Long non-coding RNA UCA1 enhances cervical cancer cell proliferation and invasion by regulating microRNA-299-3p expression

MING AN, XUEFENG XING and TONGHUA CHEN

Department of Obstetrics and Gynecology, Sanya People's Hospital, Sanya, Hainan 572000, P.R. China

Received April 27, 2021; Accepted August 16, 2021

DOI: 10.3892/ol.2021.13033

Abstract. The long non-coding RNA, urothelial cancer-associated 1 (UCA1) is an important regulator in several tumors. However, to the best of our knowledge, the clinical roles of UCA1 in cervical cancer remain unclear. Thus, the present study aimed to investigate the function and mechanism of UCA1 in cervical cancer. Reverse transcription-quantitative PCR analysis was performed to detect UCA1 and microRNA (miR)-299-3p expression in cervical cancer tissues and cell lines. The Cell Counting Kit-8 and Transwell assays were performed to assess cell proliferation and invasion, respectively. Furthermore, the dual-luciferase reporter assay was performed to confirm the association between UCA1 and miR-299-3p. Rescue experiments were performed to determine the mechanism of the UCA1/miR-299-3p axis. The results demonstrated that UCA1 expression was upregulated in cervical cancer tissues and cell lines. Furthermore, overexpression of UCA1 enhanced the proliferation and invasion of cervical cancer cells, the effects of which were reversed following UCA1 knockdown. Notably, UCA1 interacted with miR-299-3p and negatively regulated miR-299-3p expression. In addition, miR-299-3p expression was downregulated in cervical cancer tissues and cell lines. Overexpression of miR-299-3p suppressed the proliferation and invasion of cervical cancer cells, reversing the effects of UCA1 knockdown on cervical cancer cell proliferation. Taken together, the results of the present study suggest that UCA1 promotes cell proliferation and invasion by regulating miR-299-3p expression in cervical cancer.

Introduction

Cervical cancer is one of the most common types of cancer worldwide and a leading cause of cancer-associated mortality in women (1,2). In 2012, the incidence of cervical cancer was 527,600 cases and nearly 265,700 mortalities worldwide (3). According to its heterogeneity, cervical cancer is histologically divided into three subtypes, adenosquamous carcinoma, squamous cell carcinoma and adenocarcinoma (4). Currently, the main treatment strategies for patients with cervical cancer include pelvic lymph node dissection, hysterectomy and radiotherapy combined with chemotherapy (5). Although a certain degree of progress has been made in the early diagnosis, surgical treatment and application of the human papilloma-virus (HPV)16, 18, 31, 33, 45, 52 and 58 vaccine for cervical cancer, the survival rate of patients with advanced cervical cancer remains low (6,7). The 5-year survival rate for cervical cancer in China from 2012-2015 was only 59.8% (8). Thus, it is important to identify and develop biomarkers for cervical cancer and determine its disease mechanism.

Non-coding RNAs (ncRNAs) are distinctive RNA molecules that are not translated into proteins (9). ncRNAs are divided into two types, including housekeeping and regulatory ncRNAs (10). The regulatory ncRNAs include microRNAs (miRNAs/miRs), circular RNAs (circRNAs) and long ncRNAs (lncRNAs) (11). miRNAs are short ncRNAs, ~22 nucleotides in length that regulate gene expression by binding to mRNAs (12‑14) or by adjusting their stability through sponging circRNAs (15,16). lncRNAs are a group of ncRNAs with a length of >200 nucleotides (17). lncRNAs function as sponges that target miRNAs and subsequently regulate gene expression (18,19). Several studies have reported that miRNAs and lncRNAs play important roles in certain types of cancer (20-22).

Increasing evidence suggest that lncRNAs are involved in the occurrence of multiple tumors (23-25). Several lncRNAs, such as growth arrest-specific 5 (26), nuclear paraspeckle assembly transcript 1 (27), metastasis-associated lung adenocarcinoma transcript 1 (28) and small nucleolar host gene 12 (29), have been identified as important regulatory factors in cervical cancer. The lncRNA, urothelial carcinoma-associated 1 (UCA1) is located on human 19p13.12 (30) and has been reported to play an important role in different types of cancer, including colorectal (31), prostate (32), gastric (33) and bladder cancer (34). Previous studies have reported that UCA1 regulates the proliferation, migration and invasion of cervical cancer cells (35-37). However, the mechanism of UCA1 in the progression of cervical cancer remains unclear.

miRNAs have been reported to act as facilitators or suppressors in different types of cancer (38-40). According to
previous studies, miR-299-3p participates in multiple tumor processes. In hepatocellular carcinoma, miR-299-3p acts as a tumor suppressor by regulating Sirtuin 5 (41). It also plays a tumor suppressive role by targeting SHOC2 leucine-rich repeat scaffold protein in thyroid cancer (42). miR-299-3p inhibits cell proliferation and invasion by targeting vascular endothelial growth factor A in human colon carcinoma (43). In cervical cancer, miR-299-3p suppresses cell proliferation and invasion by binding to transcription factor 4 (TCF4) (44). However, the function of miR-299-3p, and the association between UCA1 and miR-299-3p in cervical cancer remain unknown.

The present study aimed to investigate the role and potential mechanism of UCA1 in cervical cancer. The association between UCA1 and miR-299-3p in the occurrence of cervical cancer was also investigated.

Materials and methods

Clinical specimens. Cervical cancer and paired adjacent normal tissues (n=30) were surgically collected from the Department of Obstetrics and Gynecology, Sanya People’s Hospital (Sanya, China) from May 2018 to May 2020. The average age of all patients was 49 years (age range, 36-69 years). The clinical information of patients with cervical cancer is presented in Table SI. No patients received adjuvant treatment prior to surgery. In addition, the patients with other tumors or a history of treatments for other gynecological tumors were excluded from the present study. All tissue samples were immediately preserved in liquid nitrogen and stored at -80°C until RNA extraction. The present study was approved by the Ethics Committee of the Sanya People’s Hospital (approval no. 2017.106; Sanya, China) and written informed consent was provided by all patients prior to the study start.

Cell lines. The Ect1/E6E7 normal human cervical epithelial cell line was purchased from the American Type Culture Collection, while the SiHa, HeLa, CaSki and ME180 human cervical cancer cell lines were purchased from the The Cell Bank of Type Culture Collection of The Chinese Academy of Sciences. All cells were maintained in DMEM (cat. no. SH30022.01B; Cytiva) supplemented with 10% fetal bovine serum (cat. no. 16140071; Thermo Fisher Scientific, Inc.), 100 U/ml penicillin (cat. no. 15140148; Thermo Fisher Scientific, Inc.) and 100 µg/ml streptomycin (cat. no. 15140122; Thermo Fisher Scientific, Inc.) at 37°C with 5% CO₂ and 100 µg/ml streptomycin (cat. no. 16140071; Thermo Fisher Scientific, Inc.), at 37˚C with 5% CO₂ and 100 µg/ml streptomycin (cat. no. 16140071; Thermo Fisher Scientific, Inc.), at 37°C with 5% CO₂ and 100% humidity.

Cell transfection. The overexpression vector pcDNA 3.1 was purchased from Thermo Fisher Scientific, Inc. All sequences were synthesized by Shanghai GenePharma Co., Ltd. The primers for the amplification of the full-length UCA1 were as follows: Forward, 5’-CCGGAATTCAGGACGCTGACTCCTTTAGGAGAATTCTCTC-3’ and reverse, 5’-GGACAACTACACCTGGAGCTGAGGAGGACG-3’. The overexpression vector pcDNA-UCA1 was produced by cloning the full-length UCA1 into pcDNA3.1. For the knockdown of UCA1, the small interfering (si)RNAs targeting UCA1 (si-UCA1) were designed.

Invasion assay. Cell invasion was assessed via the Transwell assay. The Transwell chambers were pre-coated with 100 µl Matrigel at 37°C for 1 h. Transfected cells were collected (1,000 x g for 5 min at 4°C), then the transfected cells (2x10⁵ cells/100 µl) were cultured in 96-well plates. Following incubation for 0, 24, 48 and 72 h, 10 µl CCK-8 solution was added into each well and incubated for an additional 2 h at 37°C. Cell proliferation was subsequently analyzed at a wavelength of 450 nm. The experiments were performed in triplicate.

Invasion assay. Cell invasion was assessed via the Transwell assay. The Transwell chambers were pre-coated with 100 µl Matrigel at 37°C for 1 h. Transfected cells were collected (1,000 x g for 5 min at 4°C), then the cells (1x10⁵ cells/well) were seeded into the upper chambers of Matrigel-coated Transwell plates (8-µm pore size; cat. no. 354483; Corning, Inc.) in serum-free DMEM. The DMEM (cat. no. SH30022.01B; Cytiva) medium with 10% fetal bovine serum (cat.no. 16140071; Thermo Fisher Scientific, Inc.) was plated in the lower
chambers. Following incubation for 24 h at 37˚C, cells in the lower chambers were fixed with 5% glutaraldehyde and stained with 0.1% crystal violet dye (cat. no. 548-62-9; MilliporeSigma) for 30 min at room temperature, respectively. Stained cells were counted in five randomly selected fields using a BX63 microscope (magnification, x100, Olympus Corporation).

Dual-luciferase reporter assay. LncBase Predicted v.2 software (46) was used to predict the potential binding sites between UCA1 and miR-299-3p.

The dual-luciferase reporter plasmids were constructed by inserting a UCA1 wild-type (UCA1-WT) or UCA1 mutant (UCA1-MUT) sequence into the psiCHECK-2 luciferase vector (Promega Corporation). SiHa cells were co-transfected with UCA1-WT or -MUT reporter and miR-299-3p or NC mimics, using Lipofectamine® 3000 transfection reagent (cat. no. L3000015; Thermo Fisher Scientific, Inc.). Transfected cells were subsequently cultured in 24-well plates. Following incubation at 37˚C for 24 h, luciferase activities were detected using a Dual-Luciferase Reporter assay system (cat. no. E1910; Promega Corporation). Firefly luciferase activity was normalized to Renilla luciferase activity (cat. no. E2810; Promega Corporation).

Statistical analysis. Statistical analysis was performed using GraphPad Prism 8 (GraphPad Software, Inc.). All experiments were performed in triplicate and data are presented as the mean ± SD. Paired Student's t-test was used to compare differences between tumor tissues and adjacent normal tissues, while unpaired Student's t-test was used to compare differences between unpaired groups. One-way ANOVA followed by Tukey's or Dunnett's post hoc tests were used to compare differences between multiple groups. Pearson's correlation coefficient analysis was performed to assess the correlation between UCA1 and miR-299-3p expression. P<0.05 was considered to indicate a statistically significant difference.

Results

UCA1 expression is downregulated in cervical cancer tissues and cell lines. RT-qPCR analysis was performed to detect UCA1 expression in cervical cancer tissues and adjacent normal tissues (n=30). The results demonstrated that UCA1 expression was significantly upregulated in cervical cancer tissues compared with adjacent normal tissues (P<0.01; Fig. 1A). RT-qPCR analysis was also performed to detect UCA1 expression in the SiHa, HeLa, CaSki and ME180 cervical cancer cell lines and Ect1/E6E7 human normal cervical epithelial cell line. The results demonstrated that UCA1 expression was significantly upregulated in the cervical cancer cell lines compared with the normal cell line (all P<0.01; Fig. 1B). Taken together, these results suggest that UCA1 plays a key role in the development of cervical cancer.

miR-299-3p can bind with UCA1, and its expression is negatively regulated by UCA1. To clarify the molecular mechanism of UCA1 in cervical cancer, LncBase software was used to predict the binding sites between miRNAs and UCA1. The results revealed a potential binding site between miR-299-3p and UCA1 (Fig. 3A). The dual-luciferase reporter assay was subsequently performed to confirm the association between UCA1 and miR-299-3p in SiHa cells. The results demonstrated
Figure 2. Overexpression of UCA1 promotes cervical cancer cell proliferation and invasion. (A) UCA1 expression was detected in SiHa cells transfected with pcDNA, UCA1, si-NC or si-UCA1. (B and C) The Cell Counting Kit-8 assay was performed to assess cell proliferation following transfection. (D) The Transwell assay was performed to assess cell invasion following transfection. *P<0.05; **P<0.01. UCA1, urothelial cancer-associated 1; si, small interfering; NC, negative control; OD, optical density.

Figure 3. miR-299-3p directly targets the 3′-untranslated region of UCA1 and is downregulated in cervical cancer tissues and cell lines. (A) Binding sites between UCA1 and miR-299-3p were predicted using LncBase software. (B) The association between UCA1 and miR-299-3p was confirmed via the dual-luciferase reporter assay. RT-qPCR analysis was performed to detect miR-299-3p expression in (C) cervical cancer tissues and normal tissues, and (D) cervical cancer cell lines and Ect1/E6E7. (E) UCA1 expression was negatively correlated with miR-299-3p expression in cervical cancer. (F) RT-qPCR analysis was performed to detect miR-299-3p expression in SiHa cells transfected with pcDNA, UCA1, si-NC or si-UCA1. *P<0.05; **P<0.01 vs. Ect1/E6E7 cells. miR, microRNA; UCA1, urothelial cancer-associated 1; RT-qPCR, reverse transcription-quantitative PCR; si, small interfering; NC, negative control; WT, wild-type; MUT, mutant.
that transfection with miR-299-3p mimics significantly decreased (P<0.01) the luciferase activity of UCA1-WT, but not UCA1-MUT in SiHa cells (Fig. 3B). RT-qPCR analysis was performed to detect miR-299-3p expression in cervical cancer tissues and cell lines. The results demonstrated that miR-299-3p expression was significantly downregulated in cervical cancer tissues (P<0.01; Fig. 3C) and cell lines (all P<0.01; Fig. 3D). Pearson’s correlation analysis was performed to determine the correlation between UCA1 and miR-299a-3p expression in cervical cancer tissues. The results demonstrated that miR-299-3p expression was negatively correlated with UCA1 expression (Fig. 3E). SiHa cells were transfected with pcDNA, UCA1, si-NC or si-UCA1, and miR-299-3p expression was detected. The results demonstrated that overexpression of UCA1 significantly decreased miR-299-3p expression (P<0.01; Fig. 3F). Taken together, these results suggest that UCA1 binds to miR-299-3p and negatively regulates its expression in cervical cancer.

miR-299-3p suppresses cervical cancer cell proliferation and invasion. To determine the biological function of miR-299-3p in the proliferation and invasion of cervical cancer cells, SiHa cells were transfected with miR-299-3p mimics, NC mimics, miR-299-3p inhibitor or NC inhibitor. RT-qPCR analysis was performed to detect miR-299-3p expression. The results demonstrated that miR-299-3p expression significantly increased (P<0.01) following transfection with miR-299-3p mimics, the effects of which were reversed following transfection with miR-299-3p inhibitor (P<0.01), compared with the NC groups (Fig. 4A).

The proliferation and invasion of cervical cancer cells were assessed via the CCK-8 and Transwell assays, respectively. The results confirmed that overexpression of miR-299-3p significantly decreased cervical cancer cell proliferation (P<0.01; Fig. 4B), while miR-299-3p knockdown significantly increased cervical cancer cell proliferation (P<0.01; Fig. 4C). The Transwell assay demonstrated that transfection with miR-299-3p mimics significantly decreased cervical cancer cell invasion (P<0.01; Fig. 4D), while transfection with miR-299-3p inhibitor significantly promoted cervical cancer cell invasion (P<0.05; Fig. 4D). Collectively, these results suggest that miR-299-3p acts as a tumor suppressor in cervical cancer.
Overexpression of UCA1 promotes cell proliferation and invasion by suppressing miR-299-3p expression. To further clarify the effect of the UCA1/miR-299-3p axis in cervical cancer tumorigenesis, rescue experiments were performed on SiHa cells. miR-299-3p mimics + UCA1 or miR-299-3p inhibitor + si-UCA1 were transfected into SiHa cells. RT-qPCR analysis demonstrated that overexpression of miR-299-3p partially reversed the inhibitory effects of overexpressing UCA1 on SiHa cells (P<0.05; Fig. 5A), while transfection with miR-299-3p inhibitor reversed the promoting effects of si-UCA1 on SiHa cells (P<0.01; Fig. 5B). Furthermore, transfection with miR-299-3p mimics inhibited the proliferative ability of UCA1-transfected SiHa cells (P<0.01; Fig. 5C), and si-UCA1-induced suppression in cell proliferation was recovered following transfection with miR-299-3p inhibitor (P<0.01; Fig. 5D). Furthermore, the invasive ability of UCA1-transfected SiHa cells decreased following transfection with miR-299-3p mimics (P<0.01; Fig. 5E), the effects of which were reversed following transfection with miR-299-3p inhibitor (P<0.05; Fig. 5F). Collectively, these results suggest that UCA1 promotes cervical cancer progression by regulating miR-299-3p expression.
Discussion

Cervical cancer is a common gynecological malignancy and the second most serious threat to women's health worldwide (47). Most cases of cervical cancer are caused by HPV infection (48). Although surgical treatment, chemotherapy and radiotherapy have a therapeutic effect on cervical cancer, this effect is insufficient (49). Recent studies have reported that lncRNAs play important regulatory roles in cervical cancer (50-52). Although several studies have clarified the role of UCA1 in different tumors (31-33), to the best of our knowledge, the molecular mechanism of UCA1 in cervical cancer remains unclear. In the present study, the role of UCA1 and its molecular mechanism in cervical cancer were further explored.

The lncRNA, UCA1 acts as an oncogene in different types of cancer, including pancreatic (53), gastric (54) and colorectal (26) cancers. Previous studies have demonstrated that UCA1 is upregulated in cervical cancer, which promotes cell proliferation, invasion and migration by regulating miR-145 or miR-204 expression (35,36). Consistent with these findings, the results of the present study demonstrated that UCA1 was upregulated in cervical cancer tissues and cell lines. Furthermore, overexpression of UCA1 promoted cervical cancer cell proliferation and invasion, the effects of which were reversed following UCA1 knockdown. Taken together, these findings suggest that UCA1 may play a key role in the proliferation and invasion of cervical cancer cells.

A recent study reported that miRNAs play important roles in cervical cancer. For example, miR-216a-3p inhibits cervical cancer cell proliferation and invasion by regulating actin-like 6A (55). Furthermore, miR-195-5p suppresses migration and invasion in cervical cancer by targeting ADP ribosylation factor-like GTPase 2 (ARL2) (56). Overexpression of miR-139-5p promotes cell proliferation and migration in cervical cancer by targeting TCF4 (57). miR-299-3p has been reported to act as a tumor suppressor in hepatocellular carcinoma, thyroid cancer and colon carcinoma (41-43). In addition, miR-299-3p inhibits cell proliferation and invasion by regulating TCF4 in cervical cancer (44). In the present study, miR-299-3p expression was significantly downregulated in cervical cancer, and its overexpression inhibited cervical cancer cell proliferation and invasion. Collectively, the results of the present study suggest that miR-299-3p plays an inhibitory role in cervical cancer.

Previous studies have reported that lncRNAs bind to specific miRNAs to regulate cancer progression (58,59). LncRNA UCA1 has been reported to regulate the progression of several tumors by targeting specific miRNAs. For example, UCA1 promotes cell proliferation in gastric cancer by regulating the miR-495/phosphatase of regenerating liver 3 axis (54) and mitochondrial function in bladder cancer via the miR-195/ARL2 signaling pathway (34). To further clarify the potential molecular mechanism of UCA1 in cervical cancer, the target genes of UCA1 were predicted using LncBase. The results revealed that UCA1 can bind to miR-299-3p. In addition, miR-299-3p expression was negatively correlated with UCA1 expression in cervical cancer tissues. Notably, overexpression of UCA1 decreased miR-299-3p expression in SiHa cervical cancer cells. Furthermore, transfection with miR-299-3p mimics inhibited cell proliferation and invasion, and transfection with miR-299-3p inhibitors reversed the effects of UCA1 knockdown on SiHa cells. Taken together, these results suggest that UCA1 acts as an oncogene in cervical cancer and regulates cell proliferation and invasion by inhibiting miR-299-3p expression.

The results of the present study provide novel insight into the treatment of cervical cancer. Notably, a novel target miRNA of UCA1 in cervical cancer, which has not been previously reported, was identified in the present study. In addition, the UCA1/miR-299-3p axis was revealed to regulate cell proliferation and invasion in cervical cancer, providing a novel target site for the treatment of cervical cancer. However, the present study is not without limitations. The sample size assessed was too small; thus, further studies with a larger sample size are required to investigate the mechanism of the regulatory effect of the UCA1/miR-299-3p axis on cell proliferation and invasion in cervical cancer. In addition, the present only performed in vitro experiments, and needs to be further investigated in vivo.

In conclusion, the results of the present study demonstrated that UCA1 promoted cell proliferation and invasion by targeting miR-299-3p expression in cervical cancer. These results suggest that the UCA1/miR-299-3p axis may present a potential therapeutic target for cervical cancer.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Authors' contributions

MA and XX conceived and designed the present study, analyzed the data and drafted the initial manuscript. TC only performed in vitro experiments, and needs to be further investigated in vivo.

In conclusion, the results of the present study demonstrated that UCA1 promoted cell proliferation and invasion by targeting miR-299-3p expression in cervical cancer. These results suggest that the UCA1/miR-299-3p axis may present a potential therapeutic target for cervical cancer.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of Sanya People's Hospital (approval no. 2017.106; Sanya, China) and written informed consent was provided by all patients prior to the study start.

Patient consent for publication

Not applicable.
Competing interests

The authors declare that they have no competing interests.

References


This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) License.