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Original Article

Original Article

Down-regulation of lncRNA LUADT1 suppresses cervical cancer cell growth by sequestering microRNA-1207-5p

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Abstract

Emerging evidence has proved the essential roles of long non-coding RNAs (lncRNAs) in cervical carcinoma (CC). LncRNA lung adenocarcinoma-associated transcript 1 (LUADT1) is overexpressed and plays an oncogenic role in various cancers; however, the function and clinical values of LUADT1 in CC remain unclear. In this study we found that LUADT1 is highly expressed in CC tissues and cells. Up-regulated LUADT1 is significantly correlated with the more aggressive status and poorer survival of CC patients. *In vitro* studies show that LUADT1 depletion suppresses CC proliferation, and leads to cell apoptosis and cell cycle arrest. Furthermore, the *in vivo* xenograft mouse assay demonstrates that LUADT1 knockdown remarkably suppresses tumor growth. Mechanistically, LUADT1 binds to miR-1207-5p and inhibits miR-1207-5p expression in CC cells. Septin 9 (SEPT9) is identified as a miR-1207-5p target which is negatively regulated by LUADT1. Overexpression of SEPT9 abrogates the suppressed proliferation of CC cells induced by LUADT1 knockdown. These results demonstrate that LUADT1 sponges miR-1207-5p and consequently modulates SEPT9 expression in CC. Our study suggests the possible application of LUADT1 as a prognostic and therapeutic target to inhibit CC.

Key words cervical cancer, lung adenocarcinoma-associated transcript 1, LncRNA, microRNA-1207-5p

Introduction

Cervical cancer (CC) is a common cancer that frequently occurs with a high metastasis rate in developing countries [1]. During the past decades, radiotherapy combined with chemotherapy has benefited the prognosis of CC patients. However, many patients still suffer tumor recurrence or distant metastasis after treatment [2]. Therefore, it is of significance to find novel molecules and to develop possible therapies for CC.

Long non-coding RNAs (lncRNAs) are a large group of transcripts with 200 or more nucleotides that lack protein-coding abilities [3,4]. Increasing evidence has demonstrated the critical roles of lncRNAs in fundamental and important cellular processes such as cell proliferation, angiogenesis, and migration [4,5]. LncRNA dysregulation has also been reported in cancers, which promotes cancer development [6,7]. A previous study about the function of lncRNAs in CC also uncovered their frequent dysregulation and their essential involvement in CC [8]. For example, lncRNA SNHG1 enhances the CC cell migration, proliferation, and invasion [9]. LncRNA CAR10 enhances CC development via sponging miR-125a-5p and induces the expression of PDPK1 [10]. lncRNA LUADT1 has been reported to promote tumor growth [11–14]. LUADT1 is highly expressed in lung cancer and accelerates the cell cycle by epigenetically down-regulating p27 expression [11]. The oncogenic function of LUADT1 is also found in colorectal cancer, which correlates with the patients' poorer progression [15]. Similarly, LUADT1 is also up-regulated in melanoma, which promotes the proliferation of melanoma cells [12]. Nevertheless, the clinical significance as well as the mechanism of LUADT1 in CC remain unclear.

MicroRNAs (miRNAs) are another class of single-stranded small RNAs [16,17] that bind to the 3′-untranslated region (UTR) of mRNAs to down-regulate the expressions of target genes. This physical interaction degrades the target mRNAs or causes protein translation defects [18,19]. Increasing evidence demonstrates that miRNA abundance changes significantly in CC, and the deregulated miRNAs affect the aggressive properties of CC cells, making them possible biomarkers for the clinical prognosis of CC patients [20]. Therefore, miRNAs are promising targets to inhibit cancer progression. Interestingly, as competing endogenous RNAs (ceRNAs), lncRNAs orchestrate the downstream function by sponging the target miRNAs [21–23]. LUADT1 was reported to sponge miR-28-5p

and up-regulate RAP1B in melanoma [12]. Additionally, LUADT1 sponges miR-15a-3p and consequently increases the expression of Twist in small cell lung cancer [13]. It would be interesting to identify the potential LUADT1-binding miRNAs and explore their roles in CC progression.

Septins were identified as GTP-binding proteins and proposed as key components of cytoskeleton by forming higher-order structures such as filaments and microtubules [24]. The essential roles of septins in the maintenance of cytoskeletal networks, cell proliferation and tumorigenesis have been established by increasing evidence [24]. Hyper-methylation of the *SEPT9* promoter was identified as a biomarker for colorectal cancer [25]. Additionally, overexpression of SETP9 was observed in cancers, indicating its oncogenic role in cancer progression [26,27]. Due to the key functions of SEPT9 in cellular processes, suppression of SEPT9 induces defects in cell proliferation, migration and cell cycle progression. The function and novel regulators of SEPT9 in CC remain to be explored.

In the present study, we aimed to investigate the expression and function of LUADT1 in CC, and explore the underlying molecular mechanism of LUADT1 in CC and its potential clinical significance.

Materials and Methods

Bioinformatics predictions

The binding targets of LUADT1 and miR-1207-5p were predicted with the miRDB online website (http://mirdb.org) by providing the sequence or searching by miRNA name.

CC patients and specimens

Paired CC tissues and corresponding non-cancer adjacent tissues were obtained from CC patients ($n=50$) at the First Affiliated Hospital of Jinzhou Medical University between January 2011 and October 2013. The patients underwent surgical resection, and tissues were stored in liquid nitrogen prior to analysis. The patients were grouped into stage I/II ($n=35$) and stage III ($n=15$) based on the staging system of the 7th TNM. All enrolled patients provided written informed consents. The characteristics of patients are summarized in Supplementary Table S1. The experimental protocols used in this study were approved by the Ethics Committee on Scientific Research of Jinzhou Medical University.

Cell culture and transfection

SiHa and HeLa cells were commercially purchased from the Cell Bank of Shanghai Institute of Life Sciences (Shanghai, China). CaSki, C33A, H8 and C41 were provided by American Type Culture Collection (ATCC, Manassas, USA). Cells were maintained in RPMI-1640 medium (Invitrogen, Shanghai, China) supplemented with 10% fetal bovine serum (FBS; Hyclone, Beijing, China) at 37°C with 5% CO_2.

The siRNA-control was purchased from Sigma (Cat#SIC001; St Louis, USA). siRNAs targeting LUADT1 (siRNA-LUAD1 #1, sense 5′-CACGAGCUCAAGAUAUCAATT-3′ and antisense 5′-UUGAUAUCUUGAGCUCGUGTT-3′; siRNA-LUAD1 #2, sense 5′-GGUUGCAACACACUACUAATT-3′ and antisense 5′-UUAGUAGUGUGUUGCAACCTT-3′), miR-1207-5p mimics (5′-UGGCAGGGAGGCUGGGAGGGG-3′), and corresponding control miRNA (miRNA-NC: 5′-GUUCGUACGUACACUGUUCA-3′) were synthesized by GeneCopoeia (Guangzhou, China). pcDNA3.1-vector was obtained from Invitrogen, and pcDNA3.1-SEPT9 was home made by inserting the cDNA sequence of SEPT9 into the pcDNA3.1-vector.

Transfection of these molecules was performed using lipofectamine 2000 (Thermo Fisher Scientific, Shanghai, China) following the guidelines of manufacturer. Forty-eight hours after transfection, cells were harvested for further experiments.

CCK-8 assay

The CC proliferation was detected by the CCK-8 assay. Transfected CC cells were seeded into the 96-well plate with around 500 cells per well. To determine the proliferative ability of CC cells, cells were incubated with 10 μL of CCK-8 reagent at the indicated time points for 4 h at 37°C. The absorbance at OD450 nm was detected using microplate reader (Becton Dickinson, San Jose, USA).

RT-qPCR assay

Total RNA of cells or tissues was isolated using the Trizol reagent (Qiagen, Hilden, Germany). The expression levels of LUADT1, miR-1207-5p and SEPT9 were detected with the SYBR green PCR mix (TIANGEN, Beijing, China) using the 7500 Fast PCR platform following the protocol of 95°C for 55 s, 42 cycles at 95°C for 15 s, and 60°C for 40 s. *GAPDH* and *U6* RNA levels were also detected as the endogenous controls for *LUADT1*, *SEPT9* and miR-1207-5p, respectively. The expression change of target genes was calculated according to the $2^{-\Delta\Delta Cq}$ formula. The primer sequences are as follows: *LUADT1* F, 5′-TTTCCGTTCAGCAAATCCACAC-3′ and R, 5′-TTAGGTCCAGCACTGTTATCCA-3′; miR-1207-5p F, 5′-GCCAGATCTTGATTGACTTACAGCCCAGTT-3′ and R, 5′-GCCGAATTCCACCTGTCTTTATTCCACCC-3′; *GAPDH* F, 5′-GCGCCCAATACGACCAA-3′ and R, 5′-CTCTCTGCTCCTCCTGTTC-3′; *U6* RNA F, 5′-GCTTCGGCAGCACATATACTAAAAT-3′ and R, 5′-CGCTTCACGAATTTGCGT-3′; and *SEPT9* F, 5′-ACCGGATCTCAGCCTTGAA-3′ and R, 5′-GAGAGCTCCACCCTCCGCAGG-3′.

Western blot analysis

Protein samples (20 μg) from each experimental group was separated by 15% SDS-PAGE and transferred to the nitrocellulose membrane (GE Healthcare, Beijing, China) with the semi-dry transferring instrument (Bio-Rad, Hercules, USA). After being blocked with 5% skim milk, membrane was incubated with specific anti-SEPT9 antibody (cat#10769-1-AP; Wuhan Sanying, Wuhan, China) or anti-GAPDH (ab181602; Abcam, Cambridge, USA) overnight at 4°C. The membrane was then incubated with the HRP-conjugated secondary antibody and blots were visualized with the ECL substrate (Solarbio, Beijing, China).

Analysis of cell apoptosis and cell cycle

Forty-eight hours after transfection with siRNA-control or siRNA-LUADT1, CC cells were washed with PBS twice and fixed in 70% pre-cold ethanol at –20°C overnight. Cells were then incubated with RNase A (1 mg/mL; TIANGEN) for 30 min at 37°C and stained with propidium iodide (PI; 100 μg/mL; Quanxinquanyi Biotech, Shanghai, China) at room temperature (RT) for 20 min. The DNA content of each sample was determined by flow cytometry with the FACSCalibur flow cytometer (Becton Dickinson). The CC apoptosis was analyzed using the Annexin V-fluorescein isothiocyanate (FITC) Apoptosis Detection kit (Sigma) following the procedures. Cells were washed three times with PBS and re-suspended in the provided binding buffer followed by incubating with FITC and PI for 15 min at RT. CC cell apoptosis was evaluated

with the ACSCalibur flow cytometer (Becton Dickinson). Experimental data were analyzed with the FlowJo (version 7.6.1; Flowjo, Ashland, USA).

Transwell cell invasion assay

Cells were seeded with a serum-free medium in the upper transwell chamber of a Matrigel pre-coated 24-well plate (8-μm pore size; Corning, Franklin Lakes, USA). RPMI-1640 with 10% FBS was added into the lower chamber. After 48 h of incubation, the invaded cells in the lower chamber were fixed at RT for 10 min. Cells were then stained with 0.1% crystal violet for 10 min at RT, and the stained cells were observed and counted under an inverted microscope (Olympus, Tokyo, Japan).

Luciferase reporter assay

LUADT1 oligonucleotides carrying the predicted binding site of miR-1207-5p or miRNA-NC were amplified from the cDNA of CC cells and constructed into the pmirGLO backbone (Addgene, Cambridge, USA). Site-directed mutagenesis PCR was performed on the LUADT1 sequence to induce the mutations to block miR-1207-5p binding. CC cells (1×10^4) were transfected with miR-1207-5p mimics and pmirGLO-LUADT1-WT or pmirGLO-LUADT1-Mut using PEI (MineBio Life Sciences, Shanghai, China). Forty-eight hours after transfection, luciferase activity was quantified using the Dual-Luciferase Reporter Assay kit (Promega, Madison, USA) according to the manufacturer's instructions.

In vivo xenograft mice model

Female BALB/c mice (4–6 weeks old) were obtained from the Charles River Laboratories (Beijing, China). The mice were subcutaneously injected with CC cells (1×10^7) harboring the lentivirus vector of siRNA-control or siRNA-LUADT1. Thirty days after tumor implantation, mice were sacrificed by cervical dislocation, and the tumor weight was measured. The animal experiments were performed with the approval of the Animal Care and Use Committee of Jinzhou Medical University.

Statistical analysis

All data were analyzed using the GraphPad prism (version 8) and shown as the mean ± standard deviation. Comparisons were performed by Student's *t* test or one-way analysis of variance followed by Tukey test. Spearman correlation analysis was performed to evaluate the correlation between LUADT1 and miR-1207-5p expressions. Log rank test was performed to analyze the Kaplan-Meier survival curves of CC patients based on LUADT1 expression. $P < 0.05$ was considered to be statistically significant.

Results

Overexpressed LUADT1 is correlated with the advanced status of CC

To quantify the expression of LUADT1 in CC, an RT-qPCR assay was carried out with a cohort of clinical CC tissues and paired adjacent non-cancerous tissues ($n = 50$). As shown in Figure 1A, a significantly elevated LUADT1 level was detected in CC tissues compared with that in the non-cancerous samples. To evaluate the relationship between LUADT1 and the clinical characteristics of CC patients, 50 patients were categorized into low ($n = 17$) or high ($n = 33$) LUADT1 groups according to the LUADT1 mean expression value. The Kaplan-Meier survival curve demonstrated that patients with higher LUADT1 expression have a worse 5-year overall survival rate than those with lower expression level of LUADT1 (Figure 1B). Moreover, CC patients at an advanced TNM stage exhibited a higher level of LUADT1 expression (Figure 1C). Overexpression of LUADT1 was also found in CC cell lines, consistent with the up-regulation of LUADT1 in CC tissues (Figure 1D). Collectively, these results suggested a significant positive correlation between the LUADT1 overexpression and advanced CC progression.

LUADT1 knockdown suppresses CC cell growth

To investigate LUADT1 function in CC malignancy, CC cells were transfected with siRNA-LUADT1 to knockdown LUADT1 expression. siRNA-control was also transfected as the control (Figure 2A). LUADT1 depletion significantly inhibited CC cell proliferation, as indicated by CCK-8 assay (Figure 2B,C). Transwell assay was performed to evaluate whether LUADT1 affects the invasion of CC cells, and the results showed that knockdown of LUADT1 markedly decreased the invasive capacity of both SiHa and CaSki cells (Figure 2D). Additionally, LUADT1 knockdown remarkably increased the percentage of early and late apoptotic CC cells compared with cells carrying siRNA-control (Figure 2E). Furthermore, to validate whether LUADT1 depletion-induced CC cell proliferation inhibition was due to cell cycle arrest, the cell cycle profiles of both SiHa and CaSki were determined. As shown in Figure 2F, the population of CC cells in the G1 phase was higher with LUADT1 knockdown than that in the control. This result indicated that LUADT1 depletion blocked the cell cycle progression of CC cells in the G1 phase. Taken together, these findings demonstrated the important biological significance of LUADT1 in CC.

LUADT1 acts as a sponge of miR-1207-5p in CC

To further understand how LUADT1 regulates the malignancy of CC, the possible target miRNAs of LUADT1 were predicted using the miRDB online website. A highly ranked seeding region of LUADT1 was found within the miR-1207-5p sequence (Figure 3A). The binding was further validated via luciferase assay by co-transfecting miR-1207-5p and the luciferase plasmids carrying wild-type (WT) or mutant (Mut) fragments of LUADT1. The luciferase activity of WT but not Mut LUADT1 was significantly decreased in miR-1207-5p co-transfected CC cells (Figure 3B,C). To determine whether the LUADT1 binding affects miR-1207-5p expression, miR-1207-5p level was examined in CC cells transfected with siRNA-control or siRNA-LUADT1. As shown in Figure 3D, significantly increased level of miR-1207-5p was detected in siRNA-LUADT1-transfected CC cells compared with cells transfected with the siRNA-control as detected by RT-qPCR. Furthermore, we also compared miR-1207-5p expressions in CC tissues and non-cancerous tissues. As shown in Figure 3E, decreased miR-1207-5p level was found in CC tissues compared with that in the adjacent normal tissues. Spearman test also indicated that LUADT1 and miR-1207-5p expressions in CC tissues were inversely correlated (Figure 3F). Consistent with the increased level of LUADT1, miR-1207-5p expression was also significantly lower in CC cells than that in normal H8 cells (Figure 3G). These results suggested that LUADT1 bound miR-1207-5p and suppressed miR-1207-5p expression in CC.

MiR-1207-5p targets SEPT9 in CC

Due to the miR-1207-5p dysregulation in CC, we next investigated

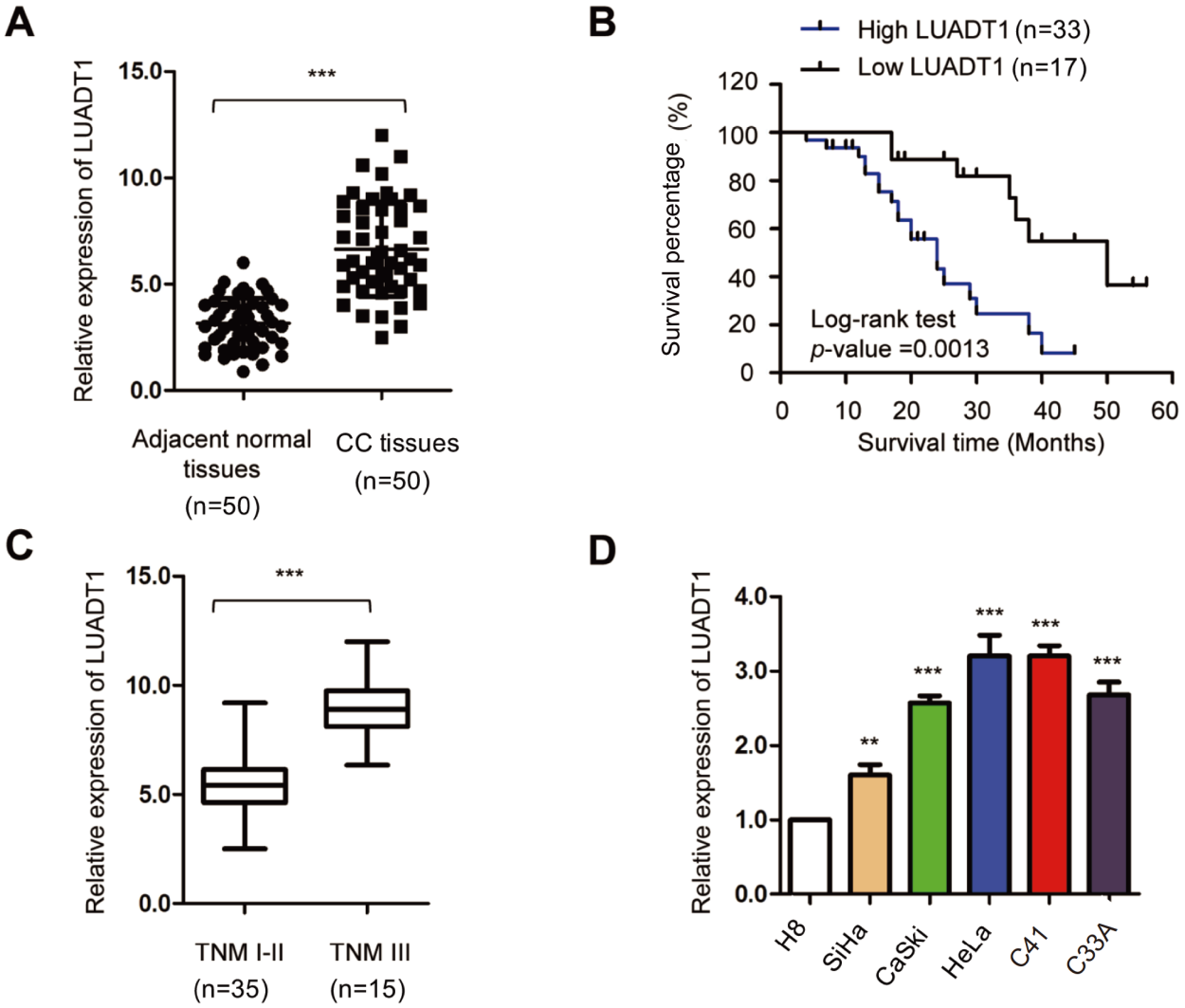

Figure 1. LUADT1 overexpression is associated with the worse prognosis of CC patients (A) RT-qPCR was carried out to quantify LUADT1 expression in CC tissues and paired normal samples. (B) CC patients with a high level of LUADT1 have a lower 5-year overall survival than those with low LUADT1 expression. Log-rank test. (C) CC patients in the higher TNM stage showed increased LUADT1 expression. (D) LUADT1 was overexpressed in CC cell lines, compared with that in normal H8 cells. $^{**}P < 0.01$, $^{***}P < 0.001$.

whether miR-1207-5p affects CC cell growth. miR-1207-5p mimics or miR-NC was transfected into SiHa and CaSki cells (Figure 4A), and CCK-8 assay was performed. Compared with cells transfected with miR-NC, the proliferation of both SiHa and CaSki cells was significantly suppressed in the presence of miR-1207-5p (Figure 4B,C). Furthermore, the bioinformatics prediction using the miRDB database indicated the binding of miR-1207-5p to the 3′-UTR of *SEPT9* (Figure 4D). Comparison of the SEPT9 expressions in CC tissues with paired non-cancerous tissues indicated increased SEPT9 mRNA levels in CC tissues (Figure 4E). Similarly, both mRNA and protein expression levels of SEPT9 were also up-regulated in CC cells compared to those in normal H8 cells (Figure 4F,G). The luciferase activity assay validated the binding of miR-1207-5p to the 3′-UTR of SEPT9. miR-1207-5p significantly repressed the luciferase activity in of cells transfected with the SEPT9 WT-3′-UTR not the SEPT9 Mut-3′-UTR (Figure 4H,I). Both RT-qPCR and western blot analysis results revealed reduced SEPT9 level in cells with miR-1207-5p overexpression compared with that in cells of the miR-NC group (Figure 4J,K). These results demonstrated that miR-1207-5p targeted and suppressed SEPT9 in CC.

Overexpression of SEPT9 abrogates the decreased CC proliferation and migration induced by LUADT1 depletion

To investigate the effect of SEPT9 regulation on CC cell proliferation, cells were transfected with pcDNA3.1-vector or pcDNA3.1-SEPT9. The results showed that overexpression of SEPT9 significantly facilitated the proliferation of CC cells (Figure 5A,B). Since LUADT1 sponged miR-1207-5p, the LUADT1 influence on SEPT9 expression was also determined. As shown in Figure 5C,D, LUADT1 depletion repressed SEPT9 expression in SiHa and CaSki cell lines. To verify the essential role of SEPT9 in growth defects of CC cells induced by LUADT1 down-regulation, *SEPT9* lacking the 3′-UTR element was constructed into the backbone of the pcDNA3.1-vector. Both SiHa and CaSki cells were co-transfected with siRNA-control or siRNA-LUADT1 and pcDNA3.1-SEPT9. Re-introduction of SEPT9 significantly recovered the proliferation of CC cells compared with siRNA-LUADT1-transfected cells (Figure 5E,F).

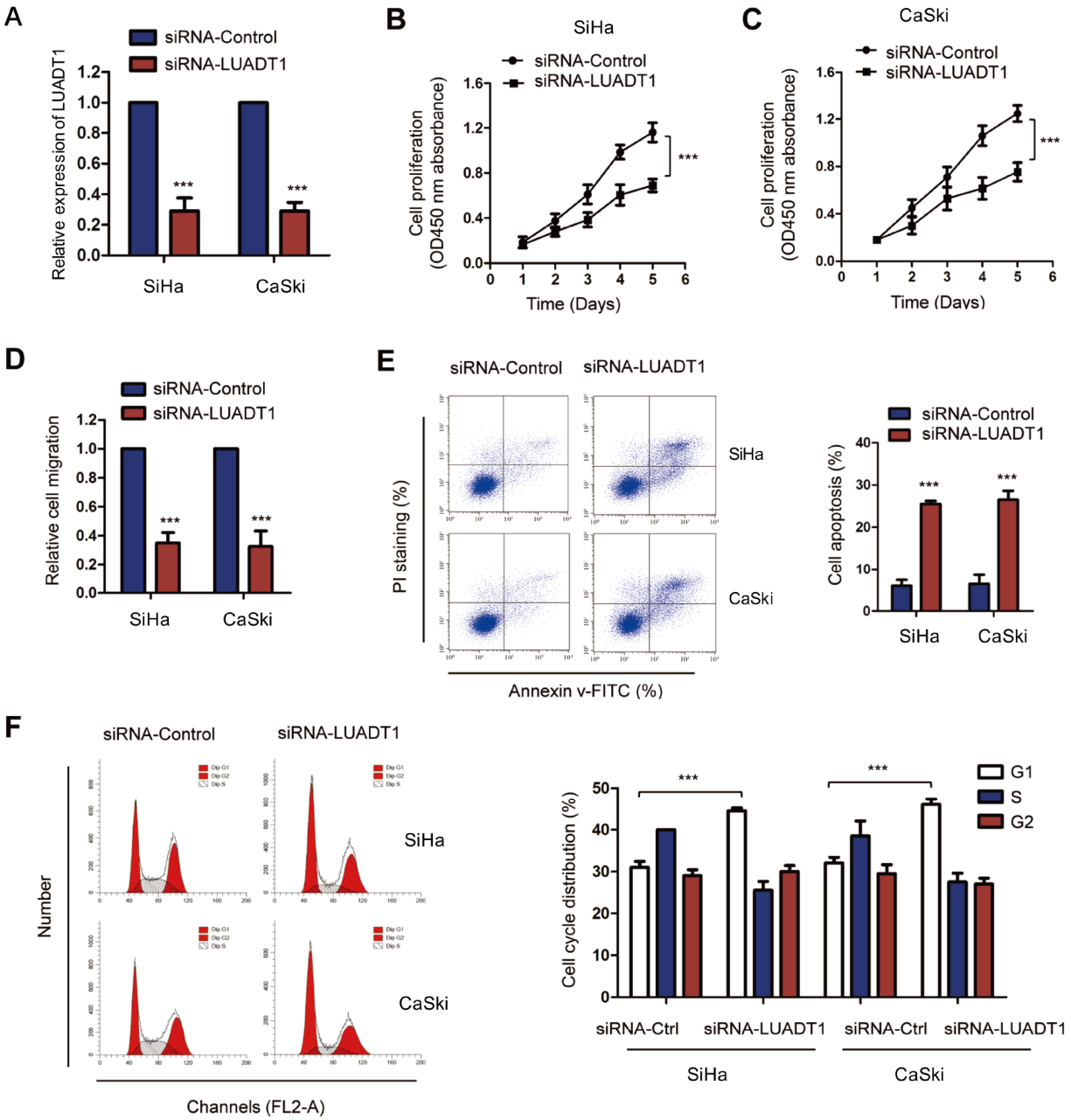

Figure 2. LUADT1 knockdown inhibits CC cell proliferation (A) Knockdown efficiency of LUADT1 in CC cells after siRNA-control or siRNA-LUADT1 transfection was confirmed by RT-qPCR analysis. (B,C) The effects of LUADT1 depletion on the proliferation of CC cells were determined by CCK-8 analysis. (D) Transwell assay suggested the suppression of the migration of CC cells with knockdown of LUADT1. (E) Up-regulated apoptosis of CC cells with the depletion of LUADT1 indicated by flow cytometry analysis. (F) Silencing of LUADT1 induced the delay of cell cycle progression. ***$P < 0.001$ vs siRNA-control group.

Similarly, co-transfection of pcDNA3.1-SEPT9 also markedly abrogated LUADT1-induced cell invasion suppression (Figure 5G). These results demonstrated that SEPT9 counteracted the inhibitory effects induced by LUADT1 knockdown on the aggressive behaviors of CC cells.

Down-regulation of LUADT1 suppresses the *in vivo* tumor growth

Given the important biological role of LUADT1 in CC, a xenograft model with nude mice was constructed by implanting CC cells expressing siRNA-LUADT1 or siRNA-control. Four weeks after tumor implantation, the mice were euthanized. As shown in Figure 6A, down-regulated expression of LUADT1 in SiHa and CaSki cells generated smaller tumors in nude mice. Tumor weight was decreased in the LUADT1-knockdown group compared with that in the control group (Figure 6C). The knockdown efficiency of LUADT1 in tumor tissues was confirmed (Figure 6B). The level of miR-1207-5p was also significantly increased in the tumors with LUADT1 knockdown (Figure 6D). Consistent with miR-1207-5p up-regulation, LUADT1 depletion markedly decreased SEPT9 expression in tumors (Figure 6E,F). In conclusion, LUADT1 knockdown repressed tumor growth and SEPT9 expression via miR-1207-5p

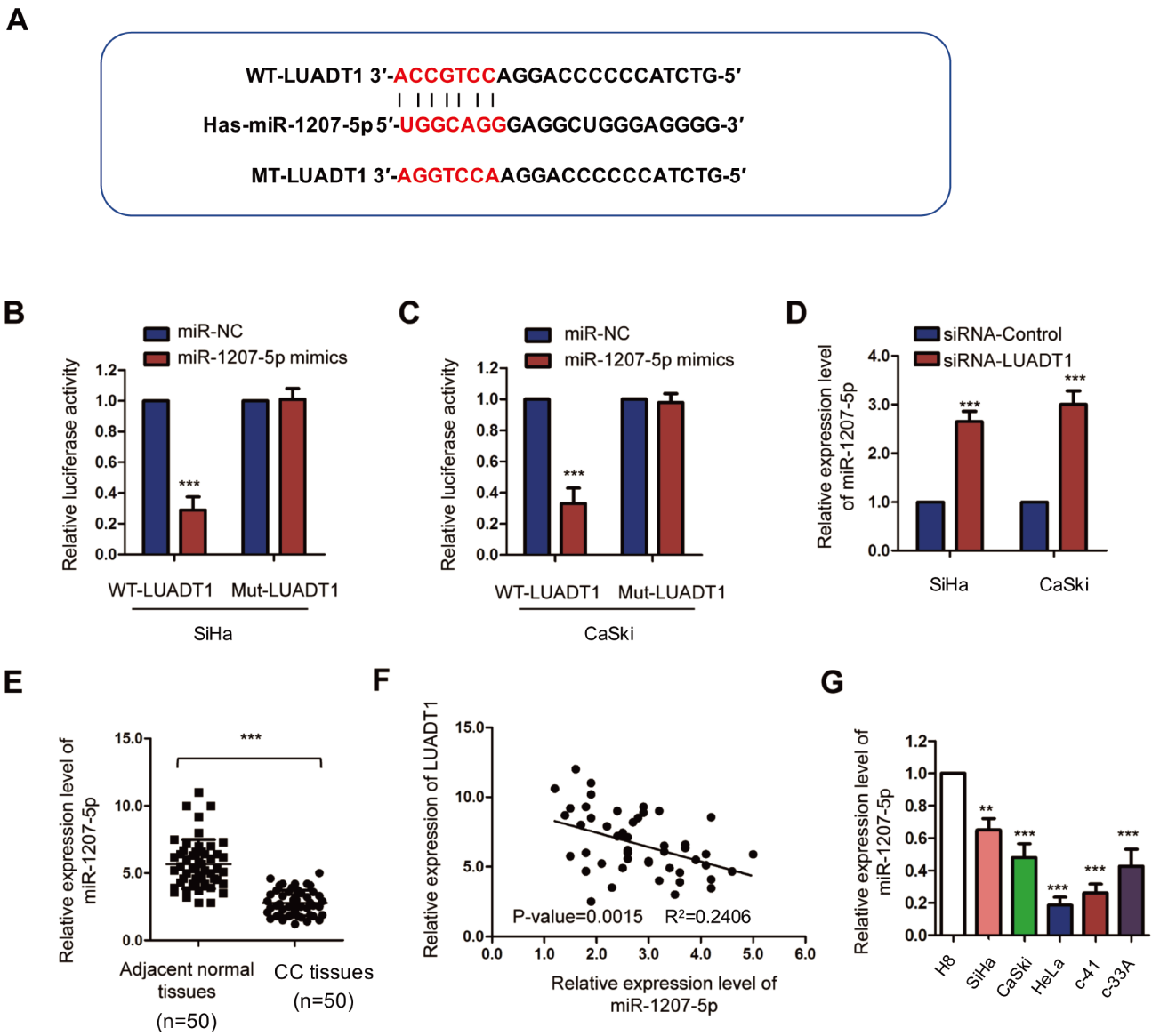

Figure 3. LUADT1 sponges miR-1207-5p in CC cells (A) The schematic diagram showing the predicted binding site of miR-1207-5p in the wild-type (WT) or mutant (MT) LUADT1 (red mark: seed region). (B,C) CC cells were co-transfected with miR-1207-5p mimics or miR-NC, and WT or Mut-LUADT1. The luciferase reporter activity was determined. (D) Down-regulated LUADT1 increased miR-1207-5p expression in CC cells. (E) RT-qPCR assay of miR-1207-5p expression in tissues. (F) Inverse correlation of miR-1207-5p and LUADT1 in CC tissues as determined by Spearman test. (G) Decreased level of miR-1207-5p in CC cell lines with H8 cells as the control. $^{**}P<0.01$, $^{***}P<0.001$ vs control group.

sequestration.

Discussion

CC is a common malignancy among female patients leading to high mortality rates [28,29]. The high metastatic potential has been considered the main reason for CC treatment failure. Abnormal expression of lncRNAs is correlated with the initiation, differentiation, and metastasis of CC [30–34]. In this study, we found that LUADT1 over-expression in CC is remarkably associated with the progression condition and poor prognosis of CC patients, suggesting a possible correlation between LUADT1 and the progression of CC.

LUADT1 has been identified as an oncogenic regulator of human cancers [11–15]. A previous study showed that LUADT1 is over-expressed and related to the clinicopathology of colorectal cancer [15]. Similarly, high expression of LUADT1, which promotes cancer cell invasion, predicts poor outcomes of lung cancer patients [11]. Recently, the oncogenic role of LUADT1 was also established in melanoma, where LUADT1 was overexpressed and promoted melanoma cell proliferation [12]. In the present study, consistent with the up-regulated LUADT1 expression in CC, LUADT1 depletion significantly inhibited CC cell proliferation and migration. Additionally, LUADT1 down-regulation dramatically promoted apoptosis and repressed the cell cycle of CC cells. The tumor growth in the xenograft nude mice model was also blocked with the depletion of LUADT1. These results indicated that blocking the LUADT1 function might be a promising strategy to inhibit the progression of CC.

The concept of ceRNAs characterizes the post-transcription

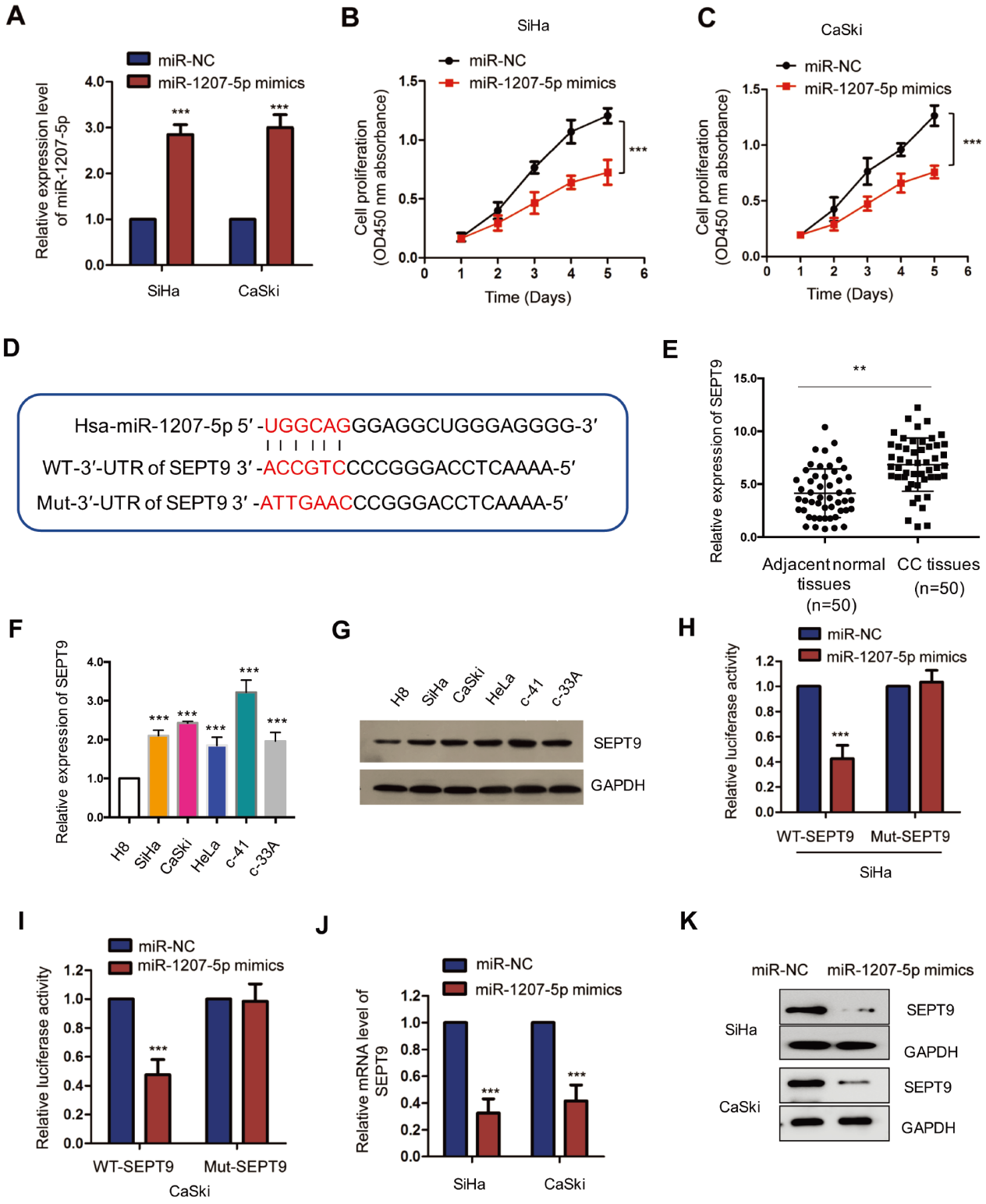

Figure 4. miR-1207-5p targets and inhibits SEPT9 expression in CC cells (A) miR-1207-5p expression was confirmed via RT-qPCR after the transfection. (B,C) The inhibited proliferation of CC cells with miR-1207-5p overexpression in contrast to the control cells. (D) The miRDB dataset predicted the potential binding site of miR-1207-5p within the 3′-UTR of *SEPT9*. (E) The mRNA level of SEPT9 was increased in CC tissues. (F) The mRNA expression of SEPT9 was up-regulated in CC cells. (G) The protein expression of SEPT9 in CC cells was increased. (H) Reduced luciferase activity of WT 3′-UTR of *SEPT9* upon the transfection with miR-1207-5p in SaHi cells. (I) Overexpression of miR-1207-5p inhibited the luciferase activity of WT 3′-UTR of *SEPT9* in CaSki cells. (J) The decreased mRNA expression of SEPT9 in CC cells after transfection with miR-1207-5p. (K) SEPT9 protein expression was reduced in CC cells with miR-1207-5p overexpression. ***$P < 0.001$ vs control group.

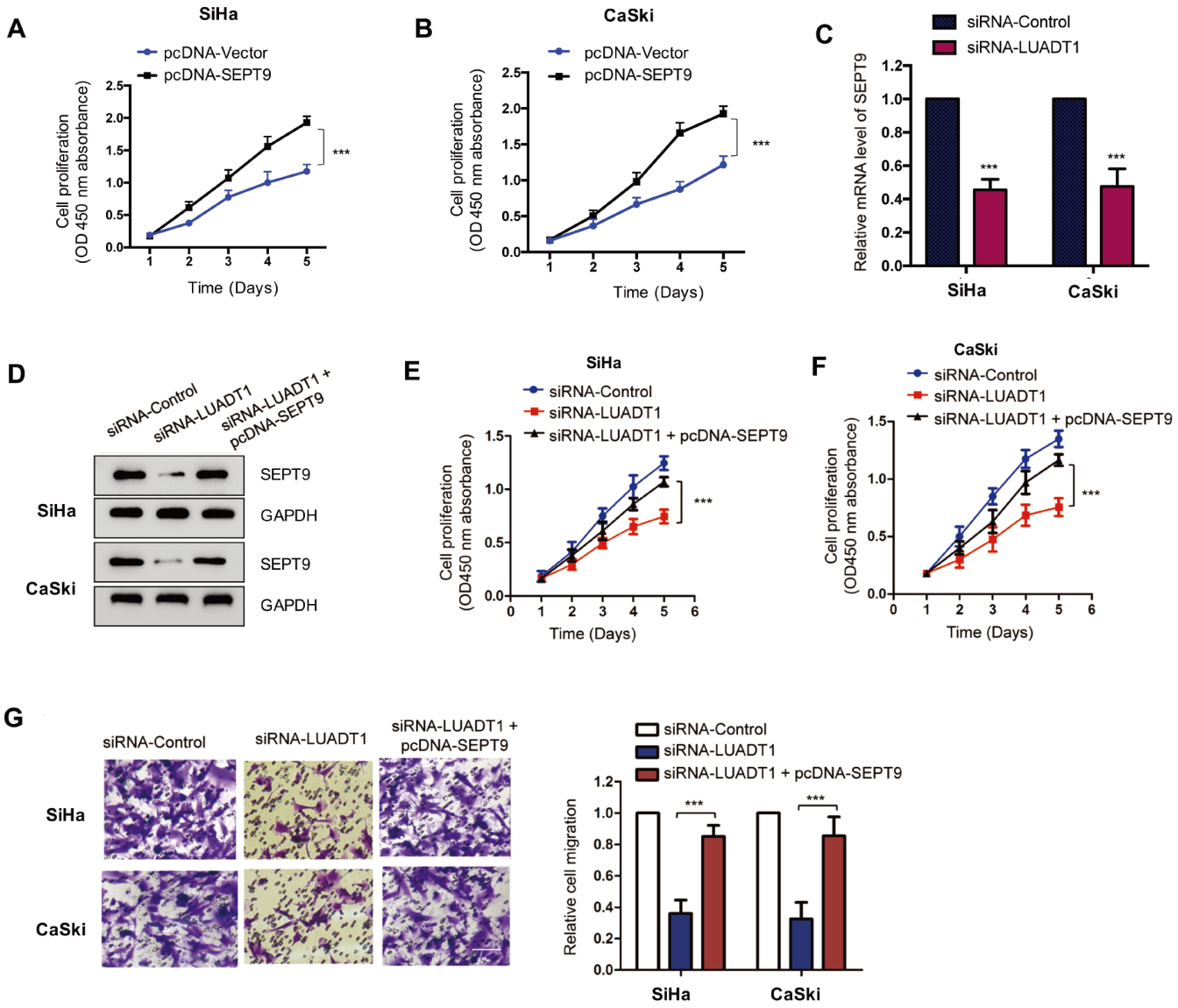

Figure 5. Overexpressed SEPT9 reversesd the decreased CC proliferation induced by LUADT1 down-regulation (A,B) Overexpression of SEPT9 promoted CC cell proliferation. (C) Down-regulation of LUADT1 suppressed SEPT9 expression in CC cells. (D) CC cells were transfected with siRNA-control, siRNA-LUADT1 alone, or co-transfected with pcDNA3.1-SEPT9. SEPT9 expression was detected by western blot analysis. (E,F) Re-introduction of SEPT9 significantly rescued LUADT1 depletion-induced proliferation defects of CC cells. (G) Re-introduction of SEPT9 recovered the migration of CC cells compared with cells transfected with siRNA-LUADT1 only. Scale bar, 50 μm. ***$P < 0.001$ vs control group.

communication network of lncRNAs, which act as miRNA sponges to suppress the function of miRNAs and regulates the expression of downstream targets [21,35–38]. LUADT1 has been reported to up-regulate Twist1 via miR-15a-3p sponging in lung cancer [13]. Similarly, LUADT1 binds to miR-28-5p in melanoma and weakens the negative effects of miR-28-5p on RAP1B expression [12]. A recent study also showed that LUADT1 modulates the level of peroxiredoxin 3 by serving as the endogenous sponge of miR-383 in diabetic retinopathy [39]. In the current study, miR-1207-5p was found to be a ceRNA of LUADT1. Down-regulation of LUADT1 induces miR-1207-5p up-regulation in CC cells. Previous studies have established the tumor-suppressive function of miR-1207-5p in gastric cancer and lung cancer [40,41]. In this study, we found that miR-1207-5p expression is remarkably reduced in CC tissues and inversely correlated with LUADT1. This observation suggests that LUADT1 is important for CC cell growth via sponging miR-1207-5p. In addition to regulating the function of targeted miRNAs, lncRNAs also bind to cellular proteins and modulate their functions. Moreover, it has been demonstrated that acetylation of USP31 suppresses its activity and plays a protective role in CC cancer cell growth [42]. Considering the essential roles of LUADT1 and USP31, it would be interesting to investigate the possible correlation between LUADT1 and USP31 in CC.

SEPT9, belonging to the septin family, is a cytoskeletal protein, which can assemble into a higher-order cytoskeletal structure [43]. In addition to the essential roles of SEPT9 in cellular functions, including chromosome segregation, cell polarization, and migration [44,45], SEPT9 is also involved in pathological events, especially

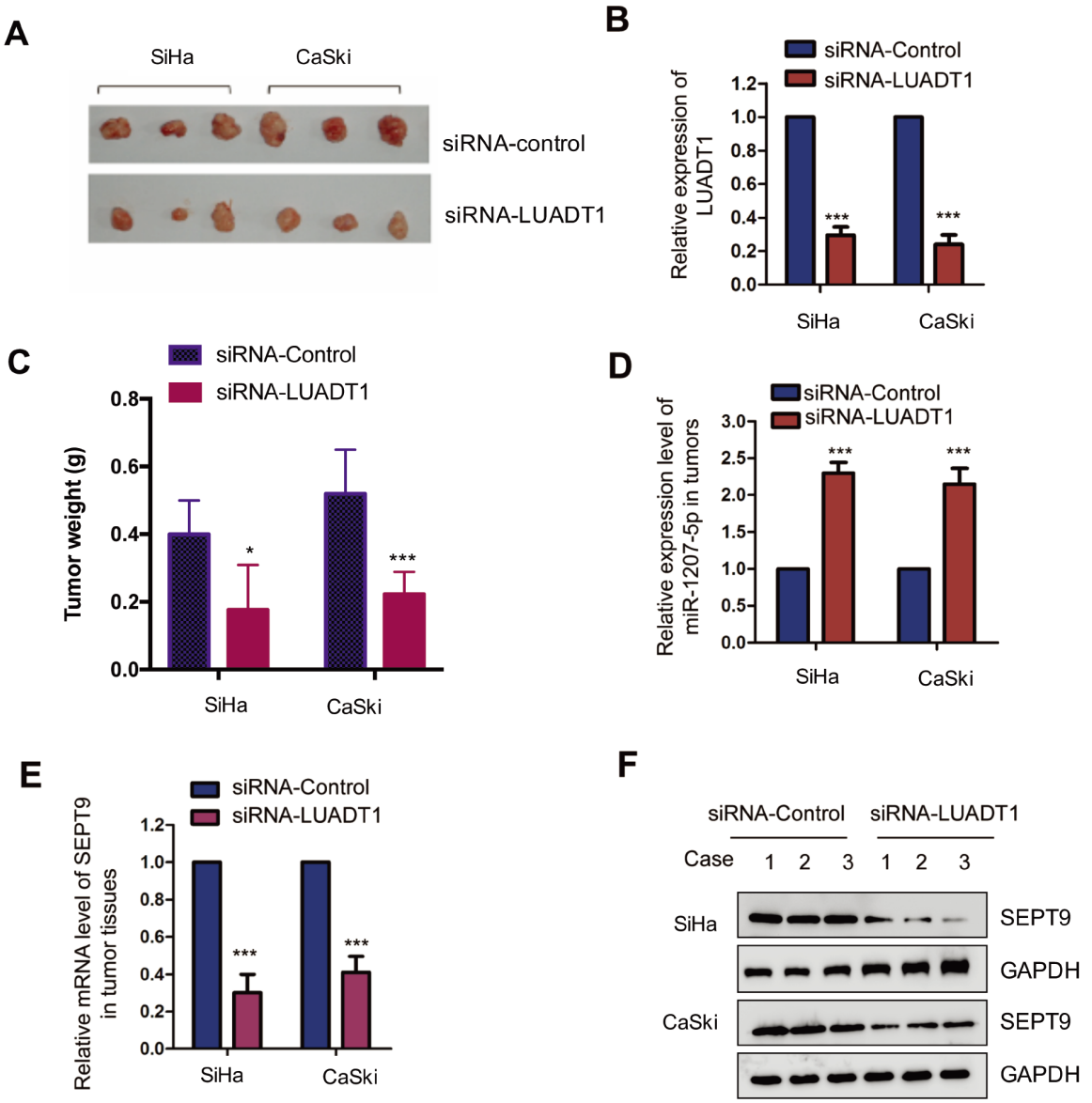

Figure 6. Knockdown of LUADT1 inhibits CC tumor growth *in vivo* (A) Mice were euthanized by cervical dislocation on day 30 after tumor implantation, and the tumors were harvested. (B) LUADT1 expression levels in tumors harboring siRNA-control or siRNA-LUADT1 were detected by RT-qPCR. (C) LUADT1 knockdown repressed the *in vivo* tumor growth. (D) Up-regulated miR-1207-5p level in tumors after LUADT1 knockdown. (E,F) The SEPT9 level in tumors was decreased at both mRNA and protein levels with down-regulated LUADT1. $^{*}P < 0.05$, $^{***}P < 0.001$ vs control group.

cancers [46]. Overexpression of SEPT9 is found in several cancers [47–50] and its overexpression is associated with the advanced clinicopathological parameters of colorectal cancer patients [49]. In this study, a higher level of SPET9 was detected in both CC tissues and cell lines. Furthermore, SEPT9 is targeted and negatively regulated by miR-1207-5p in CC cells. Consistent with the overexpression of miR-1207-5p, depletion of LUADT1 suppresses SEPT9 expression. Overexpression of SEPT9 abrogates LUADT1 depletion-induced proliferation and invasive defects of CC cells. Altogether, these data demonstrate the essential role of LUADT1 in the malignant behaviors of CC. Further studies are needed to investigate the roles and underlying mechanisms of miR-1207-5p and SEPT9 in the proliferation, migration, and invasion of CC cells. The findings of *in vitro* assays also require further validation in animal models.

In conclusion, this study reveals that elevated LUADT1 expression in CC is associated with the advanced aggressive status and poorer outcome of CC patients. Silencing of LUADT1 suppresses the growth of CC cells by inhibiting SPET9 via sponging miR-1207-5p, suggesting that targeting LUADT1 is a potential therapeutic strategy for CC.

Supplementary Data

Supplementary data is available at *Acta Biochimica et Biophysica Sinica* online.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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