein-coding genes. For instance, Fan et al[25] demonstrated that miR-185-5p reduces the gene expression of HBV of hepatoma carcinoma cells by inhibiting ELK1. In addition, it was also reported that miR-506 inhibited the growth of LSCC by the inhibition of YAP1[26]. MiR-101 inhibited autophagy and proliferation and promoted apoptosis *via* targeting the enhancer of zeste homolog 2 in LSCC[27]. Growing evidence showed the existence of a novel and extensive interaction network involving competing endogenous RNAs (ceRNAs), and in the network, lncRNAs competitively sponge miRNAs to reversely modulate these signaling pathways indirectly[28,29]. It has been revealed that lncRNAs play crucial roles in cancers through sponging miRNAs[30,31]. LncRNA UCA1 can act as a competing endogenous lncRNA to regulate cell mobility through sponging miR-185-5p in melanoma[32]. LncRNA UCA1 also regulates cell growth and apoptosis in multiple myeloma through targeting miR-331-3p/IL6R axis[33]. The present study demonstrated that UCA1 directly targeted miR-185-5p, and that miR-28-5p expression was lower in LSCC tissues but could be increased by inhibiting UCA1. Further functional investigation results suggested that the oncogenic role of UCA1 in LSCC cells was realized through the regulation of miR-185-5p.

As miRNAs realize their functions through regulating the expressions of their target genes[34,35], the potential target gene for miR-185-5p and HOXA13 was predicted by bioinformatics analysis. Shi et al[32] reported that HOXA13 increases cisplatin-resistance and promotes EMT in esophageal squamous cell carcinoma cells; however, the targeting relationship between miR-185-5p and HOXA13 remains to be confirmed. The present study discovered that HOXA13 was a direct target gene for miR-185-5p in Tu 177 cells, moreover, HOXA13 had a higher expression in LSCC tissues, but its expression could be inhibited by miR-185-5p mimic. Furthermore, we also observed that overexpressed HOXA13 significantly reduced the suppressive effects of up-regulated miR-185-5p on migration and proliferation of Tu 177 cells, suggesting that UCA1 regulates the proliferation and migration of Tu 177 cells *via* miR-185-5p/ HOXA13 axis.

Conclusions

To the best of our knowledge, the present study was the first to found that the UCA1/miR-185-5p/HOXA13 axis played a key role in the regulation of proliferation and migration of LSCC cells, and such a finding expanded the understanding of the molecular mechanism underlying LSCC progression. However, the present study did not explore the function of UCA1/miR-185-5p/HOXA13 axis in other LSCC cell lines and lacked animal experiments to support present findings. Thus, future studies should be performed to further confirm the function of this signaling pathway in multiple LSCC cell lines as well as in animals.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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