Abstract. A majority of cervical cancers are squamous cell carcinomas, arising from the squamous (flattened) epithelial cells that line the cervix. Long noncoding RNAs (lncRNAs) are a unique class of messenger RNA-like transcripts of at least 200 nucleotides in length with no significant protein-coding capacity. Aberrant lncRNA expression is emerging as a major component of the cancer transcriptome. In the present study, lncRNA microarrays were conducted to investigate the differentially expression lncRNAs in cervical cancer (CC) tissues compared with peritumoral tissues. Then, the most significantly upregulated lncRNA, which was lncRNA-AK001903 was selected to conduct further experiments. Real-time Quantitative polymerase chain reaction was conducted to investigate lncRNA-AK001903 expression in CC tissues and Hela, Siha, Ca Ski, C33a, H8 (HPV-immortalized cervical epithelial cell line) cell lines, and in situ hybridization histochemistry (ISHH) was performed to detect lncRNA-AK001903 expression level in different CC stages. The effect of lncRNA-AK001903 on cell proliferation, invasion and migration was assessed after knockdown of lncRNA-AK001903. The findings of the study confirmed that lncRNA-AK001903 was upregulated in CC cells and tissues compared with normal cell line H8 and peritumoral tissues. ISHH demonstrated that the expression level of lncRNA-AK001903 was connected with International Federation of Gynecology and Obstetrics (2018) stage of CC. Knockdown of lncRNA-AK001903 inhibited cell proliferation, invasion and migration in Ca Ski cells. In conclusion, IncRNA-AK001903 was demonstrated to be an oncogenic lncRNA that promotes tumor progression and may be an effective target for CC treatment in the near future.

Introduction

Cervical cancer is the fourth most frequently diagnosed cancer and the fourth leading cause of cancer death in women, with an estimated 570,000 cases and 311,000 deaths having occurred worldwide in 2018 (1). Persistent human papilloma virus (HPV) infection is a direct cause for CC (2). With the development of DNA examination and cytological screening for high-risk HPV and the promotion of the cervical cancer vaccine, the morbidity rate of cervical cancer has been declining (3). However, the vaccines only cover some of the cancer-causing (‘high-risk’) types of HPV, such as HPV-16 and HPV-18 (4). Women should do regular Pap smear screening even after vaccination (5). Inspite of advances in the treatment of CC, the 5-year survival rate remains <50% in China (6). Hence, it is particularly necessary to find a new and effective treatment strategy targeting CC.

Long non-coding RNAs (IncRNAs) are a group of non-coding RNAs with >200 nucleotides and are involved in various biological activities including cell proliferation, migration, invasion, apoptosis and inflammatory responses (7,8). LncRNA is a kind of non-coding RNA which modulates gene expression through epigenetic control, transcriptional regulation, and post-transcriptional regulation (9). Multiple reports have verified that abnormal expression of IncRNAs could affect the expression or function of oncogenes, tumor suppressors, metabolic enzyme genes as well as transcription factors and signaling pathways (10-14). For example, enforced expression of HOX transcript antisense RNA (HOTAIR) increased invasiveness and metastasis in breast cancer (11). Maternally expressed gene 3 was found as a tumor suppressor associated with the pathogenesis and progression in human meningiomas (12). LncRNA SNHG15 promotes colon cancer cell invasion and metastasis through blocking degradation of transcription factor Slug (13). LncRNA PVT1 facilitates tumorigenesis and progression via regulation of miR-128-3p/gremlin 1 axis and bone morphogenetic protein...
(BMP) signaling pathway in glioma (14). A growing body of evidence has indicated the importance of lncRNAs for the progression of CC. At present, the mechanisms of metastasis associated lung adenocarcinoma transcript 1 (MALAT1), HOTAIR and CDKN2B antisense RNA 1 (ANRIL) lncRNAs in cervical cancer have been characterized in detail (15-17). Guo et al (15) demonstrated that HOTAIR was upregulated in cervical cancer tissues compared with peritumoral tissues. Suppression of HOTAIR reduced autophagy and reversal of epithelial-mesenchymal transition through the inhibition of the Wnt signaling pathway, which consequently enhanced radiotherapy sensitivity in CC. MALAT1 has been reported to be generally upregulated in CC and reduces the efficiency of radiation treatment on CC cell lines by interacting with miR-143 in vitro (16). LncRNA ANRIL was significantly increased both in CC tissues and cell lines and regulated CC cell proliferation, migration and invasion through the PI3K/Akt pathway (17).

The aforementioned studies demonstrated the impacts of lncRNAs on CC progression and their potential as treatment targets. lncRNA-AK001903 has been verified as an upregulated gene in active ulcerative colitis tissues by non-coding (NC) RNA microarray (18). However, the expression and function of lncRNA-AK001903 in CC and its correlation with prognosis of patients with CC remains to be elucidated. Hence, the present study aimed to investigate the roles of lncRNA-AK001903 in the progression of cervical cancer, which may provide a new target for the treatment of CC.

Materials and methods

Cell lines and tissue samples. Cervical cancer cell lines: Ca Ski, Hela, Siha, and C33a were purchased from Procell Life Science & Technology Co., Ltd. and the normal cell line H8 was purchased from Shanghai Yu Bo Biotech Co., Ltd. Ca Ski and H8 were cultured in RPMI-1640 supplemented with 10% heat-inactivated FBS (fetal bovine serum) (Gibco; Thermo Fisher Scientific Inc.). Hela, Siha and C33a were cultured in Minimum Essential Medium (Gibco; Thermo Fisher Scientific Inc.) supplemented with 10% heat-inactivated FBS. Penicillin (100 U/ml)/streptomycin (100 µg/ml) (GE Healthcare Life Sciences) was used in the culture medium and cells were grown in a humidified atmosphere containing 5% CO₂ at 37°C. Cells were passed to the next generation every three days. In the present study, tissues were collected from Department of Gynecological Oncology in Sun Yat-sen Memorial Hospital (Guangzhou, China) between June 2016 and May 2017. The inclusion criteria of the patients were as follows: i) Females diagnosed with CC using the International Federation of Gynecology and Obstetrics (FIGO, 2018) system (19); and ii) underwent biopsy or trachelectomy surgery. The exclusion criteria of the patients were as follows: i) Diagnosed with other tumors; ii) underwent radiotherapy or chemotherapy or any other treatment prior to surgery; and iii) CC recurrence. Peritumoral tissue samples were taken at least 1 cm distal to tumor margins. Tissue histology was independently evaluated by two pathologists. A total of 29 CC tissues (mean age, 37.6 years; age range, 21-77 years) and peritumoral tissues were collected and immediately placed and stored in liquid nitrogen. All of the 29 CC tissues and peritumoral tissues were used for reverse-transcription quantitative (RT-q) PCR analysis, 3 CC tissues and corresponding peritumoral tissues were used for microarray analysis and 26 CC tissues were used for ISHH. All patients underwent biopsy or trachelectomy surgery at the Sun Yat-sen Memorial Hospital (Guangzhou, China). All specimens were obtained with the approval of the Sun Yat-sen Memorial Hospital Review Board (approval no. 2015132) (Guangzhou, China) and a written informed consent was obtained from each patient.

RNA microarray. Human 12x135K Long Non-coding RNA Array was manufactured by NimbleGen Systems Inc. Each array represented all long transcripts, both protein-coding mRNAs and lncRNAs in the human genome. In total, >23,000 lncRNAs were collected from the authoritative data sources including NCBI RefSeq (GRCh37 (hg19); http://www.ncbi.nlm.nih.gov/RefSeq/), UCSC (GRCh37 (hg19); http://genome.ucsc.edu/cgi-bin/hgTables), RNAdb 2.0 (http://research.imb.uq.edu.au/rnadb/), lncRNAs from literatures (20,21) and UCRs (NCBI Build 35 (hg17); http://users. soe.ucsc.edu/~jill/ultra.html).

RNA labeling and array hybridization. In total 3 CC tissues and 3 corresponding peritumoral tissues were used to synthesize double-stranded complementary DNA (cDNA). Double-strand cDNA (ds-cDNA) was synthesized from 5 µg of total RNA using a SuperScript ds-cDNA synthesis kit (Invitrogen; Thermo Fisher Scientific Inc.) in the presence of 100 pmol oligo dT primers according to the manufacturer's instructions. Ds-cDNA was cleaned and labeled in accordance with the NimbleGen Gene Expression Analysis protocol (NimbleGen Systems, Inc.). Briefly, ds-cDNA was incubated with 4 µg RNase A at 37°C for 10 min and cleaned using phenol:chloroform:isoamyl alcohol (25:24:1), followed by ice-cold absolute ethanol precipitation. The purified cDNA was quantified using NanoDrop ND-1000 (Thermo Fisher Scientific Inc.). For Cy3 labeling of cDNA, the NimbleGen One-Color DNA labeling kit (NimbleGen Systems, Inc.) was used according to the manufacturer's instructions detailed in the Gene Expression Analysis protocol (NimbleGen Systems, Inc.). In brief, 1 µg ds-cDNA was incubated for 10 min at 98°C with 1 optical density (OD) of Cy3-9mer primer. Next, 100 pmol of deoxyxycytosine triphosphates and 100 U of the Klenow fragment (New England Biolabs Inc.) were added and the mixture incubated at 37°C for 2 h. The reaction was stopped by 0.5 mol/l EDTA (0.1 times the volume of the previous mixture), and the labeled ds-cDNA was purified by isopropanol/ethanol precipitation. Microarrays were hybridized at 42°C for 16-20 h with 4 µg of Cy3 labelled ds-cDNA in hybridization buffer/hybridization component A (NimbleGen Systems, Inc.) in a hybridization chamber (hybridization system; NimbleGen Systems, Inc.).

Microarray wash, scanning and data extraction. Washing was performed three times using the SeqCap EZ Hybridization and Wash kit (cat. no. 05634261001; NimbleGen Systems, Inc.). After being washed in an ozone-free environment, the slides were scanned using the Axon GenePix 4000B microarray scanner (Molecular Devices, LLC.). Raw data were extracted as pair files using NimbleScan software version 2.5 (Roche
Transfection. A total of 4 AK001903-specific small interfering (si)RNAs (si-AK001903-713, siRNA-AK001903-1306, siRNA-AK001903-1051 and siRNA-AK001903-1605) were used to knock down lncRNA-AK001903, and a non-targeting siRNA (si-NC) was used as a negative control (all Shanghai Gene Pharma Co. Ltd.). Sequences were listed in Table I. siRNA sequences used in the present study.

| siRNA set                  | Sense (5'-3')                          | Antisense (5'-3')                      |
|---------------------------|----------------------------------------|----------------------------------------|
| siRNA-AK001903-713        | GCCCACACAAUCUUAGAATT                    | UUCUAGAAGUUGGUUGGGCTT                   |
| siRNA-AK001903-1306       | CCACAGUCUCAGCAGUAATT                    | AUAUGCGUGACAGAUGUGTT                   |
| siRNA-AK001903-1051       | CGGACCCAUAUAAUCAUATT                    | AUAUGAUAUUAGGCUCCGTT                   |
| siRNA-AK001903-1605       | GCAGUUCUUUAACCAUGU TT                  | ACAUUGGUAAAGAAGACG UTT                |
| siRNA-NC                  | UUCUGCCAACGUGUCACG UTT                 | ACGUGACACGUUCCGAAATT                    |

NimbleGen, Inc.) and normalized through quantile normalization and the Robust Multichip Average algorithm included in the NimbleScan software. All gene level files were imported into GeneSpring GX software version. 11.5.1 (Agilent Technologies Inc.) or further analysis. Differentially expressed lncRNAs with statistical significance between two groups were identified through volcano plot filtering. Differentially expressed lncRNAs between two samples were identified through fold change filtering. P-value was calculated using the paired t-test. The threshold set for up- and downregulated genes was a fold change ≥2.0 and a P-value ≤0.05. Hierarchical clustering was performed using the GeneSpring GX software version 11.5.1 (Agilent Technologies Inc.).

RT-qPCR. Total RNA was extracted from tissues or cell lines (Ca Ski, Hela, Siha, C33a and H8) using TRIzol reagent (Invitrogen; Thermo Fisher Scientific Inc.) according to the manufacturer’s instructions. Total RNA (500 ng) was reverse transcribed into cDNA using a PrimeScript® RT reagent kit with gDNA eraser (Takara Bio Inc.). The protocol for RT was as follows: 37°C for 15 min, 40 cycles of denaturation at 95°C for 5 sec and annealing at 60°C for 30 sec, followed by 40 cycles of denaturation at 95°C for 5 sec and annealing at 60°C for 30 sec. β-actin was used as an endogenous reference. Fold-changes of the relative expression of target genes were calculated using 2^ΔΔCq method (22). All experiments were performed in duplicate and repeated twice. Primers for quantitative PCR were presented in Table II.

**ISHH.** Tissues were fixed with 4% paraformaldehyde (with 0.1% Diethyl pyrocarbonate) at room temperature for 24 h. Then they were paraffin-embedded and sliced into 20-µm thick slices and spread over PLL-coated glass slides. The parafin sections were deparaffinized and rehydrated and treated with 0.2 mol/l HCl at 37°C for 20 min, 3 µg/ml proteinase K (PCR grade; Roche Diagnostics) at 37°C for 7 min followed by post-fixation with 4% paraformaldehyde (with 0.1% diethyl pyrocarbonate) for 5 min at room temperature. After acetylation in a solution consisting of 1.5% triethanolamine, 0.25% acetic anhydride, and 0.25% HCl at 40°C for 4 h, the slides were washed by 5X SSC once for 15 min. Pre-hybridization was performed using the pre-hybridization solution (cat. no. AR0152; Boster Biological Technology Co. Ltd.) at 37°C for 4 h. Subsequently, a digoxigenin-labeled oligonucleotide probe (5'-CCACAC CCACCTTCCCACTGAGTAT-3'; Shanghai GeneBio Co., Ltd.) was diluted to 200 nM using hybridization buffer (cat. no. AR0062; Boster Biological Technology Co. Ltd.) and hybridized with tissues for 16 h at 55°C. Slides were washed twice at 55°C for 30 min with a solution consisting of 50% formamide, 2X SSC and 0.01% Tween 20, and then 10 µg/ml RNase A was added at 37°C for 1 h in buffer (0.5 M NaCl, 10 mM Tris (pH 8.0), 1 mM EDTA, and 0.01% Tween 20). Subsequently, the samples were washed in 2X SSC wash buffer with 0.01% Tween 20 at 55°C for 30 min, followed by washing in 0.2X SSC wash buffer with 0.01% Tween 20 at 55°C for 30 min. After additional washing in Tris-buffered saline (TBS, pH 7.6) and blocking in a buffer consisting of 10% blocking Reagent (cat. no. 11096176001; Roche Diagnostics), 0.1 M maleate, 0.15 M NaCl, and 0.01% Tween 20 in TBS, DIG-labeled probes were detected by biotinylated digoxin antibody (cat. no. AR0147; Boster Biological Technology Co. Ltd.) at 37°C for 1 h, streptavidin-biotin complex-peroxoisime (SABC-POD; cat. no. AR0148; Boster Biological Technology Co. Ltd.) at 37°C for 20 min and biotin-horseradish peroxidase (HRP) (cat. no. AR0149; Boster Biological Technology Co. Ltd.) at 37°C for 20 min. The samples were stained using DAB.
Table II. Reverse-transcription quantitative PCR primers.

| Primer Set | Sequence/Assay ID |
|------------|-------------------|
| AK001903.1 | Forward: 5'-AATCTGCCCACACCAATCTT-3'  
Reverse: 5'-CAGTGTGCTGAAATTCACTTG-3' |
| AI184890   | Forward: 5'-GTTCACCTCTGGTGCTCTGG-3'  
Reverse: 5'-TGGGGACATTTGCGGAAATTTT-3' |
| AK097842   | Forward: 5'-AGGGTCTATCATGCTGGTC-3'  
Reverse: 5'-CGTTCATGGTGCGCAAAG-3' |
| BG419628   | Forward: 5'-GCACTGTGACCTCCCTGATC-3'  
Reverse: 5'-TGGGGTCACCTTCGAAATGA-3' |
| ASLNC01516 | Forward: 5'-CGGACTGTTCCTCCCTCCAC-3'  
Reverse: 5'-GGGATGGCTGAAGTGAAC-3' |
| ASLNC16271 | Forward: 5'-AGCCCTCCTTGACCAAGA-3'  
Reverse: 5'-GGTAACGAGTGCCCCCTCAA-3' |
| ASLNC14492 | Forward: 5'-AGTGGGCCCTGACTCCCTCC-3'  
Reverse: 5'-TGTTTCTTCAGAGGCCCTC-3' |
| ASLNC03532 | Forward: 5'-CACCGTCAACCAAGAACCT-3'  
Reverse: 5'-GTGGTAAGTGCTGAGTGAAC-3' |
| BF675100   | Forward: 5'-TTGCTATTTTCTCCTGCCC-3'  
Reverse: 5'-TGACCGGATGTGGCATT-3' |
| ASLNC17636 | Forward: 5'-AAATGAGGCGAGGGACAGG-3'  
Reverse: 5'-CTACAGGAGTGCCAATGAA-3' |
| β-actin    | Forward: 5'-TGGGACCCAGCACAATGAA-3'  
Reverse: 5'-CTAAGTGTCATGTCGGCTGAAGC-3' |

(Cat. no. ZLI-9019; Origene Technologies Inc.) and images were captured using light microscopy (Nikon Corporation).

**CCK-8 assay.** Cell proliferation was assessed using the CCK-8 assay. Ca Ski were plated at 1x10^3 cells/well on 96-well plates with three wells for each group. Cell viability was measured over 5 days using a Cell Counting Kit-8 (CCK-8) (Dojindo Molecular Technologies Inc.). A total of 10 µl of CCK-8 (5 mg/ml) was added to each well. After incubating for 4 h at 37°C, the absorbance was determined at 450 nm.

**Transwell assays.** Cell migration and invasion were examined using Polycarbonate Membrane Transwell inserts (Costar; Corning Inc.). The upper compartment was pre-coated with RPMI-1640 medium without serum for migration assay and Matrigel for 2 h in 37°C (Corning Inc.) for invasion assay. After 48 h of transfection, 5x10^4 Ca Ski cells were placed into the upper compartment. RPMI-1640 medium with 20% FBS was used in the lower compartment. Cells were incubated at 37°C for 4 h for the migration assay and 48 h for the invasion assay. Then the compartment were fixed at room temperature with 4% paraformaldehyde for 20 min and stained with crystal violet (1 mg/ml) for 20 min at room temperature, and cells not crossing the membrane were cleaned. Images were captured using light microscopy (Nikon Corporation).

**Statistical analysis.** Statistical analyses were performed using GraphPad Prism 8.0 software (GraphPad Software, Inc.) and SPSS 20.0 software (IBM Corp.). All experiments were performed in triplicate. Comparisons between two groups were performed by paired sample t-tests. Three or more experimental groups were compared by one-way ANOVA followed by the post hoc Bonferroni test. The median value [tissues’ CT (cycle threshold) value- corresponding β-actin CT value=7.2] of lncRNA-AK001903 expression level was used to divide patients into high and low lncRNA-AK001903 expression level groups. χ² test was used to compare the association between different clinical features and gene expression level. All data are presented as mean ± standard deviation (SD). P<0.05 was considered to indicate a statistically significant difference.

**Results**

**LncRNAs expression profile in CC.** Hierarchical clustering demonstrated systematic variations in the expression of lncRNAs between CC tissues and corresponding peritumoral tissues (Fig. 1A). Volcano Plots were used to visualize the relationship between fold-change (magnitude of change) and statistical significance (which took both magnitude of change and variability into consideration) (Fig. 1B). According to the microarray expression profiling data, 453 distinctively dysregulated lncRNAs were detected in CC tissues compared to the controls (fold change, ≥2.0 and P<0.05) (Fig. 1A and B). The top 10 of 324 significantly upregulated and 129 significantly downregulated lncRNA transcripts are summarized in Table III. To validate the microarray analysis findings, 5 upregulated and 6 downregulated lncRNAs expression were detected.
LncRNA-AK001903 is upregulated in CC tissues and cells and related to FIGO stage in patients with CC. Among the 20 most significantly differentially expressed lncRNAs (DE lncRNAs) in the CC tissues vs. CP (peritumoral tissues), the most notably upregulated one was lncRNA-AK001903 (fold change, 15.547; Table III). To verify the roles of lncRNA-AK001903 in CC cells, expression of lncRNA-AK001903 in CC cell lines and the normal cell line H8 were determined by RT-qPCR. The results demonstrated that Ca Ski exhibited significantly higher expression level of lncRNA-AK001903 compared with C33a, Hela and Siha cells. Besides, all these cervical cancer cell lines expressed higher level of lncRNA-AK001903 compared with H8 cells (Fig. 2A). In addition, lncRNA-AK001903 expression was assessed in 29 CC tissues and their corresponding peritumoral tissues to confirm the expression change of lncRNA-AK001903 in CC tissues. The result revealed significantly higher levels of lncRNA-AK001903 gene expression compared with corresponding peritumoral tissues (Fig. 2B). Clinicopathologic features of the 29 patients with CC were shown in Table IV. However, according to the results of RT-qPCR, the high and low expression of AK001903 in tumor tissues appeared to be independent of the patient's age, tumor size, FIGO stage, lymph node metastasis, differentiation, and whether the tumor was confined to the cervix (Table IV). These results suggested the probable oncogenic role of LncRNA-AK001903 in cervical cancer tumorigenesis. In addition, an ISHH probe of LncRNA-AK001903 was designed and synthesized to examine the expression differences among CC tissues. The results demonstrated that LncRNA-AK001903 was mostly located in the nucleus of cells and that high LncRNA-AK001903 expression was associated with advanced FIGO stage (Fig. 2C).

LncRNA-AK001903 regulates the cell proliferation, migration, and invasion in Ca Ski cells. As LncRNA-AK001903 was highly expressed in CC cells and tissues compared with H8 cells and peritumoral tissues and related to the FIGO stage of CC, further experimentation was performed to investigate whether LncRNA-AK001903 may be a potential oncogene during the progression of CC. Four siRNAs targeting LncRNA-AK001903 at different sites (Table I) were designed and transfected into Ca Ski cells due to the highest expression of LncRNA-AK001903 in Ca Ski. The results demonstrated that of the 4 siRNAs used siRNA-LncRNA-AK001903-1605 produced the most effective interference of LncRNA-AK001903 expression and was then used for further experimentation (Fig. 3A). Next, the effects of knockdown of LncRNA-AK001903 on the proliferation, invasion and migration of Ca Ski cells was investigated. The CCK-8 assay demonstrated that LncRNA-AK001903 knockdown significantly inhibited the proliferation of LncRNA-AK001903 compared to the si-NC group (Fig. 3B). Transwell assay results demonstrated that the ability of migration and invasion were suppressed by knockdown of LncRNA-AK001903 (Fig. 3C).

Discussion

Cancer is a complex disease, involving various changes in gene expression (23,24). These gene expression changes cause cancer development, including metastasis (25), cell proliferation (26), invasion (27), and angiogenesis (28).
Table III. Top 10 significantly up- and downregulated lncRNAs in CC vs. CP.

| Seq name                  | P-value | Fold change | MOR | CC      | CP      | Associated      | Source          | Relationship                  |
|---------------------------|---------|-------------|-----|---------|---------|-----------------|-----------------|-------------------------------|
| AK001903                  | 0.003   | 15.547      | Up  | 1,570.119 | 105.897 | misc_lncRNA     | Intergenic       |                               |
| HMlincRNA1030             | 0.046   | 7.559       | Up  | 1,336.257 | 151.717 | lincRNA         | Intergenic       |                               |
| U88892                    | 0.012   | 6.743       | Up  | 657.814  | 97.620  | misc_lncRNA     | Intron sense‑overlapping |                               |
| HMlincRNA810              | 0.015   | 6.467       | Up  | 796.874  | 136.080 | lincRNA         | Exon sense‑overlapping |                               |
| chr20:1594825-1615675     | 0.033   | 6.414       | Up  | 1,263.708 | 221.246 | lincRNA         | Intron sense‑overlapping |                               |
| HMlincRNA1030             | 0.037   | 6.212       | Up  | 1,382.065 | 221.246 | lincRNA         | Intergenic       |                               |
| uc010ldj                  | 0.019   | 5.369       | Up  | 2,010.306 | 385.026 | HSP27           | UCSC_knowngene   | Exon sense‑overlapping       |
| uc010ldld                 | 0.023   | 5.025       | Up  | 397.059  | 81.809  | TUBB6           | UCSC_knowngene   | Exon sense‑overlapping       |
| NR_024204                 | 0.009   | 4.565       | Up  | 3,247.554 | 680.789 | NCRNA00152       | RefSeq_NR       | Intergenic                   |
| DQ786233                  | 0.043   | 4.469       | Up  | 603.588  | 113.588 | misc_lncRNA     | Intergenic       |                               |
| NR_024345                 | 0.004   | 4.153       | Down| 1,615.112 | 7,101.768 | C12orf27        | RefSeq_NR       | Intergenic                   |
| AK021467                  | 0.039   | 3.649       | Down| 267.197  | 1,035.963 | misc_lncRNA     | Interon sense‑overlapping |                               |
| AY927461                  | 0.021   | 3.490       | Down| 496.854  | 1,600.906 | misc_lncRNA     | Interon sense‑overlapping |                               |
| uc001esn                  | 0.012   | 3.480       | Down| 93.238   | 318.969 | AX746564        | UCSC_knowngene   | Intergenic                   |
| chr12:92933675-92975575   | 0.007   | 3.476       | Down| 109.867  | 383.899  | lincRNA         | Exon sense‑overlapping |                               |
| AK055280                  | 0.000   | 3.327       | Down| 273.747  | 910.918  | misc_lncRNA     | Interon sense‑overlapping |                               |
| uc003wog                  | 0.021   | 3.321       | Down| 236.854  | 840.568  | LOC154822       | UCSC_knowngene   | Natural antisense            |
| chr3:64987550-65012925    | 0.002   | 3.274       | Down| 81.832   | 274.405  | lincRNA         | Intergenic       |                               |
| LIT1571                   | 0.012   | 3.110       | Down| 257.196  | 806.208  | RNAdb           | Intergenic       |                               |
| HMlincRNA1338             | 0.008   | 2.965       | Down| 115.321  | 347.049  | lincRNA         | Intergenic       |                               |

*CC, cervical cancer tissues; CP, cervical peritumoral tissues; Fold change, absolute ratio (no log scale) of normalized intensities between two conditions; MOR, mode of regulation; HSP27, heat shock protein 27; TUBB6, tubulin beta 6 class V; NCRNA00152, cytoskeleton regulator RNA; C12orf27, HNF1A antisense RNA 1.
Figure 2. LncRNA-AK001903 was upregulated in CC cells and tissues and was related to FIGO stage. (A) LncRNA-AK001903 expression level was determined in CC Cells and H8 by reverse-transcription quantitative PCR. H8 cells served as the negative control. (B) LncRNA-AK001903 had a high expression in 29 CC tissues compared with their corresponding peritumoral tissues. β-actin was an internal control. (C) A total of 26 CC tissues were fixed and sectioned into slides then used for ISHH assay. The results demonstrated that high lncRNA-AK001903 expression was associated with advanced FIGO stage. Scale bar, 100 µm. Data are shown as the mean with SD. (**P<0.01; ***P<0.001). CC, cervical cancer; lncRNA, long non-coding RNA; ISH, in situ hybridization; Tis, tumor in situ [according to FIGO (2018)].

Figure 3. Effects of lncRNA-AK001903 silencing on CC progression. (A) Ca Ski cells were transfected with siRNA-lncRNA-AK001903-713, siRNA-lncRNA-AK001903-1306, siRNA-lncRNA-AK001903-1051, siRNA-lncRNA-AK001903-1605 or siRNA-NC. LncRNA-AK001903 expression was analyzed by reverse-transcription quantitative PCR at 48 h post-infection. β-actin was an internal control. (B and C) Ca Ski cells were transfected with siRNA-lncRNA-AK001903-1605 or siRNA-NC and then analyzed by the CCK-8 assay and transwell assay, respectively. Scale bar, 1,000 µm. Data are shown as mean ± SD. *P<0.05. CC, cervical cancer; lncRNA, long non-coding RNA; si, small interfering; NC, negative control; OD, optical density.
Numerous large-scale discovery studies have demonstrated the prospect of using lncRNAs as diagnostic and prognostic biomarkers, even in successful development of RNAi-based and oligo-based drugs (29,30). For example, lncRNA highly up-regulated in liver cancer level in blood and tissues may be used to detect liver cancer (31,32). HOTAIR in tissues can be used as a prognostic marker for overall survival in breast cancer (11). Treatment with MTL-CEBPA (a small activating RNA drug) in mice lowered hepatocellular carcinoma tumor burden and improved clinically relevant parameters of liver function (29). As for the lncRNAs in cervical cancer, increasing numbers of reports have verified that lncRNAs are identified as potential biomarkers for cancer prognosis, invasion, metastasis, chemo-resistance and radio-resistance (33). Functional analysis of lncRNAs associated with CC progression may provide in depth understanding on the progression of CC (34).

Table IV. Clinicopathologic features of CC tissues and corresponding peritumoral tissues (n=29).

| Features                | Low expression | High expression | P-value |
|-------------------------|----------------|-----------------|---------|
| Age, years              |                |                 |         |
| >50                     | 4              | 9               | 0.139   |
| ≤50                     | 10             | 6               |         |
| Tumor size, cm          |                |                 |         |
| ≥3                      | 9              | 10              | 0.893   |
| <3                      | 5              | 5               |         |
| FIGO stage, R           |                |                 |         |
| ≥II                     | 4              | 8               | 0.264   |
| <II                     | 10             | 7               |         |
| Lymphatic metastasis    |                |                 |         |
| Yes                     | 3              | 3               | 0.924   |
| No                      | 11             | 12              |         |
| Differentiation         |                |                 |         |
| Low                     | 4              | 6               | 0.700   |
| Moderate or high        | 10             | 9               |         |
| Confined to the cervix  |                |                 |         |
| No                      | 8              | 8               | 0.837   |
| Yes                     | 6              | 7               |         |

CC, cervical cancer.

In the present study, the biological function of lncRNA-AK001903 in CC was explored. The CCK-8 assay demonstrated that knockdown of lncRNA AK001903 inhibited proliferation of Ca Ski cells, which indicated that lncRNA-AK001903 serves a crucial role in CC development. In the present study, transwell assays demonstrated that lncRNA-AK001903 promoted cell invasion and migration in CC. Considering Ca Ski is a cervical cancer cell line which was established from cells from a metastasis in the small bowel mesentery (39), lncRNA-AK001903 may act as an oncogene in the progression of CC and is a promising therapeutic target for the treatment of patients with CC.

The clinical application of lncRNA-AK001903 needs to be further explored due to the limitation of the present study of study and the results in 9 pairs of CC and peritumoral tissues were consistent with the microarray analysis.

It was recently reported that abnormal expression of lncRNAs, such as HAND2-AS1 and DLX6-AS1 serve an important role in the occurrence and development of CC (36-38). The change of cell phenotype depends on the influence of gene expression regulation (10). In the present study the most significantly upregulated lncRNA lncRNA-AK001903 was used to perform further experiments. The findings of the present study revealed that lncRNA-AK001903 was significantly upregulated in CC cell lines compared to normal cell line H8 and CC tissues compared to peritumoral tissues by RT-qPCR, and the level of lncRNA-AK001903 in CC tissues by ISHH was associated with FIGO (2018) stage. The aforementioned findings of the present study indicated that lncRNA-AK001903 may be a novel and effective biomarker in CC.

The clinical application of lncRNA-AK001903 needs to be further explored due to the limitation of the present study of
not tracking the prognostic differences among patients with different expression levels.

In summary, the present study demonstrated that LncRNA AK001903 is a potential oncogene in CC. LncRNA AK001903 was able to promote CC tumor proliferation, migration and invasion and may act as therapeutic target and auxiliary criteria for evaluating FIGO stage.

Acknowledgments

Not applicable.

Funding

This work was supported by the National Natural Science Foundation of China (grant no. 81572575), Guangdong province Natural Scientific Grant (grant no. 2016A020215059), Special Support for Guangdong College Students’ innovation and entrepreneurship training program (grant no. 1055813194), National College Students’ innovation and entrepreneurship training program (grant no. 20130558097) and Guangdong clinical teaching base teaching program (grant no. 2018JD004).

Availability of data and materials

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

Authors' contributions

TY, XF and GZ contributed to the conception of the study and designed the experiments. YW, QX, XF and GZ performed the experiments. GZ, XF and RL contributed significantly to analysis and manuscript preparation. ZL and YW made substantial contributions to the acquisition of patient tissues and patient data. ZL analyzed and interpreted the patient data. GZ, TY and ZL revised the manuscript critically for important intellectual content. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All specimens were obtained with the approval of the Sun Yat-sen Memorial Hospital Review Board (approval number, 2015132) (Guangzhou, China). Written informed consent was obtained from each patient.

Patient consent for publication

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Competing interests

The authors declare that they have no competing interests.

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