IncRNA PART1 and MIR17HG as ΔNp63α direct targets regulate tumor progression of cervical squamous cell carcinoma

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Abstract

Cervical cancer (CC) remains one of the leading causes of mortality of female cancers worldwide, with more than 90% being cervical squamous cell carcinoma (CSCC). ΔNp63α is the predominant isoform expressed in cervical epithelial tissues and exerts its antitumor function in CSCC. In this study, we have identified 39 long noncoding RNAs as ΔNp63α targets in CSCC through RNA sequencing and chromatin immunoprecipitation sequencing, in which we further confirmed and focused on the two tumor-related long noncoding RNAs, PART1 (lncPART1) and MIR17HG (lncMIR17HG). Experiments from stable overexpression/knockdown cell lines revealed that lncPART1 and lncMIR17HG regulated cell proliferation, migration, and invasion. In vivo experiments further showed that lncPART1 suppresses tumor growth in CSCC-derived tumors. Examinations of clinical tissues indicated that the expression of lncPART1 was positively correlated with ΔNp63α expression, while lncMIR17HG was negatively correlated with ΔNp63α expression, suggesting that ΔNp63α plays a central role via regulating its direct targets in the progression of CSCC. These findings provide novel insights in targeted therapy of cervical cancers.
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1 INTRODUCTION

Cervical cancer is the fourth most common cancer among women and ranks 2 in mortality of female cancers. More than 90% of cervical cancers are cervical squamous cell carcinoma (CSCC) with abnormal regenerative proliferation and blocked differentiation.1-4 Effective prophylactic vaccines against the most important carcinogenic human papillomavirus types are available, but the number of people receiving the vaccine remains low.5,6 Clinically, surgery, radiotherapy, and chemotherapy have been widely used to improve the overall survival rate of CSCC; however, the 5-year survival rate also remains low for advanced CSCC patients, especially for metastatic cervical cancer patients with a survival rate around 5%-15%.7,8 The underlying molecular mechanisms in tumorigenesis for CSCC need further clarification, which would promote our understanding of the clinical treatment of CSCC.

p63, a member of the p53 gene family, acts as a key transcription factor involved in cell growth, proliferation, apoptosis, and differentiation. p63 generates two different isoforms (TA isoform and ΔN isoform) from different transcription promoters. Both TA and ΔN isoforms can undergo alternative splicing to generate different carboxytermini, including α, β, γ, δ, and ε.9-11 Among them, ΔNp63α is the predominant isoform expressed in cervical epithelial tissues.12-14 As the major isotype controlling epithelium morphogenesis, aberrant expression of ΔNp63α leads to abnormal differentiation and epithelial-mesenchymal transition behavior through various mechanisms.15-17 We have previously demonstrated that ΔNp63α exerts its antitumor function in CSCC by regulation of its direct targets.18,19 Further investigations of other ΔNp63α direct targets are needed to better understand the functional mechanisms of ΔNp63α in CSCC.

Aberrant activation of RNA regulatory networks is reported to promote changes in cell state that may be involved in tumorigenesis. Long noncoding RNAs (lncRNAs) are a class of RNA molecules with over 200 nucleotides in length.20-22 With the development of RNA-sequencing (RNA-seq) techniques, more and more lncRNAs are reported to play important roles in epigenetic regulation, transcriptional regulation, and posttranscriptional regulation.23,24 lncRNAs are widely involved in the progression of various tumors, such as bladder cancer, lung cancer, hepatocellular carcinoma, etc.25-32 However, only a limited number of lncRNAs, such as MEG3, MALAT1, GAS5, HOTAIR, and EBIC, have been identified to relate to CSCC progression.2,23-37

In this study, we identified that the lncRNAs IncPART1 and IncMIR17HG are the main direct transcriptional targets of ΔNp63α by overlapping chromatin immunoprecipitation sequencing (ChIP-seq) and RNA-seq. Overexpression of IncPART1 suppressed the proliferation, migration, and invasion of CSCC cells. Knockdown of IncPART1 promoted the proliferation, migration, and invasion of CSCC cells. In vivo experiments also showed that IncPART1 suppresses tumor growth in CSCC-derived tumors. On the other hand, knockdown of IncMIR17HG suppressed the proliferation, migration, and invasion of CSCC cells, acting more like an oncogene.38,39 Our work demonstrated that ΔNp63α plays a central role via regulating its direct targets in the progression of CSCC, either upregulating or downregulating, providing new insights for the diagnosis and treatment of CSCC.

2 MATERIALS AND METHODS

2.1 Cell lines

The cervical cancer cells SiHa, ME-180, C-33A, HeLa, HaCat, and 293T were obtained from the American Type Cancer Culture (ATCC). SiHa, C-33A, HeLa, and 293T were inoculated with DMEM (HyClone), HaCat was inoculated with MEM (Gibco, Thermo Fisher Scientific), and ME-180 was inoculated with Macoy’s 5A medium (Gibco, Thermo Fisher Scientific) containing 10% fetal bovine serum (Gibco, Thermo Fisher Scientific), 100 units/mL penicillin, and
100 mg/mL streptomycin (HyClone) and cultured in an incubator at 37°C with 5% CO₂ and saturated humidity. After cells had grown along the dish wall, the medium was changed every 1-2 days and 0.25% trypsin (Sigma-Company) was used for digestion and subculture. All cells were tested for mycoplasma by a PCR-based method as well as DAPI staining to ensure the absence of contamination.

2.2 | Construction of SiHa/PART1 and ME-180/shPART1 stable cell lines

pLVX-IRES-mcherry-Inc-PART1 plasmid was constructed by inserting a full length of human Inc-PART1 cloned by RT-PCR into the EcoRI and NotI site of pLVX-IRES-mcherry. pLVX-IRES-mcherry-IncPART1 and the control plasmid with their packaging vectors pmd2g and pspx2 were cotransfected to the 293T cells. Then, virus supernatant was used to infect the SiHa cells. SiHa/PART1 stable cell lines and SiHa/Con cells were generated by selection with 100 μg/mL G418 for 2 weeks as described previously.

Knockdown of PART1 was achieved using predesigned shRNA oligonucleotides. Specific shRNA has been annealed to connect to the pLKO.1 puro vector. Lentivirus packaging plasmid Gag/Pol, Rev, VSV-G, and shNC, shPART1 were cotransfected to the 293T cells. Then, the virus supernatant infected the SiHa cells, respectively. Stable cell lines and were generated by selection with 2 μg/mL puromycin for 2 weeks.

2.3 | Construction of SiHa/si Con and SiHa/si IncMIR17HG cell lines

Transfection of control plasmid and siRNA was performed with Lipofectamine 2000 (Invitrogen) according to the manufacturer’s protocols.

2.4 | RNA extraction and qRT-PCR analyses

The total RNA samples of the cell line were isolated using TRIzol reagent according to the manufacturer’s protocol (Life Technologies) for RNA extraction and quantitative real-time polymerase chain reaction (qRT-PCR) analyses. Then, 500 ng of the total RNA was reversely transcribed in a final volume of 20 μL, using random primers and standard conditions with the GoScript Reverse Transcription System (Promega). Subsequently, we performed qRT-PCR using the SYBR Select Master Mix with 2 μL complementary DNA (cDNA) according to the manufacturer’s instructions. The qRT-PCR reaction included an initial denaturation step at 95°C for 30 seconds, which was then followed by 40 cycles at 95°C for 5 seconds and 60°C for 1 minute. All the primer sets are shown in Table S1. Note that the primers for ΔNp63α are specific for ΔNp63α, instead of TAp63α. GAPDH and specific transcript levels for each transfection condition were measured in triplicate. The ΔΔCt method was applied to quantify relative gene expression.

2.5 | Chromatin immunoprecipitation (ChIP)

ChIP assay was performed as described previously.40,41 Briefly, cells were cross-linked in a UV cross-linker (UVP) at 200 mJ. After rinsing with PBS twice, the cell pellets were lysed in 1 mL of SDS lysis buffer (1% (w/v), 10 mM EDTA, and 50 mM Tris-HCl (pH 8.1) containing Complete protease inhibitor cocktail (Roche) and were incubated for 20 minutes on ice. Cell extracts were sonicated for 5 minutes with a Vibra-Cell processor (Sonic & Materials, Inc.). A 100-μL sample of the supernatant was saved as input. The remaining sample was diluted 1:10 in a ChIP dilution buffer (0.01% (w/v) SDS, 1.1% (v/v) Triton X-100, 1.2 mM EDTA, 16.7 mM Tris-HCl, pH 8.1, and 167 mM NaCl) containing protease inhibitors. The chromatin solution was precleared and immunoprecipitated with 2 μg of p63 antibody (CST, 13109S) or normal IgG control. The immunocomplexes were eluted in 1% (w/v) SDS and 50 mM NaHCO₃, and crosslinks were reversed for 6 hours at 65°C. Samples were digested with proteinase K for 1 hour at 45°C, and the DNA was extracted with phenol/chloroform/isoamyl alcohol.

2.6 | RNA-seq preparation and sequencing

For IncRNA-seq, 5 μg extraction of the total RNA was iron-fragmented at 95°C and then subjected to end repair and 5’-adaptor ligation. Then, reverse transcription was performed with random primers containing 3’ adaptor sequences and randomized hexamers. The cDNAs were purified, amplified, quantified, and stored at −80°C until sequencing. Differentially expressed IncRNAs were identified using a t-test (P < 0.05) combined with fold change (FC) (log2(FC)>2 for upregulated IncRNAs, and log2(FC)<−2 for downregulated IncRNAs). These differentially expressed IncRNAs were visualized by a heatmap and volcano analyses using R program. All the primers are shown in Table S1.

2.7 | Cell proliferation in vitro (ACEA)

6 × 10⁵ cells (SiHa/Con, SiHa/IncPART1, ME-180/Con, ME-180/shIncPART1, SiHa/siCon, and SiHa/silIncMIR17HG cells) were resuspended in 1 mL DMEM or Myco5A containing 10% FBS. 100 μL of the cell suspension was seeded into each well of the 16-well plate (ACEA Biosciences Inc) and put into an xCELLigence RTCA DP measuring instrument (Roche). The number of cells per well was then counted at the indicated time points. Each type of cells was counted in triplicates. The cell growth plot was analyzed by xCELLigence (ACEA Biosciences Inc).

2.8 | Colony formation assay

The number of all cells per hole was strictly counted, and the cells were kept in uniform distribution. For SiHa/Con, SiHa/IncPART1, ME-180/Con, ME-180/shIncPART1, SiHa/siCon, and SiHa/silIncMIR17HG cells, 100 and 300 cells were distributed into 6-well plates separately and were run in triplicate. Cells were allowed to grow for 2 weeks.
in 5% CO₂ incubators before being stained with 0.5% crystal violet staining solution (Solarbio). The quantitation of the colony formation assays was described in a histogram. The results represent mean values of two duplicate experiments, and the error bars show SD.

2.9 | Scratch, migration, and invasion assays

For scratch assays, 400 000 cells, including SiHa/Con, SiHa/Inc-PART1, ME-180/Con, ME-180/shlncPART1, SiHa/siCon, and SiHa/silncMIR17HG cells, were plated in 6-well plates. After the cells were attached, cell monolayers were scraped by a middle pipet tip consistently and washed with PBS to gently remove cell debris. All cells were cultured in 1% FBS in DMEM or Myco5A. Photos were taken during the subsequent 12, 36, and 72 hours to monitor scratch closure. For the transwell migration assay, a cell suspension containing 4 × 10^5/mL cells was prepared in serum-free media: 1 mL of media containing 10% fetal bovine serum was added to the lower chamber, and then 500 μL of the prepared cell suspension was added to each insert (Millipore #ECM550, pore size: 8 μm). For the transwell invasion assay, 300 μL of warm serum-free media was added to the interior of the inserts and allowed to rehydrate the ECM layer for 1 hour at room temperature. Then, 2 × 10^5 cells were plated into the transwell inserts (Chemicon #P30R48, pore size: 8 μm). All the steps were performed strictly following the instructions of the transwell migration and invasion assay kit. After 24 hours, cells that did not migrate were removed by scratching the upper side of the membrane with a cotton swab before fixation in 4% methanol for 5 minutes at room temperature. Cells were then stained with crystal violet staining solution for 5 minutes. The percentage of migration was determined by calculating the sum of the area of total migrated cells on the entire membrane by using ImageJ software.

2.10 | Flow cytometry cell cycle assay

A total of 2 × 10^5 cells was plated in 6-well plates per well and cultured overnight. Then, all cells were synchronized in serum-free medium for 48 hours. After that, the cells were resuspended in complete medium for another 24 hours. The cells in each group were fixed with 75% ethanol overnight and washed with 1x phosphate buffer saline (PBS) twice. According to the protocol of the cell cycle detection kit (Multi Sciences), the cells were resuspended with 400 μL of 1x binding buffer and stained with 20 μL of propidium iodide (PI) for 20 minutes at room temperature in the dark. The cell cycle was analyzed immediately with a flow cytometer (Becton-Dickinson, FACSCalibur). The percentage of cells at each phase of the cell cycle was obtained by Cell Quest software (Becton-Dickinson, FACSCalibur).

2.11 | Xenograft and orthotopic models of cancer in mice

Animal experiments were performed as described previously. Five-week-old female nude mice (Experiment Animal Center of Shanghai) (each group, n = 5) were subcutaneously injected with 6 × 10^6 SiHa/Con, SiHa/IncPART1, ME-180/Con, or ME-180/shlncMIR17HG cells in 0.1 mL PBS containing 20% matrigel, respectively. The growth of solid tumors of SiHa/Con, SiHa/IncPART1, ME-180/Con, or ME-180/shlncMIR17HG cells after injection were measured every 5 days for up to 30 days. All of the animals were sacrificed to take away the tumors for analysis. The use of mice was approved by the Animal Care and Use Committee of the UST University (USTCACUC1801017).

2.12 | Clinical samples

A total of 15 clinical samples were obtained from patients at Anhui Provincial Hospital, Hefei, China, including 5 cervical cancer patients and 10 uterine myoma patients. The detailed patients’ information is listed in Table S2. This study was reviewed and approved by the Ethics Review Board of Anhui Provincial Hospital. Written informed consent was obtained from each patient for this study.

2.13 | Statistical evaluation

SPSS16.0 and GraphPad Prism 7 software were used for all statistical analyses. Data of all the experiments were presented as mean ± SD, which were replicated at least three times. Student’s two-tailed t-test or analysis of variance (ANOVA) was used to assess the statistical significance of the difference. A P-value <0.05 was considered statistically significant.

2.14 | Data availability

The accession number for the RNA-seq and ChIP-seq data reported in this paper is GEO: GSE135257.
RNA-seq
355
RNA-seq
39
ChIP-seq
6466

(C) Relative expression of IncPART1

Relative expression of IncPART1 (normalized to GAPDH)

IncPART1

ME-180/Con

ME-180/shp63

***

(E) Expression of IncPART1

Expression of IncPART1

Normal
Cancer

P = 0.0468
n = 15

(G) Expression of IncMIR17HG

Expression of IncMIR17HG (normalized to GAPDH)

IncMIR17HG

ME-180/Con

ME-180/shp63

***

(F) P63 mRNA level

R = 0.7902
P = 0.0005
n = 15

(H) P63 mRNA level

R = -0.6934
P = 0.0042
n = 15

(l) IncPART1

IncMIR17HG

ME-180

p63 ChIP-seq

Input
3 | RESULTS

3.1 Identification of target genes of ΔNp63α in CSCC

We first established stable ME-180 cells with ΔNp63α shRNA knockdown (ME-180/shp63) as reported previously. To investigate the transcriptional regulatory mechanisms of ΔNp63α in CSCC, we performed RNA-seq in ME-180/shp63 cell lines. Totally, 394 lncRNAs were significantly affected (cutoff of two FCs and $P < 10^{-5}$) compared with the no-knockdown control. To determine global direct targets of ΔNp63α, we performed ChIP-seq of endogenous p63 in ME-180 cells. Bioinformatics analyses of the ChIP-seq data identified that 6505 genes were directly regulated by ΔNp63α. Among the 394 genes significantly affected by ΔNp63α in the RNA-seq analyses, 39 possessed p63 ChIP-seq binding sites, and thus should be direct targets of ΔNp63α (Figure 1A,B). These direct targets could be either activated or suppressed by ΔNp63α. All the 39 candidate lncRNAs were then analyzed by qRT-PCR in ME-180/shp63 cells (Figure S1).

Among the 39 directly regulated lncRNAs, previous studies indicated that IncPART1 and IncMIR17HG are associated with tumorigenesis. Besides, IncPART1 and IncMIR17HG demonstrated significant differential expression in ME-180/shp63 cells. IncPART1 showed significantly decreased expression levels, while IncMIR17HG showed significantly increased levels in ME-180/shp63 cells (Figure 1C,D). We then detected the expression correlation of the two lncRNAs and ΔNp63α in clinical tissues. A total of 15 clinical samples were collected for analyses, including five cervical cancer patients and ten uterine myoma patients. The expression of IncPART1 was significantly lower than that in normal tissues (Figure 1E). The correlation analyses revealed that IncPART1 expression was positively correlated with ΔNp63α expression (Figure 1F). On the other hand, the expression of IncMIR17HG was significantly higher than that in normal tissues (Figure 1G). The correlation analyses revealed that MIR17HG expression was negatively correlated with ΔNp63α expression (Figure 1H). We hence focused on IncPART1 and IncMIR17HG for downstream examination. ChIP-seq peaks for both lncRNAs and ChIP efficiency are shown in Figure 11.

3.2 Overexpression of IncPART1 suppresses cell proliferation and migration

To investigate the functions of IncPART1 in cervical squamous tumorigenesis, we screened five cervical squamous cancer cell lines (HaCat and ME-180 with high expression of ΔNp63α, and C-33A, HeLa, and SiHa with low expression of ΔNp63α). Among them, SiHa cells showed the lowest expression levels of IncPART1 (Figure S2A,B). We hence selected SiHa (low expression of ΔNp63α) for the following in-depth study. We first established stable SiHa cells with overexpression of IncPART1 (SiHa/IncPART1). The overexpression efficiency of IncPART1 was confirmed using qRT-PCR analyses (Figure 2A). Compared with the control, cell proliferation and colony formation were significantly decreased in SiHa/IncPART1 cells (Figure 2B,C). Overexpression of IncPART1 also decreased the proportion of cells in S phase and increased the proportion of cells in G1 phase in SiHa cells (Figure 2D). To investigate the role of IncPART1 in cell migration, we performed scratch assays and transwell assay. SiHa/IncPART1 cells migrated significantly slower compared with the control cells (Figure 2E). Moreover, SiHa/IncPART1 cells showed weaker migration and invasion capability in matrigel (Figure 2F).

3.3 Knockdown of IncPART1 promotes cell proliferation and migration

ME-180 cells harbor the highest expression level of IncPART1 of all five tested cell lines (Figure S2B); hence, we established stable ME-180 cells with IncPART1 shRNA knockdown (ME-180/shIncPART1-1 and ME-180/shIncPART1-2). The knockdown efficiency of IncPART1 in the above two knockdown cell lines was confirmed using qRT-PCR analyses (Figure 3A). Compared with the control, cell proliferation and colony formation were significantly increased in ME-180/shIncPART1-1 and ME-180/shIncPART1-2 cells (Figure 3B,C). Knockdown of IncPART1 increased the proportion of cells in S phase and decreased the proportion of cells in G1 phase in ME-180 cells (Figure 3D). To investigate the role of IncPART1 in cell migration, we performed scratch assays and transwell assay. ME-180/shIncPART1-1 and ME-180/shIncPART1-2 cells migrated significantly faster compared with the control cells (Figure 3E). Moreover, ME-180/shIncPART1-1 and ME-180/shIncPART1-2 cells showed weaker migration and invasion capability in matrigel (Figure 3F).
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(A) Expression of lncPART1 (normalized to GAPDH)

(B) Cell number over time

(C) Colony number

(D) S+G2/M Cell percentage (%)

(E) Healing rate of scratch

(F) Migration and Invasion
(A) Expression of IncPART1 (normalized to GAPDH)

(B) Cell Index vs. Time (in Hour)

(C) ME-180/Con, ME-180/shlncPART1-1, ME-180/shlncPART1-2

(D) ME-180/Con, ME-180/shlncPART1-1, ME-180/shlncPART1-2

(E) ME-180/Con, ME-180/shlncPART1-1, ME-180/shlncPART1-2

(F) ME-180/Con, ME-180/shlncPART1-1, ME-180/shlncPART1-2
3.4 | LncPART1 suppresses tumor growth in CSCC-derived tumors

We then investigated the role of LncPART1 on tumorigenesis of cervical cancer cells in vivo. SiHa/LncPART1, ME-180/shlncPART1-1, and ME-180/shlncPART1-2 cells, along with their corresponding control cells, were subcutaneously injected into 5-week-old female athymic nude mice. The tumors from SiHa/LncPART1 cells grew significantly slower than those from control cells (Figure 4A-C). On the other hand, tumors in the ME-180/shlncPART1 and ME-180/shlncPART1 groups grew significantly faster as compared with their control (Figure 4D-F).
**A)**

Expression of lncMIR17HG

- **SiHa/Con**
- **SiHa/shMIR17HG**

**B)**

Healing rate of scratch

- **SiHa/Con**
- **SiHa/shlncMIR17HG**

**C)**

Colony number

- **SiHa/Con**
- **SiHa/shlncMIR17HG**

**D)**

Migration

- **SiHa/Con**
- **SiHa/shMIR17HG**

**E)**

Invasion

- **SiHa/Con**
- **SiHa/shMIR17HG**
LncMIR17HG promotes proliferation of cervical squamous cells in vitro. A, Expression levels of lncMIR17HG in SiHa/shlncMIR17HG cells (SiHa cells with knockdown of lncMIR17HG). B, Proliferation curves (as detected by RTCA assay) of SiHa/shlncMIR17HG cells compared with the corresponding controls. C, Colony formation assays of SiHa/Con and SiHa/shlncMIR17HG cells. Quantification of colony formation number is also shown. D, Representative images of wound healing in SiHa/Con and SiHa/shlncMIR17HG cells. Quantification of healing rate is also shown. E, Representative images of transwell migration (up) and Matrigel invasion assays (down) of SiHa/Con and SiHa/shlncMIR17HG cells. Quantification of migrating cells is also shown. All the experiments were performed in triplicates. *P < 0.05, **P < 0.01, ***P < 0.001, based on the Student’s t-test.

3.5 Knockdown of lncMIR17HG suppresses cell proliferation and migration in CSCC

In investigation of the functions of lncMIR17HG in cervical squamous tumorigenesis, of all five tested CSCC cell lines, SiHa cells harbor the highest expression level of lncMIR17HG; hence, we selected SiHa (low expression of ΔNp63α) for the following in-depth study (Figure S2C). We first established stable SiHa cells with the knockdown of lncMIR17HG (SiHa/shlncMIR17HG). The knockdown efficiency of lncMIR17HG was confirmed using qRT-PCR analyses (Figure 5A). Compared with the control, cell proliferation and colony formation were significantly decreased in SiHa/lncMIR17HG cells (Figure 5B,C). To investigate the role of lncMIR17HG in cell migration, we performed scratch assays and transwell assay (Figure 5D,E). SiHa/shlncMIR17HG cells migrated significantly slower compared with the control cells. Moreover, SiHa/shlncMIR17HG cells showed weaker migration and invasion capability in matrigel (Figure 5E).

4 DISCUSSION

Cervical cancer is the fourth most common cancer and the second leading cause of cancer deaths among women in the world. The discovery of critical diagnostic and therapeutic markers against CSCC would broaden our understanding on the molecular basis of CSCC. Besides, high-throughput RNA-seq has greatly enabled us to detect and quantify the entire transcriptome in CSCC. ΔNp63α plays a fundamental role in the regulation of cervical squamous tumorigenesis. We have previously identified that ΔNp63α acts as a tumor suppressor by regulating a cohort of downstream targets in CSCC. In this study, we found 39 potential ΔNp63α target IncRNAs by overlapping ChIP-seq and RNA-seq. ΔNp63α is the predominant isotype expressed in the cervix and ME-180 cell line, while other isoforms (eg, TAp63α) are hardly detectable; thus, here we used p63α antibody for ChIP in ME-180 cell line. All the potential targets were verified by qRT-PCR and the results were in accordance with those in the above sequencing data. Among the 39 directly regulated IncRNAs, we found the expression level of IncPART1, a tumor-associated IncRNA, was significantly decreased in ME-180/shp63 cells, as compared with the control cells. Overexpression of IncPART1 suppressed, while knockdown of IncPART1 promoted proliferation, migration, and invasion of cervical cancer cell lines. We found that IncPART1 suppresses tumor growth in CSCC-derived tumors. Correlation analysis of cervical cancer tissues revealed that IncPART1 expression was positively correlated with ΔNp63α expression. The above results indicated that IncPART1 might act as a tumor suppressor in CSCC. The detailed molecular mechanism of how IncPART1 functions remains for further investigation.

The functions of IncPART1 were previously reported to be positively involved in cell migration and proliferation in multiple cancers. For example, IncPART1 promoted cell proliferation ability and apoptosis via the inhibition of Toll-like receptor pathways in prostate cancer and promoted gefitinib resistance in esophageal squamous cell carcinoma by functioning as a competing endogenous RNA. Our study demonstrates that, at least in CSCC cell lines examined and in the contexts of our experimental setup, IncPART1 is a tumor suppressor in CSCC, which broadens our insights into the function of IncPART1 in tumorigenesis.

Another core target of ΔNp63α is lncMIR17HG, a miR-17-92 cluster host gene IncRNA that is involved in cell proliferation and growth by modulating cell growth phenotype. However, the biological function of most Inc-miR-HGs in tumor progression is not well understood. In this study, we found that the level of lncMIR17HG was significantly increased in cultured cells and clinical tissues. Knockdown of lncMIR17HG markedly suppressed the proliferation, migration, and invasion of SiHa cells. The expression level of lncMIR17HG in cervical cancer tissues was significantly higher than in normal tissues. Correlation analysis of cervical cancer tissues revealed that lncMIR17HG expression was negatively correlated with ΔNp63α expression. The above results indicated that lncMIR17HG may be suppressed by ΔNp63α and functions as a protumor gene in CSCC. Previous studies indicated that lncMIR17HG normally functions through regulating its host miRNAs. For instance, lncMIR17HG promoted colorectal cancer progression via mir-17-5p, and downregulation of MIR17HG-miR-18a/miR-19a axis expression and attenuating Wnt/β-catenin signaling inhibited gastric cancer metastasis. We have analyzed the association of lncMIR17HG and six lncMIR17HG cluster members (miR-17, miR-18a, miR-19a, miR-19b-1, miR-20a, and miR-92a-1). All of the members, except miR-20a, showed positive correlation with lncMIR17HG (data not shown). The detailed molecular mechanism of how lncMIR17HG functions in CSCC needs further investigation.

Taken together, we have identified two direct transcriptional targets of ΔNp63α, IncPART1 and lncMIR17HG. We showed that IncPART1 suppressed the proliferation, migration, and invasion of CSCC cells and acted as a tumor suppressor gene in CSCC. On the other hand, lncMIR17HG is a protumor gene. Knockdown of lncMIR17HG suppressed the proliferation, migration, and invasion of CSCC cells. ΔNp63α, as a transcription factor, can both activate the expression of target genes (eg, IncPART1) and inhibit the expression of some other target genes.
(eg, IncMIR17HG), which reveals its combinatory effects of transcriptional regulators. Further identification and characterization of ΔNp63α targets in tumorigenesis are of fundamental significance in the understanding, prognosis, and treatment of CSCC.

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DISCLOSURE
The authors have no conflict of interest to declare. All authors have read the journal's authorship agreement. The manuscript has been reviewed by and approved by all named authors.

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**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section.

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