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# Upregulation of lncRNA CALML3-AS1 promotes cell proliferation and metastasis in cervical cancer via activation of the Wnt/β-catenin pathway

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**Abstract. – OBJECTIVE: It is well verified that lncRNA are emerging as imperative regulators in various tumors. LncRNA CALML3-AS1 (CALML3-AS1), a freshly discovered lncRNA, has been confirmed as a tumor promoter in bladder cancer. This present study aimed to explore the biological functions and molecular mechanisms of CALML3-AS1 in cervical cancer (CC).**

**PATIENTS AND METHODS: We analyzed RNA sequencing data from The Cancer Genome Atlas (TCGA) datasets to determine dysregulated lncRNAs in CC. Real Time-Polymerase Chain Reaction (RT-PCR) was applied for the assays of CALML3-AS1 amplification in CC samples and cell lines. Kaplan-Meier analysis and multivariate assays were carried out for determination of the prognostic values. The functions of CALML3-AS1 on cell proliferation, invasion, migration, and apoptosis were determined by a series of cells experiments by knocking down CALML3-AS1. MRNA and protein expressions of signaling pathways were examined using Western blot.**

**RESULTS: We found that CALML3-AS1 was upregulated in CC tissues and this upregulation was associated with FIGO stage, histological grade, and reduced overall survival. Multivariate assays indicated that high CALML3-AS1 expression was an independent prognostic parameter indicating poorer clinical outcome for CC patients. Functional assays suggested that knockdown of CALML3-AS1 suppressed the proliferation, migration, and invasion of CC cells, and induced apoptosis. Mechanistic investigations revealed that inhibiting the expression of CALML3-AS1 decreased the levels of β-catenin, cyclin D1, and c-myc via Western blot.**

**CONCLUSIONS: Our study revealed that CALML3-AS1 could be an oncogenic lncRNA promoting the growth and metastasis of CC by modulating Wnt/β-catenin pathway, suggesting that CALML3-AS1 may be an important contributor to CC progression.**

Key Words
LncRNA CALML3-AS1, Cervical cancer, Prognosis, Metastasis, Wnt/β-catenin pathway.

## Introduction

Cervical cancer (CC) is one of the common cancers following breast cancer and a leading cause of cancer-related mortality in women worldwide, especially in many backward countries[1]. The incidence and mortality of CC in women from China have been increasing in recent years, making it a serious public-health problem[2]. Despite operative treatments have been used in CC patients and the development of cancer diagnosis and treatment have been achieved in recent decades, the clinical outcome of CC patients diagnosed at a late stage is still dismal due to higher incidences of metastasis and recurrence for those patients[3,4]. Up to date, due to the extreme complexity of oncogenesis, the complete scope of underlying mechanisms of CC remains largely unclear despite continuous exertion in studying this problem in recent years[5]. Thus, for better management of CC patients, the exploration of the underlying mechanisms involved in CC progression and metastasis abilities in different ways is urgently necessary.

The in-depth studies for the functions of the human genomes have resulted in the surprising achievement. Matsui and Corey[6] suggest that 75-90% of the DNA is transcribed but about 3% was transcribed into mRNAs and further translated into proteins. Long non-coding RNAs (lncRNAs), lacking the abilities of protein-coding, are a group of transcribed RNA possessing more than 210 nucleotides[7]. LncRNAs are broadly divided in sense, intronic, long intergenic, and some other types based on their associations with the nearest genes. An increasing number of works[8,9] have described the regulators' effects of lncRNAs in various cellular processes, such as imprinting, immune response, and genes modulations. The regulatory effects of lncRNAs in tumor-related genes highlight their potential functions in tumor progres-

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sion. Moreover, emerging evidence has confirmed the fact that lncRNAs act as tumor suppressors or oncogenes which were involved in almost every tumor-related progression, such as cells growth, metastasis, and tumor recurrence[10,11]. In addition, their dysregulated levels in tumors, arresting tissues specificity, and multipurpose modulation networks provided great potential that lncRNAs, especially some higher expressed lncRNAs, may represent a new class of diagnostic and prognostic biomarkers for tumor patients[12,13].

LncRNA CALML3-AS1 (CALML3-AS1) is a newly discovered lncRNA and is transcribed from the CALML3 gene on 10p15.1. Up to date, the functional studies of CALML3-AS1 in tumors remain largely unknown. Wang et al[14] showed that CALML3-AS1 upregulation may contribute to the progression of bladder cancer. In this research, we first reported the overexpression of CALML3-AS1 in CC and further explored its prognostic value, finding that CALML3-AS1 has the potential to act as a novel prognostic biomarker. In addition, functional assays were also performed. We first provided evidence that CALML3-AS1 plays a functional role in CC, and may have an important clinical application for CC treatments.

## Patients and Methods

### Patients and Specimens

Surgical tumor specimens were obtained from 184 patients at Linyi Cancer Hospital, and their matched normal samples were obtained. All collected specimens proximately frozen by the use of liquid nitrogen, followed by being preserved at –80°C for extracting tissues RNAs. Patients had not received chemotherapy or other anti-neoplasm treatments and were aged between 33.7 and 71.6 years (average age, 46.8 years). The clinical diagnosis of all patients was demonstrated by two experienced pathologists and all specimens were classified according to the standard of the WHO Classification of Tumors. All the patients signed informed consent. The study protocol and consent procedures were approved by the Ethics Committee of Linyi Cancer Hospital. Detail clinical information from 184 patients was presented in Table I.

### Cell Transfection

Four CC cells (HeLa, CaSKi, SiHa, SW756, and C33A) were bought from the Department of Cell Biology at China Medical University. Standard cervical epithelial cells (Ect1/E6E7) were purchased from the CASCB (Xuhui, Shanghai, China). Above cells were cultured using Dulbecco's Modified Eagle's Medium (DMEM; Gibco, Kunming, Yunan, China) supplemented with 10% fetal bovine serum (FBS; Hyclone, Xuhui, Shanghai, China) at 37°C with 5% $CO_2$.

SiRNA (100 nM) targeting CALML3-AS1 (si-CALML3-AS1) and siRNA-NC (si-NC) were synthesized by Jima Technology (Pudong, Shanghai, China). Transient transfections for HeLa and CaSKi cells were performed using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA) by si-CALML3-AS1 or si-NC.

### Real Time-PCR

Total RNAs were extracted employing TRIzol reagents (Invitrogen, Carlsbad, CA, USA). cDNA was reversely transcribed from collected RNAs (2 mg) by the use of M-MLV reverse transcriptase (Invitrogen, Carlsbad, CA, USA). The levels of CALML3-AS1 in CC specimens and cells were examined and analyzed by qPCR assays which were conducted in Cycler 520 SYBR Green kits (Roche, Pudong, Shanghai, China). The qPCR conditions were: 95°C for 20 s; 39 cycles of 95°C for 10 s, 60°C for 30 s. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was applied as an endogenous control. The Real Time-Polymerase Chain Reaction (RT-PCR) primers for CALML3-AS1 and GAPDH were synthesized and purchased from GeneCopoeia (Hangzhou, Zhejiang, China). The sequences for primers were: CALML3-AS1 (F: 5'-ACTcAAACTCACAGTGAAG-3'; R: 5'-CCCGTTCGACGCAATATCC-3'); GAPDH (F: 5'-GGAGCGAGATCCCTCCAAAATC-3'; R: 5'-GGCTGTTGTCATACTTCTCATG-3'). All the data from our experiments were finally calculated using the $2^{-\Delta\Delta Ct}$ methods.

### MTT Assays and Colony Formation

Cell viability was examined using the Methyl Thiazolyl Tetrazolium (MTT) assays. Briefly, 150 μL of HeLa and CaSKi cells which grew exponentially were placed in nighty-six-well plates and added with 10% serum for 0-96 h. Thereafter, every well was supplied with thirty microliters of MTT solutions and then treated CC cells were incubated for 4 h. After discards of the medium, the supplements of 150 μ l of dimethyl sulfoxide (DMSO) were carried out. Absorbance was determined at a wavelength of 490 nm.

**Table I.** Correlations between CALML3-AS1 expression and the clinical features in patients with CC.

| Factors | No. of patients | CALML3-AS1 expression | | *p*-value |
|---|---|---|---|---|
| | | Low | High | |
| ***Age (years)*** | | | | 0.352 |
| <65 | 80 | 36 | 44 | |
| ≥ 65 | 104 | 54 | 50 | |
| ***HPV*** | | | | 0.415 |
| (+) | 73 | 33 | 40 | |
| (−) | 111 | 57 | 54 | |
| ***Tumor histology*** | | | | 0.187 |
| Squamous | 95 | 42 | 53 | |
| Adenocarcinoma | 89 | 48 | 41 | |
| ***Tumor Size (cm)*** | | | | 0.273 |
| <4 | 107 | 56 | 51 | |
| ≥4 | 77 | 34 | 43 | |
| ***Distant metastasis*** | | | | 0.005 |
| Yes | 50 | 16 | 34 | |
| No | 134 | 74 | 60 | |
| ***FIGO stage*** | | | | 0.032 |
| I/II | 123 | 67 | 56 | |
| III/IV | 61 | 23 | 38 | |
| ***Histological grade*** | | | | 0.198 |
| Well/moderate | 114 | 60 | 54 | |
| Poor | 70 | 30 | 40 | |

For colony formation assay, 800 cells were put into each well of a 6-well plate and incubated for 14 days. When the colonies could be seen with naked eyes, the colonies were treated by 10% formaldehyde for 5 min, and crystal violet for 30 s. Finally, the concrete number of visible colonies was manually counted by the use of a microscope.

### Wound-Healing Assay

HeLa and CaSKi cells treated with si-CALML3-AS1 or si-NC were cultured as monolayers and were starved for relative synchronization in DMEM (Gibco, Weijia Technology, Guangzhou, Guandong, China) encompassing centesimal FBS. Then, a standard two hundred microliter pipette tip was used to remove a widespread strip of cells for wounding the above cells. After 56 h of culture, an inverted microscope was used to photograph all cells. An Image J software was applied for the calculation of the distances between all groups.

### Invasion Assay

For the cell invasion experiments, 24-well transwell coated with Matrigel (1 mg/ml) was purchased from BD Biosciences (Pudong, Shanghai, China). Treated cells were placed in media without serum and they were planted in the upper sides of the chambers. Six hundred microliter DMEM medium containing 25% FBS was then put in the lower chamber. After incubation for 24 h, the cells that finished invasion were then fixed with 6% paraformaldehyde and stained with hematoxylin. In addition, cotton swabs were used to remove other cells that remain unchanged from the upper 24-well. Finally, an inverted microscope was used to photograph invaded cells and their number was calculated using five random views.

### Annexin V/PI Staining

The possible roles of CALML3-AS1 in cells apoptosis of HeLa and CaSKi was determined using Annexin V/PI staining detection. Briefly, Trypsin-Ethylene Diamine Tetraacetic Acid (EDTA) solution was used for the digestion of above cells and it was then centrifuged on 2500 rpm for 5 min. Next, $2\times10^4$ cells which received washing with phosphate-buffered saline (PBS) were further suspended using 600 μl Binding Buffer. Then, the Apoptosis analyses kits (BestBio, Haidian, Beijing, China) was used to double-stain collected cells and the assays of the apoptosis rate (%) were carried out using flow cytometry (BD Biosciences, Franklin Lakes, NJ, USA ) within 1 h. Our operation was in line with the manufacturers' protocols.

### Luciferase Report Assay

HeLa and CaSKi cells after transfection were placed in each well of a plate. TOP flash or FOP flash and luciferase plasmid were purchased from Jilin Technology (Pudong, Shanghai, China). Then, the plasmids transfection was conducted by the use of Lipofectamine 2000 reagents (Invitrogen, Carlsbad, CA, USA). The dual Luciferase reporter detection kits (Promega, Madison, WI, USA) were used for the performance of the luciferase assays 48 h after transfections.

### Western Blot Assay

The related proteins in this study were detected using Western blot assays. Briefly, for the extraction of total cell lysates, the cells were treated using radioimmunoprecipitation assay (RIPA) buffer (Solabio, Tongzhou, Beijing, China) and Bradford methods were used to quantify proteins concentrations. Equal quantities (40 μg) of proteins were loaded on 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and subsequently transferred onto polyvinylidene difluoride (PVDF) membranes (JianWei, Guangzhou, China). The membranes were incubated with rabbit anti-β-Catenin (LoT No. ab106523, Abcam, China), anti-cyclin D1(LoT No. ab123799, Abcam, Pudong, Shanghai, China), anti-c-myc (LoT No. ab109521, Abcam, Pudong, Shanghai, China ) or anti-b-actin antibody (Abcam, Pudong, Shanghai, China ) at 4°C for 10-12 h, followed by being incubated using horseradish peroxidase (HRP)-conjugated goat anti-mouse antibodies (LoT No. ab109442, Abcam, Pudong, Shanghai, China) which was used as secondary antibodies for 1 h. Finally, proteins were scanned using enhanced chemiluminescence (ECL) kits (Thermo Fisher, Kunming, Yunnan, China).

### Statistical Analyses

All statistical analyses were carried out by SPSS 18.0 (SPSS Inc., Chicago, IL, USA). The Student *t*-test or One-way analysis of variance (ANOVA) was carried out for the determination of statistical significances. The Chi-square test was utilized to analyze the clinical significance of CALML3-AS1 with various clinicopathological parameters of CC patients. Survival curves were analyzed by the Kaplan-Meier methods. Univariate and multivariate analyses were used to estimate the prognostic factors for the above factors. The difference was considered significant at *p*-value less than 0.05.

## Results

### CALML3-AS1 is Upregulated in CC Cell Lines and Specimens

Identification of dysregulated lncRNA is the first step to study the roles of specific lncRNAs in tumors. Using TCGA datasets, we downloaded microarray data of CC samples and normal cervical tissues. Then, statistical methods were performed and the results were presented in Figures 1A and 1B using Heat Map and VOLCANO plots: a large number of abnormally expressed lncRNAs were shown. In this study, one of the most up-regulated lncRNA CALML3-AS1 attracted our attention (Figure 1C). for the demonstration of online findings, we further applied RT-PCR to determine the levels of CALML3-AS1 in CC tissues from our hospital. Figure 1D showed that CALML3-AS1 expressions were increased in the majority of CC specimens compared with the corresponding non-cancerous tissues ($p < 0.01$). In addition, the cells experiments using RT-PCR also revealed that CALML3-AS1 also exhibited increased levels in five CC cell lines compared with that in the Ect/E6E7 cells (Figure 1E). Overall, our experiments firstly identified a CC-related lncRNA CALML3-AS1 which may be involved in the development of CC.

### Clinical Prognostic Values of CALML3-AS1 Expression Levels in CC

For the exploration of the clinical significance of CALML3-AS1 in CC, CALML3-AS1 expressions in CC specimens were separated into high expressions (> 5.37-fold) and low expressions (< 5.37-fold). Then, the Chi-square test was performed and the results showed that CALML3-AS1 upregulations were distinctly correlated with distant metastasis ($p = 0.005$) and FIGO stage ($p = 0.032$) (Table I). However, CALML3-AS1 expressions were not related to other factors. ($p > 0.05$). Then, the relation between CALML3-AS1 expression and clinical survival were analyzed using Kaplan-Meier assays. As shown in Figure 1F, our data showed that CC patients with high CALML3-AS1 expressions had distinctly shorter overall survival time than those with low CALML3-AS1 expressions ($p = 0.0015$). Cox regression assays at the univariate level suggested that CALML3-AS1 expression, distant metastasis, and FIGO stage were associated with overall survival of CC patients (Table II). More importantly, the result by analyzing multivariate assays revealed that CALML3-AS1 expression (HR=2.729, 95% CI: 1.137-4.562, $p =$

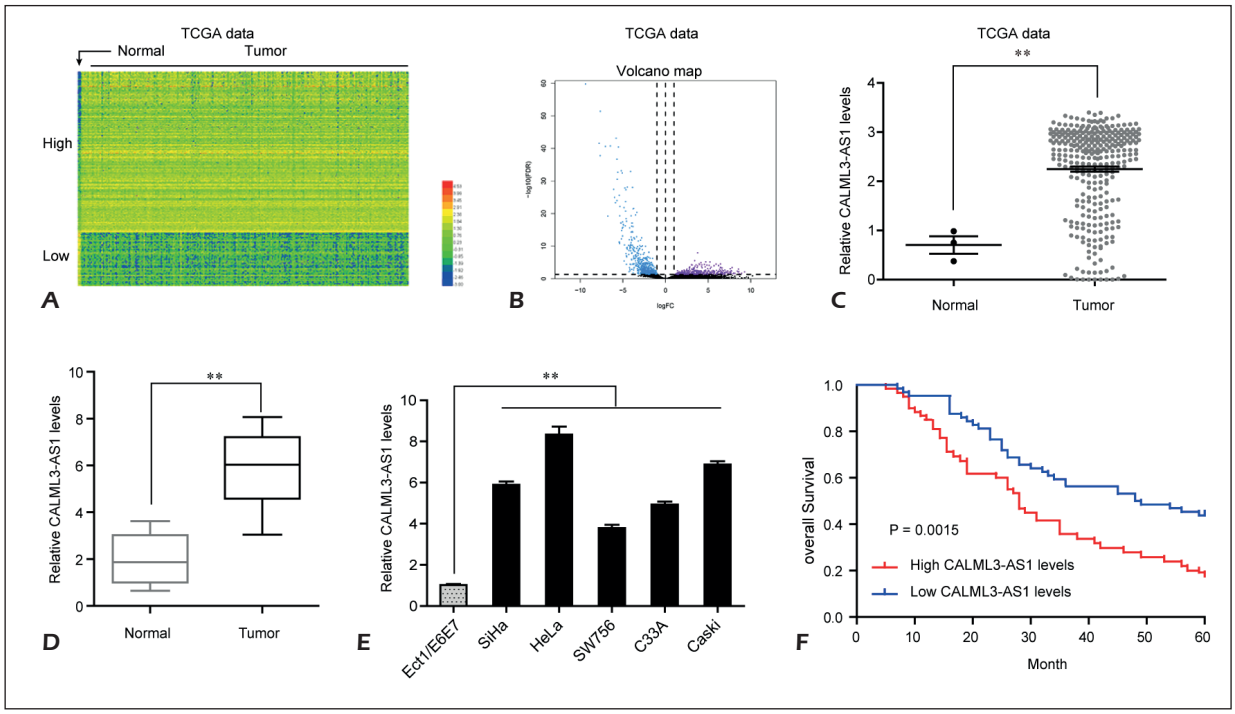


**Figure 1.** Up-regulation of CALML3-AS1 in CC and its clinical value in CC patients. **A**, Heat map diagram depicting expressions of dysregulated lncRNAs in CC samples by analyzing microarray data from TCGA datasets. **B**, Dysregulated lncRNAs in CC tissues were displayed using the VOLCANO plot. **C**, Expression levels of CALML3-AS1 in the TCGA cohorts. **D**, QRT-PCR experiments revealed that the expressions of CALML3-AS1 were increased in CC specimens. **E**, QRT-PCR assays of CALML3-AS1 in five CC cell lines and Ect1/E6E7. **F**, Kaplan-Meier plots exhibited overall survivals based on the levels of CALML3-AS1 in CC specimens. $^{**}p<0.01$, $^{*}p<0.05$.

0.009) was an independent prognostic factor for CC patients, in addition to distant metastasis and FIGO stage (Table II).

### *Effect of CALML3-AS1 on Cells Growth and Apoptosis Ability*

To study the potential influence of CALML3-AS1 on CC cellular functions, a siRNA specifically targeting CALML3-AS1 was designed for the down-regulation of CALML3-AS1. The results of RT-qPCR experiments detected the knockdown efficiencies of CALML3-AS1 in HeLa and CaSKi cells (Figure 2A). Then, Cell-proliferation assay (MTT) was performed and the results revealed that cell proliferation of HeLa and CaSKi after transfection was distinctly increased (Figure 2B). Consistent with the above findings, the number of cell colony formation was distinctly reduced in

**Table II.** Summary of univariate and multivariate Cox regression analyses of overall survival duration.

| Variables | Univariate analysis | | | Progression-free analysis | | |
|---|---|---|---|---|---|---|
| | HR | 95% CI | *p* | HR | 95% CI | *p* |
| Age | 0.985 | 0.437-1.664 | 0.231 | - | - | - |
| HPV | 1.218 | 0.672-1.994 | 0.186 | - | - | - |
| Tumor histology | 1.045 | 0.854-2.148 | 0.138 | - | - | - |
| Tumor Size | 1.138 | 0.582-2.194 | 0.113 | - | - | - |
| Distant metastasis | 3.237 | 1.374-5.019 | 0.003 | 2.895 | 1.194-4.372 | 0.013 |
| FIGO stage | 2.985 | 1.482-4.877 | 0.008 | 2.672 | 1.219-4.367 | 0.021 |
| Histological grade | 1.482 | 0.885-2.362 | 0.118 | - | - | - |
| CALML3-AS1 expression | 3.362 | 1.374-5.274 | 0.002 | 2.729 | 1.137-4.562 | 0.009 |

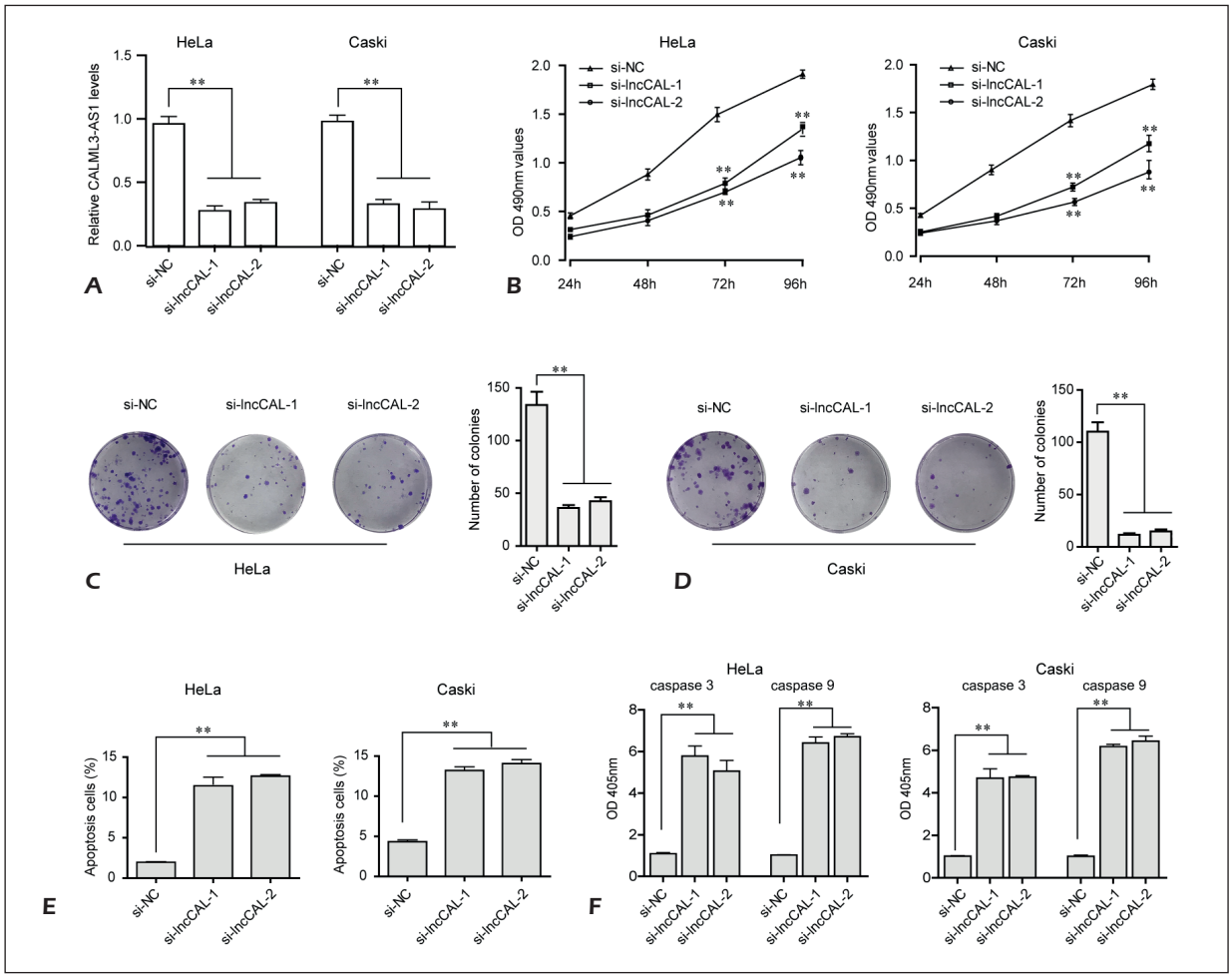


**Figure 2.** Downregulation of CALML3-AS1 suppressed the growth of CC cells. **A**, HeLa and Caski cells were transfected with siRNAs against CALML3-AS1. qRT-PCR was used for the determination of CALML3-AS1 levels. **B**, MTT assays were used to determine cell proliferation in HeLa and Caski cells. **C-D**, Shown are classic photographs of colony formation assays of HeLa (**C**) and Caski (**D**) cells for 14 days (Magnification: 10×). **E**, Flow cytometric analysis of apoptosis in CC cells. **F,** Activity detections of caspase 3 and caspase 9. $^{**}p<0.01$, $^{*}p<0.05$.

cells treated with si-CALML3-AS1. However, the control cells were shown to have an opposite trend (Figures 2C and 2D). Then, we further explored whether CALML3-AS1 functions as a suppressor in cellular apoptosis in CC by flow cytometric analyses. The data suggested that down-regulation of CALML3-AS1 in HeLa and CaSKi cells distinctly increased cells apoptosis by 6.13-fold compared to controls (Figure 2E). For a better understanding of the potential mechanism involved in increased apoptosis ability, we performed Western Caspase 3/9 activity detection to explore the influence of CALML3-AS1 apoptosis-related proteins, finding that the activity of Caspase 3/9 was elevated in cells transfected with si-CALML3-AS1 (Figure 2F). Overall, our finding revealed that CALML3-AS1 has a functional effect on CC growth.

### *Effect of CALML3-AS1 on CC Cell Invasion and Migration*

To investigate whether CALML3-AS1 possessed promotive abilities to influence CC cells metastasis, we further performed Wound-healing assays and transwell assays using HeLa and CaSKi cells. As shown in Figures 3A and 3B, the result of Wound-healing assays suggested that the migratory capacities of HeLa and CaSKi cells were distinctly reduced after the suppression of CALML3-AS1. In addition, transwell assays also revealed that silencing CALML3-AS1 inhibited the invasive cells number compared with negative control transfections (Figure 3C). Overall, our findings showed the tumor-promotive roles of CALML3-AS1 metastasis ability of CC cells.

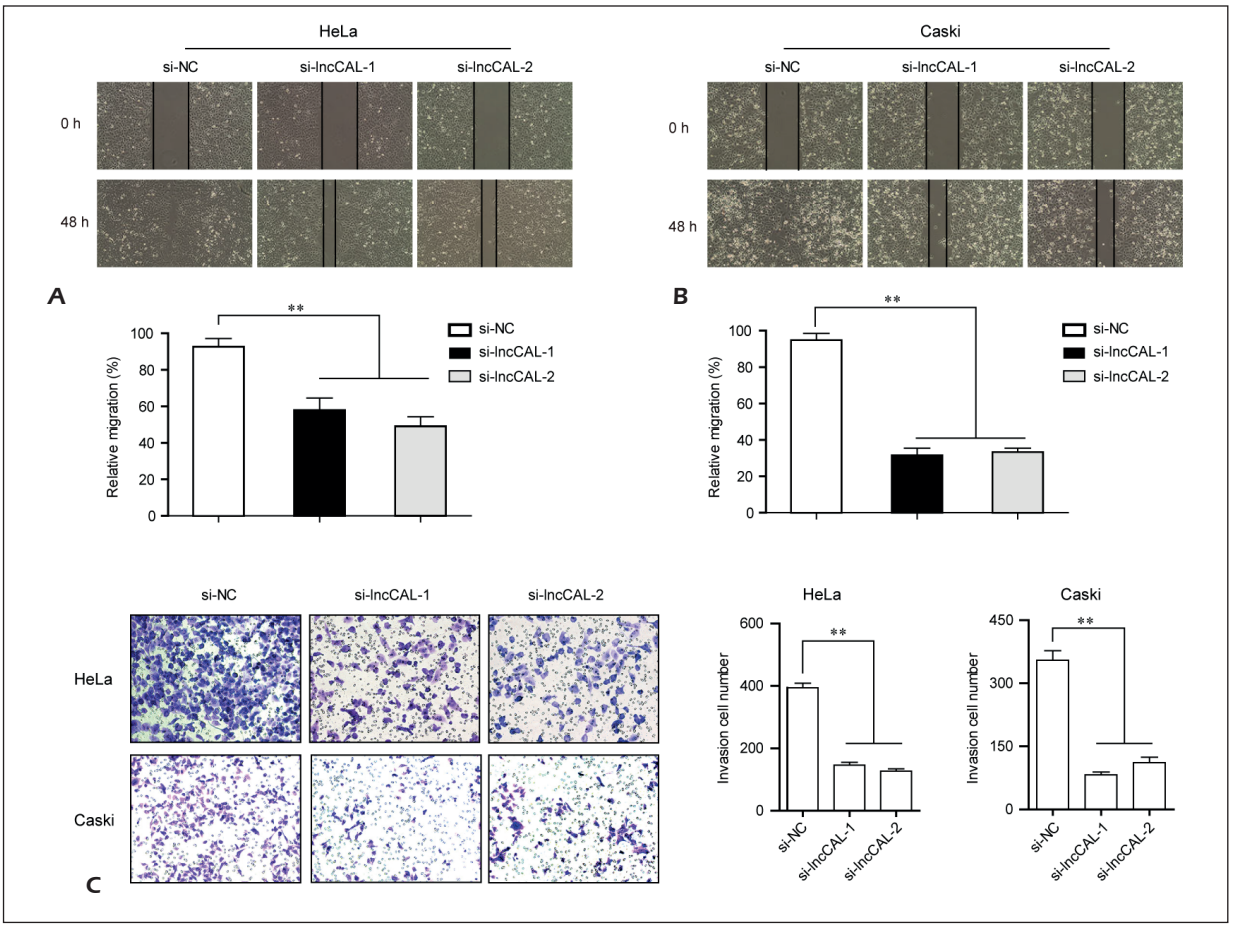


**Figure 3.** Knockdown of CALML3-AS1 inhibited CC cellular invasion and migration. A, B, Wound-healing assays were used to examine HeLa (**A**) and Caski (**B**) cells migration capacities (Magnification: 10×). **C**, Transwell assays were applied to examine HeLa (**A**) and Caski (**B**) cells invasion capacities (Magnification: 40×). $^{**}p<0.01$, $^{*}p<0.05$.

### *CALML3-AS1 Modulate the Wnt/β-Catenin Pathway in CC Progression*

There are many studies showing that Wnt/β-catenin signaling played key roles in cells growth and metastasis in various tumors, including CC[15,16]. To understand the potential mechanisms by which CALML3-AS1 contributed to the development of CC, our group focused on the associations between CALML3-AS1 and Wnt/β-catenin pathway. Using the TOP-Flash reporter assays, it was observed that CALML3-AS1 down-regulation led to a distinct suppression of TOP-Flash reporter activity in CC cells (Figures 4A and 4B). Then, RT-PCR assays and Western blot were performed for the detection of the related proteins of the Wnt/β-catenin pathway. As shown in 4C, the mRNA levels of β-catenin, cyclin D1, and c-myc were distinctly suppressed in CC cells treated with si-CALML3-AS1. In addition, the results of Western blot revealed that the protein levels of the above genes also displayed a similar trend (Figure 4D). Taken together, our data demonstrated that CALML3-AS1-mediated progression of CC may be mediated by the Wnt/β-catenin pathway,

## Discussion

CC is considered to be one of the most serious cancers in women with more than 535,200 CC patients diagnosed annually[17]. The early detections of tumors can distinctly reduce cancer mortality and promote long-term survival. Although generous efforts have been devoted to the identification of novel biomarkers which can be used diagnosis and prognosis prediction, suitable and sensitive biomarkers remain difficult to discover[18,19]. In recent years, growing works supported the great potential of lncRNAs as novel biomarkers. In this

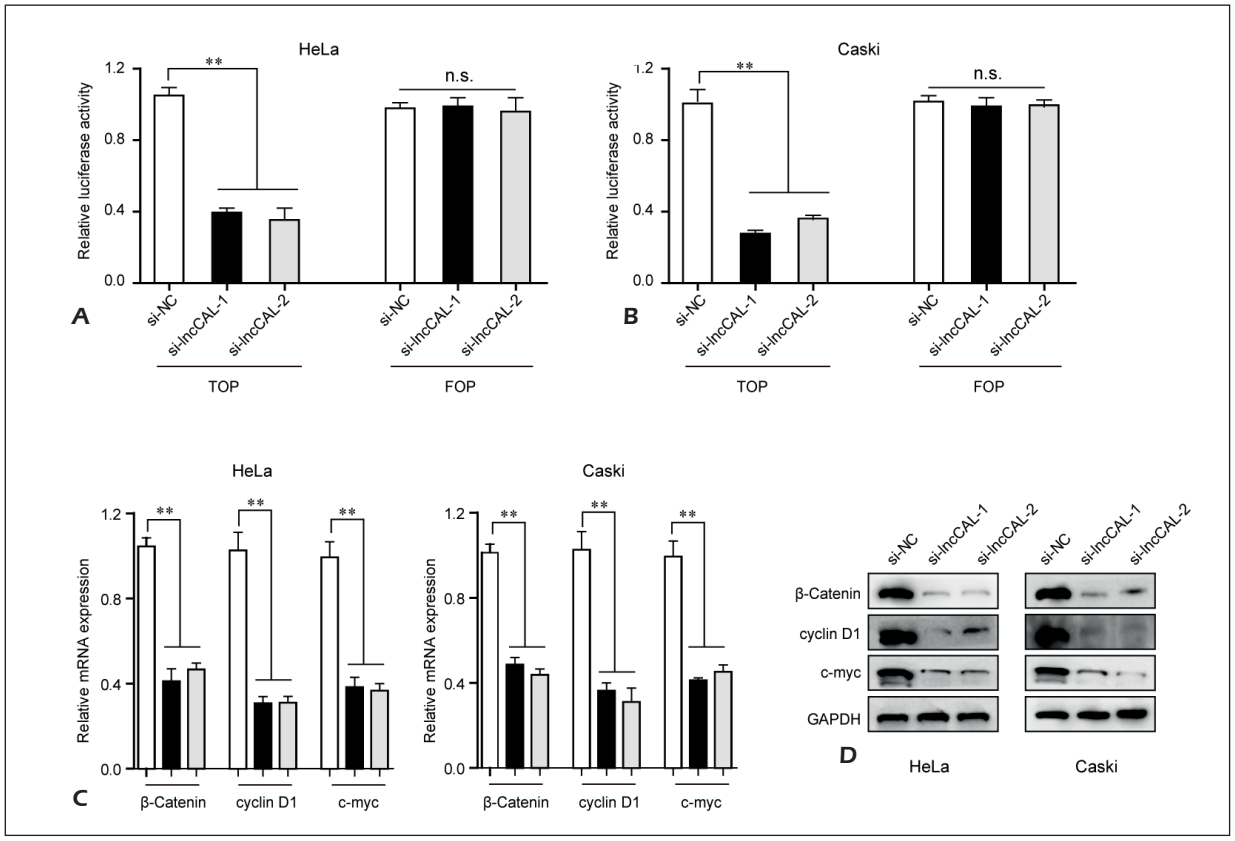


**Figure 4.** Down-regulation of CALML3-AS1 suppressed the activity of the Wnt/β-catenin pathway. **A**-**B**, TOP-flash luciferase activity was determined for the study of influence of si-CALML3-AS1 on Wnt/β-catenin pathway in HeLa (**A**) and Caski (**B**) cells. **C**-**D**, MRNAs (**C**) and proteins (**D**) levels of related proteins in si-CALML3-AS1 transfected HeLa and Caski cells were measured using Western blot assays. $^{**}p<0.01$, $^{*}p<0.05$.

research, we used microarray data from TCGA to identified CC-related lncRNAs and identify CALML3-AS1 as a potential candidate. The collected data by the use of RT-PCR also revealed CALML3-AS1 as an overexpressed lncRNA in both CC tissues and cell lines. In addition, the correlation and survival assays demonstrated that patients with higher CALML3-AS1 expressions exhibited advanced clinical stages and poorer overall survival. Moreover, by the use of multivariate assays, CALML3-AS1 was an independent prognostic factor for patients. Previously, several lncRNAs have been demonstrated to possess prognostic value in CC, such as lncRNA HCP5, lncRNA PANDAR and lncRNA PVT1[20-22]. Thus, our findings added novel lncRNA which may act as a biomarker for CC again.

Growing studies have revealed lncRNAs acted as contributors in a diversity of biological functions in CC. For instance, Feng et al[23] reported that lncRNA LOC105374902 which was induced by TNF-α was overexpressed in CC and its reduced expressions suppressed the metastasis and EMT by sponging miR-1285. LncRNA XIST, a newly identified regulator in tumors, was found to be up-regulated in CC by Zhu et al[24] and serve as a tumor promoter because of its knockdown suppressing cells proliferation and migration by the modulation of miR-200a. Jiang et al[25] suggested that higher levels of lncRNA TPT1-AS1 were associated with shorter overall survival, and its knockdown using si-TPT1-AS1 resulted in suppressor abilities of cells growth and migration in CC. Recently, the effects of CALML3-AS1 in bladder cancer was also reported. Increasing levels of CALML3-AS1 were firstly demonstrated in this disease using RT-PCR. Clinical investigations also confirmed its prognostic values in bladder cancer patients. Functional experiments with a series of cells experiments suggested that CALML3-AS1 served as a regulator by promoting cells proliferation and metastasis of bladder cancer by regulating miR-4316. Previous findings have confirmed CALML3-AS1 as an

overexpressed lncRNA in CC. Thus, we wondered whether CALML3-AS1 also displayed tumor-promotive roles in this cancer. As expected, suppression of CALML3-AS1 displayed anti-cancer effects in CC cells, suggesting it as a novel tumor promoter involved in CC tumorigenesis and progression.

Since the preliminary breakthrough of the original member of the Wnt family thirty-six years ago, the interest of clinical and fundamental studies in Wnt signaling has gradually risen[26]. Wnt/β-catenin pathway, conserved in evolutions and extensive in its activities, is one of the well-studied biological pathways which is implicated in cellular processes[27,28]. Given the regulator values of Wnt signaling for cells development, it is not amazing that the dysregulations of the Wnt/β-catenin pathway are always detected in various tumors[29]. A large number of tumor-related genes were found to exhibit their tumor-promotive or anti-cancer roles by modulating this pathway. In addition, several lncRNAs were also reported to play a functional role via the regulation of Wnt/β-catenin pathway[30,31]. In this study, our group using TOP/FOP assays found that knockdown of CALML3-AS1 suppressed the activity of the Wnt/β-catenin pathway. Moreover, in CC cells transfected with si-CALML3-AS1, the levels of the related proteins of Wnt/β-catenin pathway including β-catenin, cyclin D1, and c-myc were observed to be distinctly down-regulated, suggesting that the activity of this pathway was suppressed. Our data revealed CALML3-AS1 serve as a tumor promoter via promoting Wnt/β-catenin pathway.

## Conclusions

We identified, for the first time, CALML3-AS1 as an overexpressed lncRNA in CC and provided clinical evidence that higher levels of CALML3-AS1 predicted a poorer outcome in CC patients. The functional study confirmed that CALML3-AS1 served as an anti-metastatic lncRNA by modulating Wnt/β-catenin pathway in CC. Those findings suggested that CALML3-AS1would be a potential prognostic biomarker and therapeutic target for CC.

### Conflict of Interests

The Authors declared that they have no conflict of interests.

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