lncRNA DSCAM-AS1 facilitates the progression of endometrial cancer via miR-136-5p

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Abstract. Previous studies have indicated that long non-coding RNA (lncRNA) down syndrome cell adhesion molecule antisense 1 (DSCAM-AS1) serves an oncogenic role in numerous cancer types. However, its role in endometrial cancer (EC) remains largely unknown. In the present study, DSCAM-AS1 expression levels in EC tissues and cells and their normal counterparts were analyzed using reverse transcription-quantitative. *In vitro* and *in vivo* experiments were conducted to validate the functions of DSCAM-AS1 in EC. It was revealed that DSCAM-AS1 was expressed at a high level in EC tissues and cells after analyzing patient data and data obtained from The Cancer Genome Atlas. Notably, it was also revealed that high DSCAM-AS1 expression was associated with a less favorable overall survival in patients with EC. Knockdown of DSCAM-AS1 was able to suppress EC cell proliferation by upregulating cell apoptosis *in vitro*. Furthermore, it was revealed that DSCAM-AS1 acted as a microRNA (miR)-136-5p sponge to exert its oncogenic roles in EC. Collectively and to the best of our knowledge, the current results provided first evidence that DSCAM-AS1 stimulated EC progression by regulating miR-136-5p, which may improve the understanding of the roles of ncRNAs in EC, and may help identify novel targets for anticancer treatment.

Introduction

Endometrial cancer (EC) accounts for around 76,000 deaths in females worldwide each year (1,2). Abnormal gene expression regulation results in numerous types of human diseases, including cancer. Non-coding RNA (ncRNA) represents a large proportion of human genome transcripts (3). Long non-coding RNA (lncRNA) is a large family of ncRNAs that accounts for 80% of all RNA transcripts. Emerging evidence has indicated that lncRNAs serve vital roles in carcinogenesis and cancer progression (4,5). Multiple studies have also proposed different mechanisms of action for lncRNAs, the key mechanism of which is named the competing endogenous RNAs (ceRNAs) theory (6).

Down syndrome cell adhesion molecule antisense 1 (DSCAM-AS1) was reported to serve as an oncogenic lncRNA in different cancer types (7-9). High DSCAM-AS1 levels were identified as a predictor for the overall survival of patients with colorectal cancer (7). Moreover, DSCAM-AS1 was determined to stimulate non-small cell lung cancer progression by regulating high mobility group box protein 1 expression via sponging microRNA (miR)-577 (8). Another study indicated that DSCAM-AS1 promoted breast cancer progression by regulating the miR-204-5p/ribonucleotide reductase M2 axis (9). However, it is unclear whether DSCAM-AS1 serves a role in affecting EC carcinogenesis and progression.

The aim of the present study was to analyze DSCAM-AS1 expression level in EC tissues and cells and to explore its biological roles in regulating EC progression. In addition, the detailed acting mechanism of DSCAM-AS1 in EC was analyzed using both *in vitro* and *in vivo* experiments.

Materials and methods

Patient tissues. EC tissues and adjacent non-cancerous tissues were collected from 34 patients (mean age, 59.3 years; age range, 47-68 years) who underwent treatment at The Second Affiliated Hospital of Fujian Medical University (Fujian, China) between January 2015 and December 2015. Non-cancerous tissues were collected at 2 cm distant from tumor tissues. The study protocol was approved by the Ethics Committee of The Second Affiliated Hospital of Fujian Medical University. Inclusion criteria for patients were as follows: i) Diagnosed as EC by medical examination; ii) did not receive any anticancer treatments; and iii) without other malignancies or chronic diseases. Additionally, patients who did not have complete clinical information were excluded. The treatment protocols of patients with EC were in accordance with the Diagnosis and Therapy Guideline for Endometrial cancer 4th edition (10). After surgery, patients were treatment with radiotherapy, chemotherapy, or combination

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therapies. Estrogen receptor (ER), Lymph-vascular space invasion (LVSI) and histology type were classified by pathologists at our hospital in a blinded manner according to the criteria of previous literature (1-13). Written informed consent was obtained from all enrolled patients.

Cell culture and treatment. The EC cells (HEC-1-B, HEC-1-A and KLE) used in the present study were purchased from the American Type Culture Collection, while normal uterine endometrial epithelial cells (NUEEC) were obtained from Chi Scientific, Inc. RPMI-1640 (Invitrogen; Thermo Fisher Scientific, Inc.) supplemented with 10% fetal bovine serum (Invitrogen; Thermo Fisher Scientific, Inc.), 100 U/ml penicillin, and 0.1 mg/ml streptomycin were used to incubate cells. Cell culture was conducted at 37˚C in an incubator filled with 5% CO_2.

Cell transfection. Small interfering (si)RNA against DSCAM1-AS1 (si-DSCAM1-AS1, 5'-GUUCUGGUCUCA UCAUGAUTT-3'), control siRNA (si-con, 5'-AUAGAC UCGCUUGUUGUUCTT-3'), miR-136-5p mimic (5'-CAU CAUCGUCUCAAAUGAGUCU-3'), miR-136-5p inhibitor (5'-AGACUCAUUUGAGACGAUGAUG-3') and control miRNA (mimic-con, 5'-ACAUUAACGUAUCGUCACUCU G-3'; or inhibitor-con, 5'-GCGAGAUCGCUGAUAUGAAUA U-3') were synthesized by Shanghai GenePharma Co., Ltd. miRNA or siRNA (50 nM) transfection was conducted using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) at 37˚C according to the manufacturer's instructions. After 48 h transfection, cells were collected for subsequent analyses.

Cell Counting Kit-8 (CCK-8) assay. Cell proliferation rate was measured using a CCK-8 assay (Sigma-Aldrich; Merck KGaA). In brief, cells were seeded into 96-well plate at a density of $4x10^3$ cells/well. Plates were maintained at 37˚C in the incubator, as aforementioned. A total of 10 μl CCK-8 reagent was added after 24, 48 and 72 h incubation and then incubated for a further 2 h at 37˚C. Optical density at the wavelength of 450 nm was measured using microplate reader.

Flow cytometry assay. Cell apoptosis was measured using the Annexin-V-FITC/PI cell apoptosis kit (Beyotime Institute of Biotechnology). After treatment with 0.25% trypsin at 37˚C for 2 min, $5x10^6$ cells were collected, suspended in binding buffer and stained with 5 μl Annexin-V-FITC at 4˚C for 15 min in the dark. Then, 5 μl PI was added to cells and incubated at 4˚C for 5 min. Finally, cell apoptosis rate was measured using LSRFortessa™ (BD Biosciences) and analyzed with FlowJo 10.7 software (BD Biosciences). The cells with the Annexin-FITC label were regarded as apoptotic cells.

Reverse transcription-quantitative (RT-qPCR) analysis. RNA samples of tissues and cultured cells were isolated with TRIzol® kit (Invitrogen; Thermo Fisher Scientific, Inc.) and reverse transcribed into complementary DNA using the PrimeScript® RT Reagent kit (Takara Biotechnology Co., Ltd.) according to the provided protocols. RT-qPCR was performed using an ABI 7500 PCR instrument (Applied Biosystems; Thermo Fisher Scientific, Inc.) using SYBR Green (Takara Biotechnology Co., Ltd.). Primers were synthesized by Sangon Biotech Co., Ltd. and the sequences were as follows: DSCAM-AS1 forward, 5'-GTGACACAGCAAGACTCC CT-3' and reverse, 5'-GATCCGTCGTCCATCTCTGT-3'; GAPDH forward, 5'-AAGGTGAAGGTCGGAGTCAA-3' and reverse, 5'-AATGAAGGGGTCATTGATGG-3'; miR-136-5p forward, 5'-ACACTCCAGCTGGGACTCCATTTGTTTT-3' and reverse, 5'-CCAGTGCAGGGTCCGAGGT-3'; and U6 small nuclear (sn)RNA (U6 snRNA) forward, 5'-TCCGAT CGTGAAGCGTTC-3' and reverse, 5'-GTGCAGGGTCCG AGGT-3'. Gene expression levels were calculated with the $2^{-\Delta\Delta Cq}$ method (14). The thermocycling conditions used were as follows: 95˚C for 30 sec (1 cycle), 95˚C for 5 sec; and 60˚C for 30 sec (40 cycles).

Target prediction. miRNA targets for DSCAM-AS1 were analyzed using the Encyclopedia of RNA Interactomes (ENCORI) (http://starbase.sysu.edu.cn/agoClipRNA.php?source=lncRNA&flag=target&clade=mammal&genome=human&assembly=hg19&miRNA=all&clipNum=1&deNum=0&panNum=0&target=DSCAM-AS1). Among all predicted targets results in ENCORI, miR-136-5p ranked first and therefore was selected for subsequent analyses.

Dual-luciferase activity reporter assay. Chemically synthesized wild-type (WT) 3'-untranslated region sequence of DSCAM-AS1 was inserted into a pMIR-reporter (Promega Corporation) to generate a WT-DSCAM-AS1 construct. A site-direct mutagenesis kit (Takara Biotechnology Co., Ltd.) was used to generate MT-DSCAM-AS1 construct from WT-DSCAM-AS1. WT-DSCAM-AS1 or MT-DSCAM-AS1 and the aforementioned miRNAs were co-transfected into EC cells using Lipofectamine® 2000 according to the manufacturer's instructions. After 48 h, cells were collected to measure relative luciferase activity using the Dual-luciferase activity system (Promega Corporation) with *Renilla* luciferase activity used as the internal control.

DSCAM-AS1 expression level analysis in EC using online database. DSCAM-AS1 expression level in EC tissues and normal tissues was measured using ENCORI.

RNA immunoprecipitation (RIP) assay. A Magna RIP RNA-Binding Protein Immunoprecipitation kit (EMD Millipore) was utilized to detect the potential interactions of DSCAM-AS1 and miR-136-5p based on supplier's instructions. Cells were lysed with RIP buffer and then incubated with anti-Argonaute 2 (anti-Ago2, MA5-23515, Thermo Fisher Scientific, Inc.) or normal immunoglobulin G (IgG)-conjugated magnetic beads. RNA samples were then extracted using TRIzol® and subjected to RT-qPCR analysis (as described in the RT-qPCR section) to detect relative DSCAM-AS1 and miR-136-5p expression levels using the protocols described above.

In vivo tumorigenesis assay. The animal experiment protocol was approved by the Ethics Committee of The Second Affiliated Hospital of Fujian Medical University. BALB/C nude female mice (4 weeks old; n=5 for each group, 10 in total; 18-20 g) were purchased from the National Laboratory Animal Center and cultured in specific pathogen-free conditions with

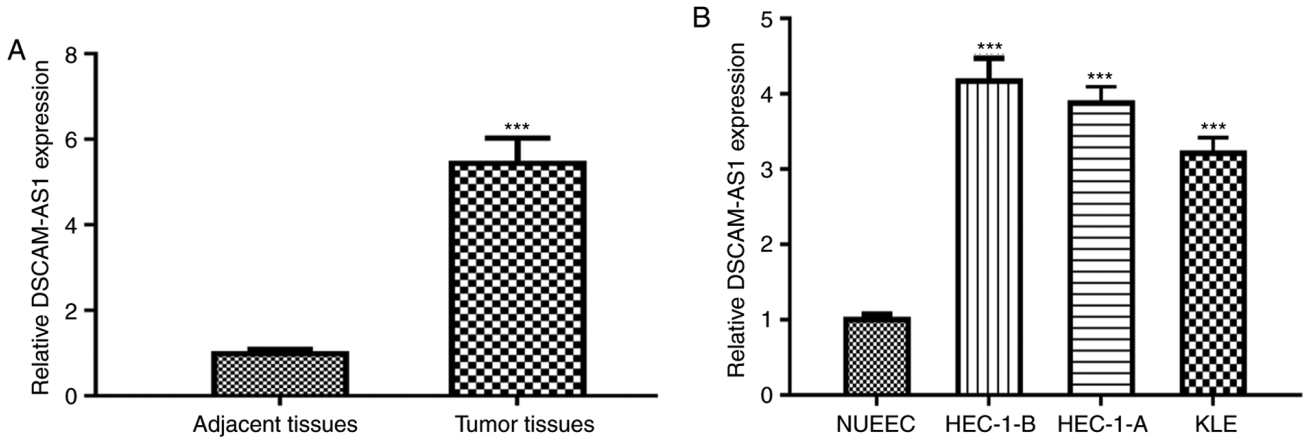

Figure 1. DSCAM-AS1 is upregulated in EC tissues and cell lines. (A) The expression of DSCAM-AS1 was measured in 34 pairs of EC and adjacent normal tissues. ***P<0.001 vs. normal tissues. (B) The expression of DSCAM-AS1 was measured in EC cells (HEC-1-B, HEC-1-A and KLE) and NUEECs by reverse transcription-quantitative PCR (***P<0.001 vs. NUEEC). DSCAM-AS1, down syndrome cell adhesion molecule antisense 1; EC, endometrial cancer; NUEEC, normal uterine endometrial epithelial cells.

controlled temperature (23±3˚C) and humidity (40±10%), and under a 12 h light/dark cycle. Mice had free access to laboratory food and water. Sample size calculation was accompanied using an online calculation tool (https://clincalc.com/). sh-DSCAM-AS1 (5'-CCGGGCTGCAGTGAGCTGAGATCATCTCGAGATGATCTCAGCTCACTGCAGCTTTTTG-3') or sh-con 5'-CCGGGCTGTTAAGCCGTGAGAGCTACTCGAGTAGCTCTCACGGCTTAACAGCTTTTTG-3; both Sangon Biotech Co., Ltd. were cloned into pLKO.1 and packaged into lentivirus particles together with psPAX2 and pMD2.G through co-transfecting 293T cells with Polyethylenimine (PEI, 1 mg/ml, Sigma-Aldrich; Merck KGaA) in DMEM supplemented with 10% fetal bovine serum w at 37˚C incubator filled with 5% CO_2. After 48 h, lentivirus particles were collected from supernatant and then transduced into HEC-1-B cells. Stably infected DSCAM-AS1-silenced cells were selected via puromycin (0.5 mg/ml) for 7 days. The successful transduction of sh-DSCAM-AS1 was confirmed by RT-qPCR. Then, $1x10^7$ cells in PBS were injected into the right flank of each mouse. On the 7th day after injection, tumor width and length were measured weekly to calculate tumor volume using the following formula: length x $width^2$ x 0.5. After 4 weeks, mice were sacrificed via cervical dislocation. In accordance with the IACUC guidelines, the tumor diameter was maintained at <1.5 cm throughout the experiment period. Finally, tumor tissues were collected and weighed.

Statistical analysis. Data obtained from three independent experiments were analyzed using SPSS 21.0 software (IBM Corp.) and presented as the mean ± SD. Paired Student's t-test was conducted to analyze differences between two groups, while one-way ANOVA followed by Dunnett's post hoc test was conducted to analyze differences among three or more groups. Log-rank test was used to analyze survival difference between high- and low-DSCAM-AS1 groups using the mean expression value as cut-off value. The χ^2 test was used to calculate associations between DSCAM-AS1 expression and clinicopathological features of patients with EC. Spearman's correlation co-efficient for DSCAM-AS1 and miR-136-5p was calculated. P<0.05 was considered to indicate a statistically significant difference.

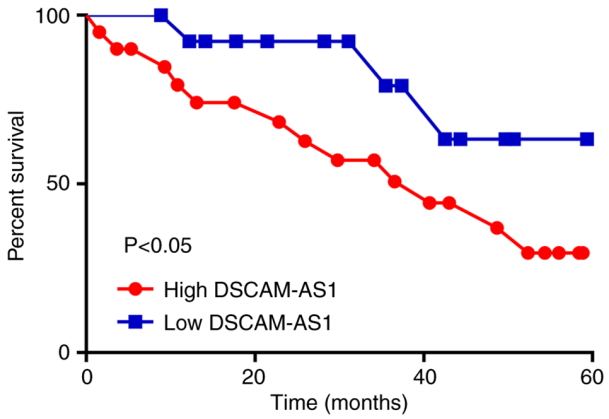

Figure 2. Comparison of overall survival between patients with endometrial cancer harboring high or low DSCAM-AS1 expression. DSCAM-AS1, down syndrome cell adhesion molecule antisense 1.

Results

DSCAM-AS1 is highly expressed in EC. DSCAM-AS1 expression levels were analyzed in EC tissues. The results revealed that DSCAM-AS1 was highly expressed in EC tissues compared with normal tissues (Fig. 1A). Additionally, analysis of TCGA data revealed that DSCAM-AS1 expression was also elevated in clinical EC samples (Fig. S1). Moreover, the DSCAM-AS1 expression level was higher in EC cells (HEC-1-B, HEC-1-A and KLE) compared with in NUEEC cells (Fig. 1B).

High DSCAM-AS1 expression is associated with poor overall survival of patients with EC. Patients were classified into two groups based on mean DSCAM-AS1 expression level (2.78). Patients with low DSCAM-AS1 expression levels exhibited better overall survival than those with high expression (Fig. 2). In addition, it was demonstrated that high DSCAM-AS1 levels were significantly associated with FIGO stage and lymph node metastasis, but were not associated with other clinical features (Table I).

Table I. Association of DSCAM-AS1 expression and clinicopathological features of EC patients.

Variable	n	DSCAM-AS1 expression		χ^2-value	P-value
		High	Low		
Age (years)				2.835	0.092
≥50	18	13	5		
<50	16	7	9		
FIGO stage				5.247	0.022[a]
I-II	14	5	9		
III	20	15	5		
Grade				0.486	0.485
G1/G2	17	11	6		
G3	17	9	8		
Lymph node metastasis				3.927	0.048[a]
No	15	6	9		
Yes	19	14	5		
Myometrial invasion				0.064	0.800
<1/2	21	12	9		
<1/2	13	8	5		
Estrogen receptor				0.105	0.746
Positive	16	8	8		
Negative	18	8	10		
Lymph-vascular space invasion				0.007	0.933
Positive	13	7	6		
Negative	21	10	11		
Histological type				1.574	0.455
Serous	12	5	7		
Endometrioid	15	8	7		
Mixed serous and endometrioid	7	5	2		

[a]P<0.05. DSCAM-AS1, down syndrome cell adhesion molecule antisense 1.

Knockdown of DSCAM-AS1 suppresses EC cell growth. Subsequently, si-DSCAM-AS1 was transfected into HEC-1-B and HEC-1-A cells and it was revealed that DSCAM-AS1 levels were significantly decreased by si-DSCAM-AS1 in both cells (Fig. 3A). The CCK-8 assay revealed that the proliferation rate of HEC-1-B and HEC-1-A cells was decreased following si-DSCAM-AS1 transfection (Fig. 3B and C). Furthermore, flow cytometry revealed that knockdown of DSCAM-AS1 stimulated HEC-1-B and HEC-1-A cell apoptosis (Fig. 3D and E).

DSCAM-AS1 directly interacts with miR-136-5p. miR-136-5p was identified as the top DSCAM-AS1 potential target (Fig. 4A). The transfection of the miR-136-5p mimic significantly increased, while miR-136-5p inhibitor decreased miR-136-5p levels in both cell lines (Fig. 4B). It was also revealed that miR-136-5p overexpression significantly decreased relative luciferase activity in WT-DSCAM-AS1-transfected HEC-1-B and HEC-1-A cells (Fig. 4C and D). Moreover, miR-136-5p exhibited a decreased expression in EC samples and cells compared with the normal counterparts (Fig. 4E and F). The results of the RIP assay revealed that DSCAM-AS1 and miR-136-5p can directly interact with each other (Fig. 4G and H). Subsequently, DSCAM-AS1 was enriched in the miR-136-5p mimic-transfected cells following anti-Ago2 treatment (Fig. 4I). In addition, si-DSCAM-AS1 transfection significantly increased miR-136-5p levels in HEC-1-B and HEC-1-A cells (Fig. 4J and K). Notably, miR-136-5p levels were negatively correlated with DSCAM-AS1 levels in EC tissues (Fig. 4L).

DSCAM-AS1 regulates EC cell growth by targeting miR-136-5p. To validate the functional associations of DSCAM-AS1 and miR-136-5p in EC, rescue experiments were conducted. RT-qPCR analysis revealed that miR-136-5p levels were elevated following si-DSCAM-AS1 transfection, which was partially abrogated by miR-136-5p knockdown (Fig. 5A). The CCK-8 assay revealed that the inhibitory

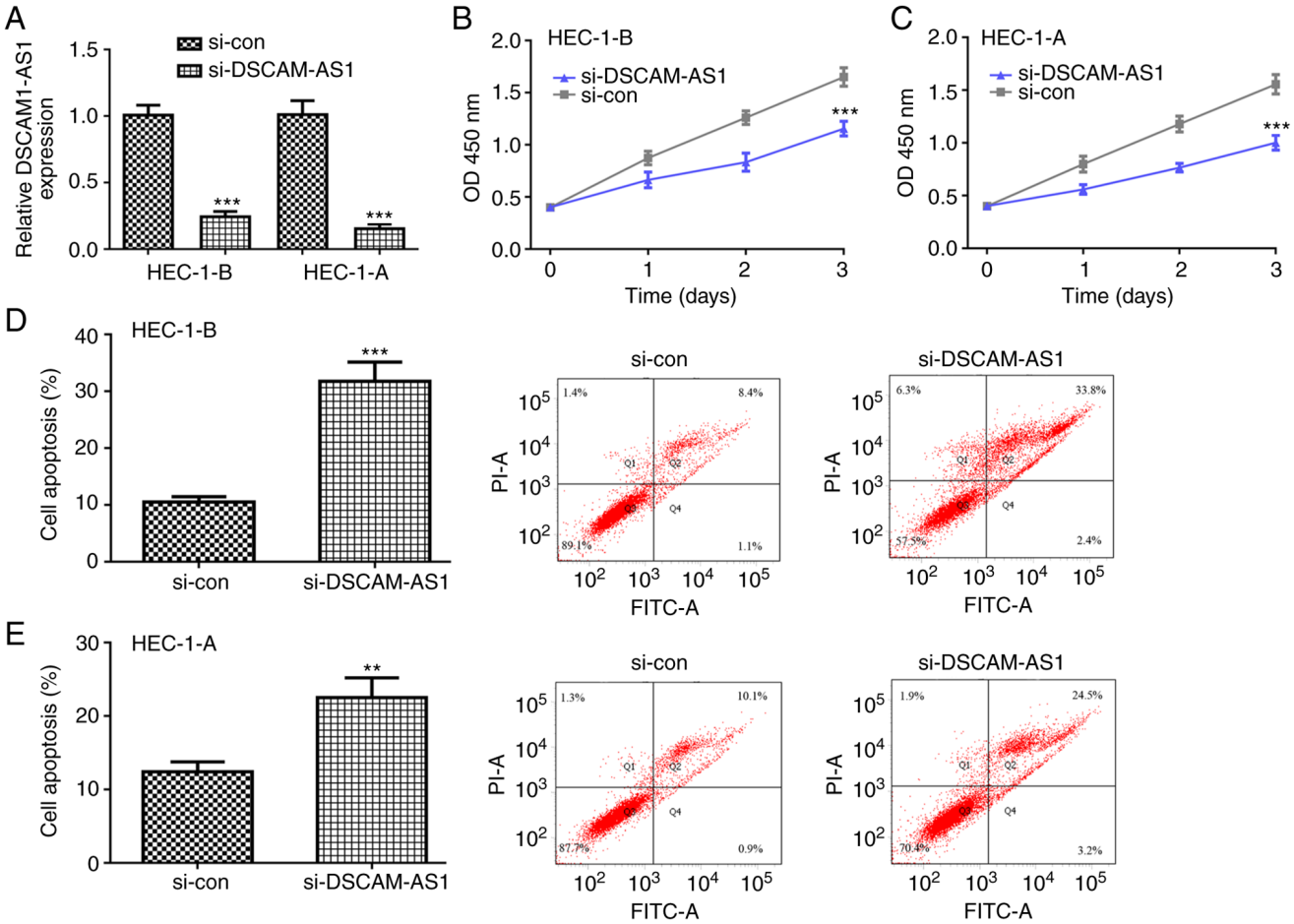

Figure 3. Inhibiting expression of DSCAM-AS1 suppresses growth in EC cells. (A) The transfection efficacy of si-DSCAM-AS1 was measured by reverse transcription-quantitative PCR assay. Cell proliferation was evaluated in (B) HEC-1-B and (C) HEC-1-A cells using the Cell Counting Kit-8 assay. The percentage of cell apoptosis was evaluated in (D) HEC-1-B and (E) HEC-1-A cells using flow cytometry. **P<0.01 and ***P<0.001 vs. si-con. DSCAM-AS1, down syndrome cell adhesion molecule antisense 1; EC, endometrial cancer; si-DSCAM-AS1, small interfering RNA against DSCAM-AS1; si-con, negative control siRNA.

effects of DSCAM-AS1 knockdown on HEC-1-B cell proliferation were attenuated by miR-136-5p knockdown (Fig. 5B). Meanwhile, the flow cytometry assay indicated that inhibition of miR-136-5p partially reversed the stimulatory effects of DSCAM-AS1 knockdown on regulating cell apoptosis (Fig. 5C).

Silencing of DSCAM-AS1 suppresses EC tumor growth. Finally, the effects of DSCAM-AS1 on EC tumor growth were explored. It was revealed that mice injected with EC cells with stable knockdown of DSCAM-AS1 had smaller tumors compared to the sh-con group (Fig. 6A). The decreased expression of DSCAM-AS1 following sh-DSCAM-AS1 transfection was also confirmed by RT-qPCR (Fig. 6B). In addition, it was demonstrated that the weight of tumors from the sh-DSCAM-AS1 group were significantly lower than those from the sh-con group (Fig. 6C).

Discussion

In the present study, DSCAM-AS1 was found to be expressed at a high level in EC samples, and strongly associated with a less favorable overall survival, higher FIGO stage and poorer lymph node metastasis in patients with cancer. EC is prone to metastasis and this typically results in a poor prognosis; therefore, it is necessary to better understand the mechanisms underlying EC progression. Notably, recent studies have revealed that lncRNAs can affect the malignant behavior of cells (15,16). In addition, numerous studies have revealed that DSCAM-AS1 can affect tumorigenesis, but its role in EC remains unclear (7-9,17,18). Therefore, the aim of the present study was to investigate the biological functions of DSCAM-AS1 in EC.

Functional experiments were conducted in the present study, the results of which revealed that DSCAM-AS1 silencing inhibits EC cell proliferation via promoting cell apoptosis *in vitro*. In addition, *in vivo* animal experiments revealed that knockdown of DSCAM-AS1 suppressed EC tumor growth. Previous studies have reported that DSCAM-AS1 serves as oncogenic lncRNA in cancers including colorectal cancer, non-small cell lung cancer, breast cancer, cervical cancer and hepatocellular carcinoma (7-9,17,18). Consistent with these findings, the present study also indicated that DSCAM-AS1 could stimulate EC progression.

The ceRNA theory helps explain the underlying mechanisms of ncRNAs, with the hypothesis that lncRNAs

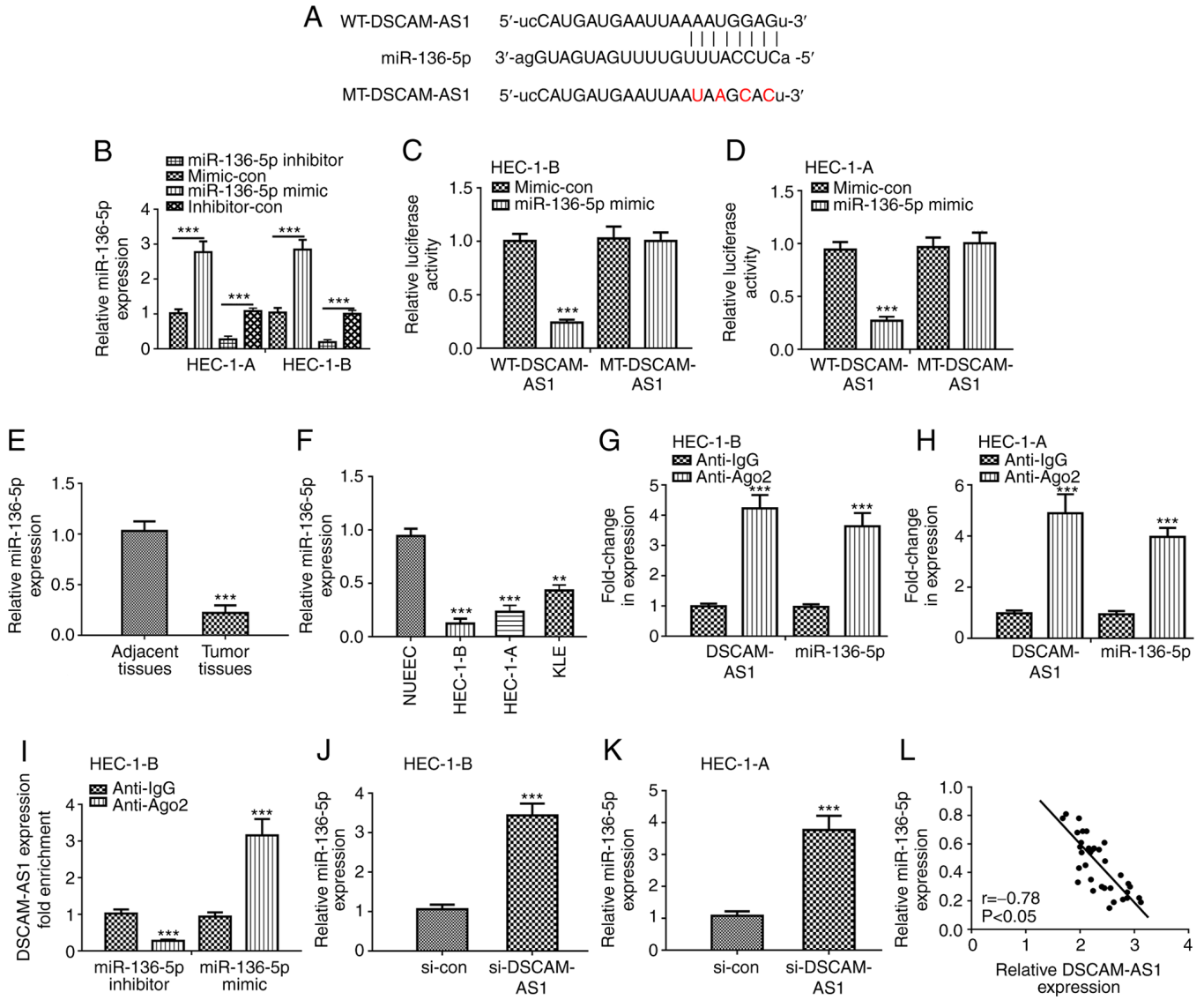

Figure 4. DSCAM-AS1 specifically binds miR-136-5p and regulates miR-136-5p expression. (A) The putative binding sites between DSCAM-AS1 and miR-136-5p and the mutant sequences of DSCAM-AS1 are presented. (B) miR-136-5p levels were assessed in HEC-1-B and HEC-1-A cells after miR-136-5p mimic or inhibitor transfection. Luciferase activity was detected in (C) HEC-1-B and (D) HEC-1-A cells co-transfected with WT-DSCAM-AS1 or MT-DSCAM-AS1 and miR-136-5p mimic or mi-con. ***P<0.001 vs. mi-con. DSCAM-AS1 expression was measured in (E) 34 pairs of EC tissues and adjacent normal tissues. ***P<0.001 vs. normal tissues. (F) DSCAM-AS1 expression was measured in EC cells (HEC-1-B, HEC-1-A and KLE) and NUEECs by RT-qPCR assay. **P<0.01 and ***P<0.001 vs. NUEEC. Relative enrichment of DSCAM-AS1 and miR-136-5p in (G) HEC-1-B and (H) HEC-1-A cells was detected by RIP assay. ***P<0.001 vs. anti-IgG. (I) Fold enrichment of DSCAM-AS1 in miR-136-5p mimic or miR-136-5p inhibitor group. ***P<0.001 vs. anti-IgG. The expression of miR-136-5p in (J) HEC-1-B and (K) HEC-1-A cells after si-DSCAM-AS1 transfection was detected by RT-qPCR. ***P<0.001 vs. si-con. (L) Correlation analysis between DSCAM-AS1 and miR-136-5p expression was conducted by Pearson analysis in EC tissues. DSCAM-AS1, down syndrome cell adhesion molecule antisense 1; EC, endometrial cancer; RT-qPCR, reverse transcription-quantitative PCR; si-DSCAM-AS1, small interfering RNA against DSCAM-AS1; si-con, negative control siRNA; mimic-con, negative control miRNA for miR-136-5p mimic; inhibitor-con, negative control for miR-136-5p inhibitor; miR-136-5p, microRNA-136-5p; RIP, RNA immunoprecipitation; NUEEC, normal uterine endometrial epithelial cell.

compete with miRNAs to bind mRNA (19). Notably, this theory connects ncRNAs and mRNAs and results in a better understanding of the role of gene regulatory networks in regulating normal development and disease progression (19). Based on this theory, emerging studies have indicated that DSCAM-AS1 can sponge miRNAs to affect tumorigenesis and tumor progression (7-9,17,18). miRNAs including miR-384, miR-577, miR-204-5p, miR-877-5p and miR-338-3p were previously identified as DSCAM-AS1 targets in various types of cancer (7-9,17). However, whether DSCAM-AS1 sponges miR-136-5p remains unclear. miR-136-5p was identified as a tumor suppressive miRNA in numerous types of cancer (20-23). Notably, miR-136-5p was found to be regulated by several ncRNAs, including circular RNA TLK1, forkhead box P4 antisense RNA, non-coding RNA activated by DNA damage and family with sequence similarity 83 member H antisense RNA 1 in renal cell carcinoma, cervical cancer, retinoblastoma, and triple-negative breast cancer (20-23). In the current study, it was demonstrated that miR-136-5p expression was regulated by DSCAM-AS1 in EC. Rescue experiments demonstrated that the effects of DSCAM-AS1 silencing on EC cell proliferation can be

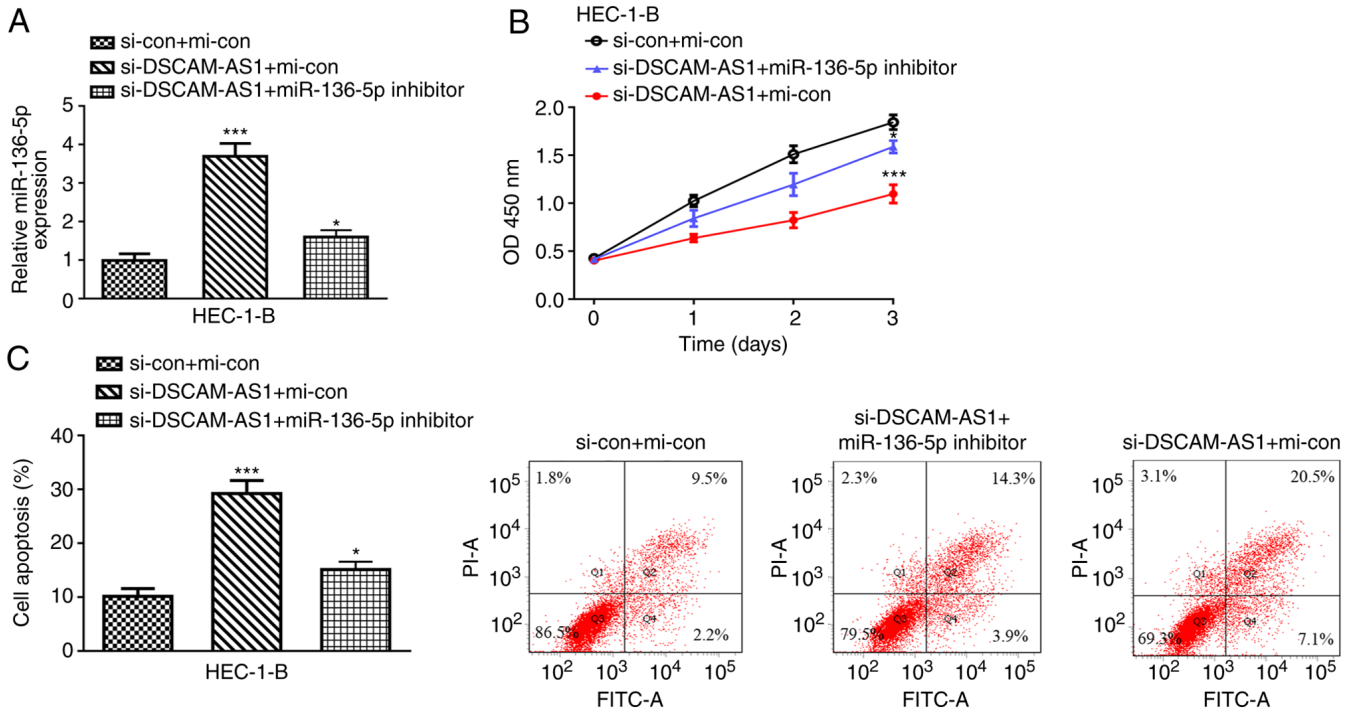

Figure 5. DSCAM-AS1 regulates EC cell growth via miR-136-5p. (A) The transfection efficacy of si-con + mi-con, si-DSCAM-AS1 + mi-con, si-DSCAM-AS1 + miR-136-5p inhibitor was measured by reverse transcription-quantitative PCR assay. (B) Cell viability was evaluated in HEC-1-B after si-con + mi-con, si-DSCAM-AS1 + mi-con, si-DSCAM-AS1 + miR-136-5p inhibitor transfection by Cell Counting Kit-8 assay. (C) Cell apoptosis percentage was evaluated in HEC-1-B after si-con + mi-con, si-DSCAM-AS1 + mi-con, si-DSCAM-AS1 + miR-136-5p inhibitor transfection by flow cytometry assay. *P<0.05 and ***P<0.001 vs. si-con + mi-con. DSCAM-AS1, down syndrome cell adhesion molecule antisense 1; EC, endometrial cancer; si-DSCAM-AS1, small interfering RNA against DSCAM-AS1; si-con, negative control siRNA; mi-con, negative control miRNA.

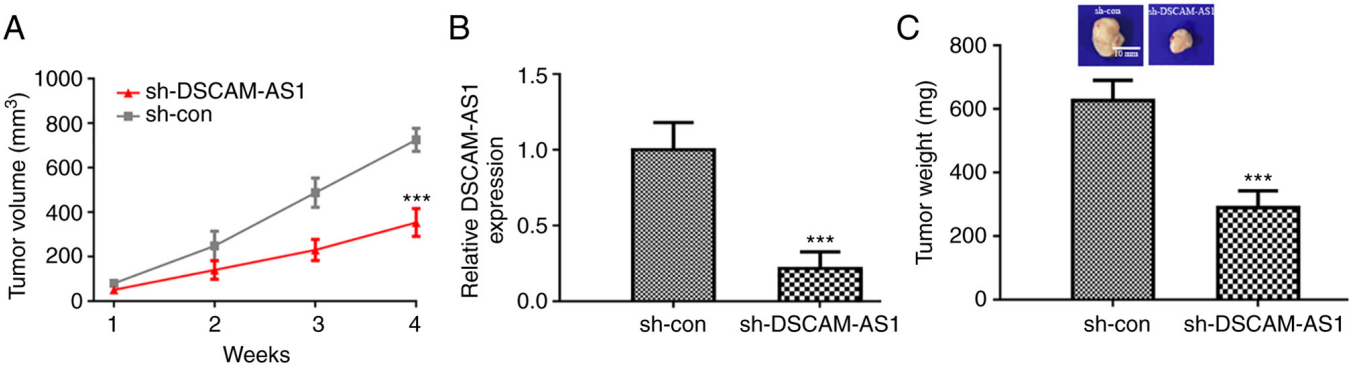

Figure 6. DSCAM-AS1 knockdown impairs EC tumor growth *in vivo*. (A) Tumor volume was examined in mice injected with HEC-1B cells transfected with sh-DSCAM-AS1 or sh-con every week. (B) DSCAM-AS1 expression level in the sh-DSCAM-AS1 or sh-con groups. (C) Tumor weight in the mice injected with cells transfected with sh-DSCAM-AS1 or sh-con after 4 weeks. ***P<0.001 vs. sh-con. DSCAM-AS1, down syndrome cell adhesion molecule antisense 1; EC, endometrial cancer; sh-DSCAM-AS1, short hairpin RNA against DSCAM-AS1; sh-con, negative control shRNA.

partially abolished by miR-136-5p knockdown. These results implied that DSCAM-AS1 regulates EC progression by affecting miR-136-5p.

Collectively, the current study revealed that DSCAM-AS1 stimulates EC progression by regulating miR-136-5p. A limitation of the present work was that the detailed mechanisms of DSCAM-AS1 in EC were not fully investigated. Another limitation is that the enrolled population size was small in the present study and the background of these patients varied. In the present study only the prognostic value of DSCAM-AS1 in all patients with EC were collected and the effect of different clinicopathological characteristics was not investigated. In the future, a large cohort study should be conducted by co-operating with other research groups to further validate the conclusions of the present study.

In conclusion, it was demonstrated that DSCAM-AS1 expression was upregulated in EC tissues and cell lines. Moreover, DSCAM-AS1 may bind with miR-136-5p to affect EC progression. To the best of the authors' knowledge, the present study provided the first evidence to highlight the importance of the DSCAM-AS1/miR-136-5p axis in the regulation of cancer progression.

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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

LHL designed the study. LHL, PPC, BBH and PYC performed the experiments and collected the data. LHL wrote the manuscript. LHL and PPC confirm the authenticity of all the raw data. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

The study protocol was approved by the Ethics Committee of The Second Affiliated Hospital of Fujian Medical University. Written informed consent was obtained from all patients prior to enrollment.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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