Article

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Tumor Suppressor *LINC02487* Inhibits the Progression of Cervical Cancer *in Vitro* by Regulating the PTEN/Akt/mTOR Pathway

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Objective: Cervical cancer (CC) ranks among the most prevalent malignant tumors affecting the female reproductive system. Nonetheless, various shortcomings exist within current treatment approaches for CC. Therefore, the quest for new intervention targets holds significant importance. Research has demonstrated that long non-coding RNA (lncRNA) long intergenic non-protein coding RNA 2487 (*LINC02487*) can suppress the development of oral squamous cell carcinoma (OSCC). However, its function and potential mechanisms in CC remain unclear, therefore, this study aims to investigate the role and potential mechanism of *LINC02487* in CC.

Methods: *LINC02487* and phosphatase and tensin homolog (*PTEN*) expression were assessed using real-time quantitative polymerase chain reaction (RT-qPCR) in CC tissue samples and constructed cell models. *LINC02487* was either knocked down or overexpressed, and PTEN was knocked down in the CC (SiHa) cell line via transfection technology. The expression levels of *LINC02487* and *PTEN* in SiHa cell lines were examined using RT-qPCR after various treatments. Cell proliferation ability was determined through Cell Counting Kit (CCK)-8 and colony formation assays, while the ability to invade and migrate was assessed via Transwell experiments. Western blot analysis was employed to measure the levels of key proteins in the PTEN/Akt/mechanistic target of the rapamycin (mTOR) signaling pathway.

Results: A positive correlation was observed between *LINC02487* and *PTEN*, both of which were found to be downregulated in CC cells and tissues ($p < 0.05$). *In vitro* experiments demonstrated that overexpression of *LINC02487* significantly inhibited colony formation ($p < 0.01$), invasion ($p < 0.01$), migration ($p < 0.01$), and proliferation ($p < 0.01$) of SiHa cells. Furthermore, *LINC02487* overexpression led to upregulation of *PTEN* expression ($p < 0.01$) and inhibition of the Akt/mTOR signaling pathway ($p < 0.01$), while knockdown of *LINC02487* produced the opposite effect ($p < 0.01$). Additionally, knocking down *PTEN* counteracted the inhibitory effects of *LINC02487* overexpression on CC progression ($p < 0.01$) and the Akt/mTOR signaling pathway ($p < 0.01$).

Conclusion: *In vitro* findings suggest that *LINC02487* may impede the progression of CC by suppressing the Akt/mTOR signaling pathway through the upregulation of *PTEN* expression. Consequently, *LINC02487* holds promise as a potential therapeutic target for the treatment of CC.

Keywords: cervical cancer; *lncRNA LINC02487*; *PTEN*; PTEN/Akt/mTOR signaling pathway

Introduction

Cervical cancer (CC) ranks as the fourth most common malignancy worldwide [1]. According to statistics from the International Agency for Research on Cancer (IARC), approximately 600,000 women are newly diagnosed with CC each year, with over 340,000 deaths attributed to the disease [2]. Current clinical treatment methods for CC typically involve radical surgery, radiotherapy and chemotherapy, each with its own drawbacks. Radical surgery often necessitates the removal of a significant portion, if not all, of the uterus, resulting in loss of fertility for patients. Meanwhile, radiotherapy and chemotherapy can lead to permanent damage to the reproductive system and pelvic organs, as well as systemic adverse reactions such as nausea, vomiting, alopecia and immunosuppression [3]. In recent years, targeted therapy has emerged as a promising treatment scheme for CC. While offering advantages such as individualization and fewer side effects [4], there is currently a lack of targeted drugs specifically developed for CC. Besides, most targeted therapies are adapted from targeted treatment regimens for breast cancer, colorectal cancer, gastric cancer and other diseases [5], posing challenges for their widespread application in clinical practice. Therefore, the quest for a simple, effective, and low-side-effect method to treat CC is of paramount importance. Such an advancement would not only enhance the quality of life of CC women but also extend their lifespan.

Long non-coding RNA (lncRNA) comprises a vast group of functional RNA molecules exceeding 200 nu-

cleotides in length, yet lacking protein-coding capability [6]. With over 15,000 genes in the human genome capable of producing approximately 28,000 lncRNA transcripts, these molecules constitute nearly 14% of all transcripts [7]. Functionally, lncRNA interacts with various RNA molecules and proteins, thus participating in physiological processes such as gene expression, RNA maturation, protein translation, and post-translation modification [8]. Increasing evidence suggests a close association between abnormal lncRNA expression and the onset and progression of various malignancies [9].

The long intergenic non-protein coding RNA 2487 (*LINC02487*) gene, located on human chromosome 6, spans 2557 base pairs in length. Its transcription product, lncRNA *LINC02487*, typically localizes in the cytoplasm around the nuclear envelope [10]. While limited studies have focused on *LINC02487* available research suggests its potential role as a tumor suppressor. Clinical studies by Kozłowska *et al*. [11] and Li *et al*. [12], indicate that decreased expression of *LINC02487* is a common feature among patients with oral squamous cell carcinoma (OSCC) compared to healthy individuals. Moreover, reduced *LINC02487* expression correlates with an increased risk of poor prognosis and tumor recurrence, suggesting its potential utility as a biomarker for auxiliary diagnosis and prognosis of OSCC. Additionally, basic research by Feng *et al*. [13] suggests that *LINC02487* can directly bind to the deubiquitinating enzyme USP17, thereby inhibiting invasion, migration, and the epithelial-to-mesenchymal transition (EMT) of OSCC cells *in vitro*. However, to date, no study has explored the effect of *LINC02487* in CC.

Phosphatase and tensin homolog (*PTEN*), located on human chromosome 10q23.3, is a tumor suppressor gene. It encodes a PTEN protein consisting of 403 amino acids. As a bispecific phosphatase, the PTEN protein primarily engages in various physiological processes by antagonizing various phosphorylases [14]. Under normal circumstances, the PTEN protein can inhibit excessive cell proliferation, impede unordered cell migration, improve energy metabolism, and maintain genome stability [15]. *PTEN* gene mutations or deletions are prevalent in malignant tumors such as prostate cancer, breast cancer, colorectal cancer, and are closely associated with the poor prognosis of patients with these malignancies [16]. Similarly, studies have indicated that the pathogenesis of CC involves decreased expression and abnormal function of the PTEN protein due to *PTEN* gene mutations [17,18]. However, the relationship between *LINC02487* expression and *PTEN* expression, as well as the potential impact of this relationship on CC, remains to be elucidated. Therefore, this study was designed to address a series of unresolved issues and build upon previous findings.

In this study, the levels of *LINC02487* and *PTEN* expression were found to be significantly decreased in clinical tissue samples of CC and in *in vitro* cell models, and their expressions were positively correlated. Moreover, we further confirmed that overexpression of *LINC02487* negatively regulated the PTEN/Akt/mechanistic target of the rapamycin (mTOR) signaling pathway by upregulating *PTEN* expression levels, thereby exerting inhibitory effects on colony formation, invasion, proliferation, and migration of CC cells *in vitro*. Consequently, *LINC02487* emerges as a potential therapeutic target for CC, offering a novel avenue for future CC treatment strategies.

Materials and Methods

Clinical Tissue Sample Acquisition

During the surgical procedure, the clinician excised tumor tissue (CC group, n = 20) and an appropriate amount of adjacent normal tissue (Normal group, n = 20) from patients. Upon rapid pathological examination of frozen sections during surgery, typical CC cell morphology was observed in the tissues of the CC group under a microscope, while cancer cells were not evident in the tissues of the Normal group. The collected tissue samples were preserved in a refrigerator at –80 °C for future use.

Cell Culture

The Human cervical squamous cell carcinoma cell line SiHa (SCSP-5058) and the normal immortalized human epidermal cell line HaCaT (SCSP-5091) were provided by the Type Culture Collection of the Chinese Academy of Sciences in Shanghai, China, as cell models. All cells were characterized by Short Tandem Repeat (STR) analysis and tested negative for mycoplasma contamination. Dulbecco's Modified Eagle's Medium (DMEM) (cat. no. 12491015, Thermo Fisher Scientific, Waltham, MA, USA), fetal bovine serum (FBS, C0226S, Beyotime Biotechnology Co., Ltd., Shanghai, China), was utilized for culturing the aforementioned cell types. The cells were maintained in a cell incubator set at a temperature of 37 °C with a CO_2 concentration of 5%.

Cell Transfection and Grouping

In this study, small interfering RNA (siRNA) technology and pcDNA3.1 vector technology were employed to knock down and overexpress related genes, respectively. GenePharma Company (Shanghai, China) designed and synthesized siRNAs targeting *LINC02487* (sense: 5′-GCGCAGUGUGGAUGUUCAACA-3′, anti-sense: 5′-UUGAACAUCCACACUGCGCUG-3′) and *PTEN* (sense: 5′-CCUCAGUUUGUGGUCUGCCAGCUAA-3′, anti-sense: 5′-UUAGCUGGCAGACCACAAACUGAGG-3′), as well as negative control siRNA not targeting any gene (sense: 5′-UUCUCCGAACGUGUCACGUTT-3′, anti-sense: 5′-ACGUGACACGUUCGGAGAATT-3′), and pcDNA3.1 plasmids overexpressing *LINC02487* and negative control pcDNA3.1 plasmids lacking any foreign gene.

SiHa cells were cultured under standard conditions until 70%–80% confluence. Subsequently, Lipofectamine 3000 (cat. no. L3000008, Invitrogen, Carlsbad, CA, USA) was utilized to transfect the aforementioned siRNA and pcDNA3.1 plasmids into the cells following the kit instructions. After transfection, the cells were incubated for 48 hours before collection for subsequent experiments.

SiHa cells were divided into the following groups based on different treatments: (1) siNC group: SiHa cells transfected with negative control siRNA; (2) si-*LINC02487* group: SiHa cells transfected with *LINC02487* siRNA; (3) vector group: SiHa cells transfected with negative control plasmid pcDNA3.1; (4) *LINC02487* group: SiHa cells transfected with pcDNA3.1 plasmid overexpressing *LINC02487*; (5) si-*PTEN* group: SiHa cells transfected with PTEN siRNA; (6) vector+siNC group: SiHa cells transfected with negative control siRNA and pcDNA3.1 plasmids; (7) *LINC02487*+siNC group: SiHa cells transfected with pcDNA3.1 plasmid overexpressing *LINC02487* and negative control siRNA; (8) vector+si-*PTEN* group: SiHa cells transfected with negative control pcDNA3.1 plasmid and *PTEN* siRNA; (9) *LINC02487*+si-*PTEN* group: SiHa cells transfected with pcDNA3.1 plasmid overexpressing *LINC02487* and *PTEN* siRNA.

Real-Time Quantitative PCR

The TRIzol kit (cat. no. 15596026, Invitrogen, Carlsbad, CA, USA) was utilized to thoroughly extract total RNA from clinical tissue samples, the HaCaT cell line, and the SiHa cell line. Subsequently, reverse transcription was conducted to generate cDNA from the total RNA using the PrimeScript RT Reagent Kit (cat. no. RR037A, Takara Biotechnology Ltd., Dalian, China). Real-time quantitative polymerase chain reaction (RT-qPCR) was then performed on the Real-Time PCR system (cat. no. ABI7500, Applied Biosystems, Waltham, MA, USA) using the SYBR Premix EXTaq kit (cat. no. DRR041A, Takara, Tokyo, Japan) to detect and analyze the cDNA. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) served as the internal control, and the GAPDH expression level was utilized to normalize the *LINC02487* and PTEN expression levels to obtain relative values. The $2^{-\Delta\Delta CT}$ method was employed for quantitative calculation and analysis. Each sample was subjected to three repeated experiments, and the final result was determined by averaging the values. Table 1 provides a list of the primers used in the PCR.

Table 1. Human RT-qPCR primers.

RNA	Sequences (5′ to 3′)
LINC02487	5′- TAGGAGGCCACGGCTTTTAC -3′ (forward)
	5′- TGGGCCTCATCACAGATTCG -3′ (reverse)
PTEN	5′- CTCAGCCGTTACCTGTGTGTGT -3′ (forward)
	5′- AGGTTTCCTCTGGTCCTGGT -3′ (reverse)
GAPDH	5′- GGGAAACTGTGGCGTGAT -3′ (forward)
	5′- GAGTGGGTGTCGCTGTTGA -3′ (reverse)

RT-qPCR, real-time quantitative polymerase chain reaction; *LINC02487*, long intergenic non-protein coding RNA 2487; *PTEN*, phosphatase and tensin homolog; *GAPDH*, glyceraldehyde-3-phosphate dehydrogenase.

CCK-8

During the logarithmic growth phase, SiHa cells (2×10^3 cells/well) treated with various interventions were seeded into 96-well plates, with 10 µL of Cell Counting Kit-8 (CCK-8) solution (cat. no. CK04, Dojindo, Mashiki, Japan) added to each well. Cell incubation continued for 72 hours under standard conditions. Throughout the incubation period, the optical density (OD) was measured at 450 nm using a multifunctional microplate reader (cat. no. VL0000D0, Multiskan SkyHigh, Thermo Fisher Scientific, Waltham, MA, USA) at 12, 24, 48, and 72 hours after the addition of the CCK-8 solution. Subsequently, the number of viable cells was assessed. Each treatment group for SiHa cells was conducted in triplicate to obtain the average value as the final outcome.

Cell Colony Formation Assay

Following various treatments, SiHa cells in the logarithmic growth phase were seeded into a 6-well plate at a density of 1×10^3 cells/well. They were cultured in DMEM supplemented with 1% penicillin/streptomycin solution and 10% FBS for 12 days under standard conditions. The medium was replaced every 3 days during the culture period. After 12 days, the culture medium was carefully aspirated, and the cells were fixed with 2 mL of 4% paraformaldehyde for 15 minutes. Subsequently, the cells were stained with 2 mL of 1% crystal violet solution (cat. no. A100528-0100, Sangon Biotech, Shanghai, China) for 5 minutes. The number of cell colonies was then assessed and recorded under a microscope (BX53, Olympus, Tokyo, Japan), with a colony defined as a cluster of more than 50 cells. The experiment with SiHa cells was repeated in triplicate for each group, and the average value was considered the final outcome.

Transwell

The Transwell experiment was conducted following the method proposed by Justus *et al*. [19]. Briefly, prior to the Transwell experiment, cells were cultured in 500 µL of FBS-free DMEM medium for 12 hours to minimize serum interference with the experimental results. For assessing cell migration ability, 4×10^4 SiHa cells with various treatments were seeded into the upper chamber of the Transwell. The volume of DMEM without FBS in the upper chamber was increased to 200 µL, while 600 µL of DMEM with 15% FBS was added to the lower chamber. After 24 hours of incubation under standard conditions, the culture solution in the lower chamber was discarded, and the chamber was rinsed with calcium-free PBS. Unmigrated cells were

gently removed with cotton swabs. The cells were then fixed with 4% paraformaldehyde for 20 minutes, followed by staining with 1% crystal violet for 10 minutes. Subsequently, treated cells were assessed and counted under a microscope (BX53, Olympus, Tokyo, Japan) in five randomly selected fields.

For evaluating cell invasion ability, the steps were similar to those described above, with the exception that the bottom of the upper chamber of the Transwell was coated with 100 µL of matrigel evenly before cell seeding. The assay was repeated in triplicate for SiHa cells in each group to obtain the average value as the final outcome.

Western Blot

Total proteins from SiHa cells in each group were obtained using RIPA buffer (cat. no. 89900, Thermo Fisher Scientific, Waltham, MA, USA) containing phosphatase and protease inhibitors. The protein concentration in each sample was determined using the bicinchoninic acid (BCA) protein concentration assay kit (cat. no. E-BC-K318-M, Elabscience, Wuhan, China). Protein separation was carried out by 6–10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), followed by electrotransfer onto polyvinylidene fluoride (PVDF) membranes (Millipore, Burlington, MA, USA). The PVDF membranes were then sealed with 5% skimmed milk at room temperature for 1 hour, followed by overnight incubation with primary antibodies at 4 °C. The primary antibodies used were as follows: PTEN (1:2000, #22034-1-AP, Proteintech, Rosemont, IL, USA); p-mTOR (1:5000, #80596-1-RR, Proteintech, Rosemont, IL, USA); mTOR (1:2000, #28273-1-AP, Proteintech, Rosemont, IL, USA); Akt (1:2000, #10176-2-AP, Proteintech, USA); p-Akt (1:2000, #28731-1-AP, Proteintech, Rosemont, IL, USA); GAPDH (1:8000, #10494-1-AP, Proteintech, Rosemont, IL, USA).

The following day, the PVDF membranes were incubated with the secondary antibody HRP-conjugated goat anti-rabbit immunoglobulin G (IgG) (1:6000, #ab6721, Abcam, Cambridge, UK) for 1 hour at room temperature. Finally, the membranes were visualized using an imaging analysis system (Tanon, Shanghai, China), and protein expression levels were semi-quantitatively analyzed by Image J (version 1.8.0, NIH, Bethesda, MD, USA) software. GAPDH protein was used as the internal control to normalize the levels of mTORp-mTOR, p-Akt, PTEN and Akt, protein expression.

Statistical Treatment

Since the quantitative data obtained in this study generally followed a normal distribution, comparisons between the two groups were conducted using the independent sample *T*-test. Differences among multiple groups were analyzed using one-way analysis of variance (ANOVA), with post-hoc pairwise comparisons between groups performed using the Tukey method. Furthermore, the correlation between *LINC02487* and PTEN expression was assessed using Pearson correlation analysis. The data were expressed as mean ± standard deviation. Statistical analysis was performed using SPSS 25 software (IBM, Armonk, NY, USA), with a significance threshold set at $p < 0.05$.

Results

LINC02487 Lowly Expresses in Cell Models of Cervical Cancer and Clinical Tissue Samples

To examine *LINC02487* expression in CC, we utilized RT-qPCR technology to analyze clinical tissue samples and cell models. Our results revealed a significant decrease in *LINC02487* expression in the CC group compared to the Normal group ($p < 0.01$) (Fig. 1A). Additionally, *LINC02487* expression was markedly lower in SiHa cells compared to HaCaT cells ($p < 0.05$) (Fig. 1B). These findings suggest that *LINC02487* is downregulated in CC tissue samples and cell models, implicating a potential role in CC inhibition. Consequently, further verification is necessary to elucidate the impact of *LINC02487* in CC.

LINC02487 Participates in Inhibiting the Colony Formation, Proliferation, Invasion and Migration of Cervical Cancer Cells

The impact of *LINC02487* expression levels on the malignant biological behavior of CC cell models (SiHa) was further investigated. Initially, we conducted knockdown and overexpression experiments of *LINC02487* in SiHa cells. Following RT-qPCR analysis, it was evident that the *LINC02487* expression level in cells from the si-*LINC02487* group was significantly reduced compared to the siNC group ($p < 0.01$). Conversely, in the *LINC02487* group, *LINC02487* expression levels were markedly elevated compared to the vector group ($p < 0.01$) (Fig. 2A). These results confirm the successful knockdown and overexpression of *LINC02487* in SiHa cells.

Subsequently, the malignant biological characteristics of SiHa cells were observed across the aforementioned four groups. Through colony formation assays and CCK-8 analysis, it was evident that the proliferative capacity of cells was significantly enhanced in the si-*LINC02487* group compared to the siNC group ($p < 0.01$), while it was notably reduced in the *LINC02487* group compared to the vector group ($p < 0.01$) (Fig. 2B,C). Furthermore, in the Transwell experiment, the invasive and migratory abilities of cells were substantially higher in the si-*LINC02487* group compared to the siNC group ($p < 0.01$), whereas they were markedly diminished in the *LINC02487* group compared to the vector group ($p < 0.01$) (Fig. 2D,E). Consistent with these findings, *LINC02487* appears to inhibit the proliferation, invasion, and migration of SiHa cells.

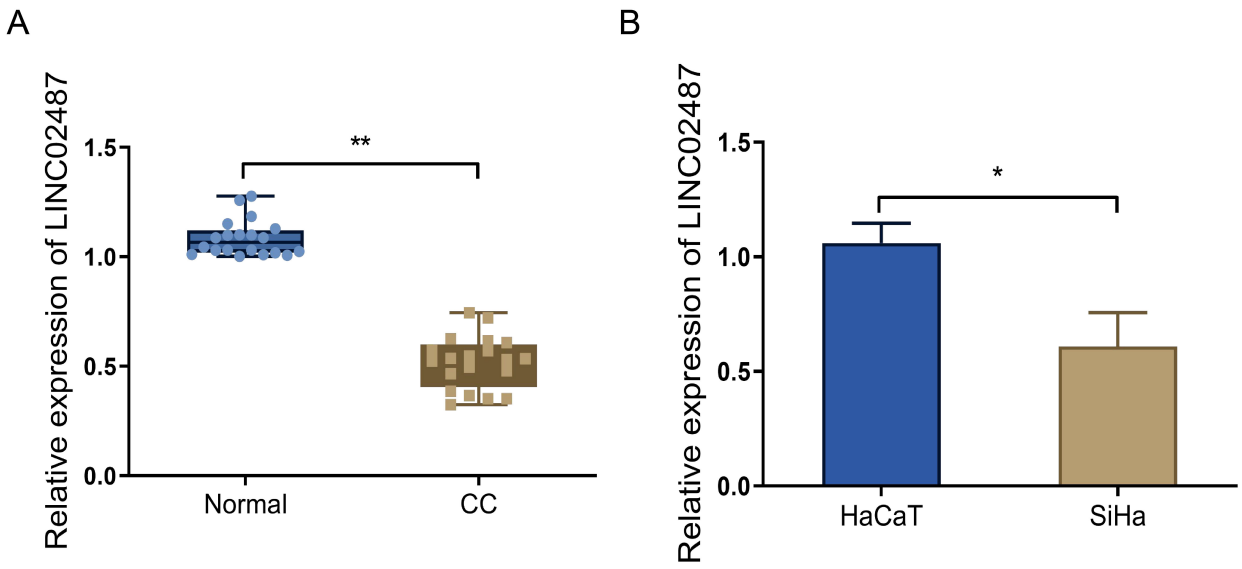

Fig. 1. Low *LINC02487* expression in clinical tissue samples and cell models of cervical cancer. (A,B) RT-qPCR for checking the expression level of *LINC02487* in tissue samples of the CC group (n = 20) and the Normal group (n = 20) (A) as well as the HaCaT group (n = 3) and the SiHa group (n = 3) (B). $^{*}p < 0.05$, $^{**}p < 0.01$. RT-qPCR, real-time quantitative polymerase chain reaction; CC, cervical cancer.

LINC02487 Upregulates the Expression of PTEN and Inhibits the Akt/mTOR Signaling Pathway

We investigated the potential association between *LINC02487* and *PTEN* in CC. The RT-qPCR results indicated a significant decrease in *PTEN* expression levels in the CC group compared to the Normal group ($p < 0.01$) (Fig. 3A). Additionally, Pearson correlation analysis revealed a positive correlation between *LINC02487* and *PTEN* expression levels (Fig. 3B). Furthermore, *PTEN* expression levels were notably lower in SiHa cells compared to HaCaT cells ($p < 0.01$) (Fig. 3C). Moreover, RT-qPCR analysis demonstrated a significant decrease in *PTEN* expression levels in the si-*LINC02487* group compared to the siNC group ($p < 0.01$), while *PTEN* expression levels were markedly elevated in the *LINC02487* group compared to the vector group ($p < 0.01$) (Fig. 3D). These findings suggest that *LINC02487* may positively regulate *PTEN* expression.

Following the discovery of *LINC02487*'s upregulatory effect on *PTEN* expression and considering previous studies demonstrating *PTEN*'s negative regulation of the Akt/mTOR signaling pathway [20], we further examined the influence of *LINC02487* expression levels on the PTEN/Akt/mTOR signaling pathway. Based on western blot results, the *PTEN* protein level in the si-*LINC02487* group was significantly decreased compared to the siNC group ($p < 0.01$), while the levels of p-Akt and p-mTOR proteins were markedly elevated ($p < 0.01$). Additionally, the ratios of p-Akt/Akt and p-mTOR/mTOR were remarkably increased ($p < 0.01$). Conversely, in the *LINC02487* group, the *PTEN* protein level increased significantly ($p < 0.01$), accompanied by notable decreases in the levels of p-mTOR and p-Akt proteins. Furthermore, the ratios of p-mTOR/mTOR and p-Akt/Akt dropped significantly ($p < 0.01$) compared to the vector group (Fig. 3E,F). These findings suggest that *LINC02487* may inhibit the Akt/mTOR signaling pathway in the SiHa cell line.

Hence, *LINC02487* upregulates *PTEN* expression and inhibits the Akt/mTOR signaling pathway in cervical cancer cells.

Knocking Down PTEN can Counteract the Inhibitory Effect of LINC02487 Over-Expression on Malignant Biological Behavior of Cervical Cancer Cells

The previous experiment unveiled the potential connection between *LINC02487* and *PTEN*. We further investigated whether the intervention effect of *LINC02487* on the CC cell (SiHa) models is primarily achieved by influencing *PTEN* expression levels. *PTEN* expression was reduced in SiHa cells through siRNA transfection to observe if *PTEN* knockdown could counteract the anticancer effect induced by *LINC02487* overexpression. Initially, RT-qPCR results demonstrated a significant downregulation of *PTEN* expression in the si-*PTEN* group compared to the siNC group ($p < 0.01$) (Fig. 4A). Similarly, based on the western blot results, the *PTEN* protein level in the si-*PTEN* group markedly decreased relative to the siNC group ($p < 0.01$) (Fig. 4B). These outcomes affirm the successful knockdown of *PTEN* expression via siRNA transfection.

Subsequently, we observed the malignant biological behaviors of SiHa cells after *PTEN* knockdown. The re-

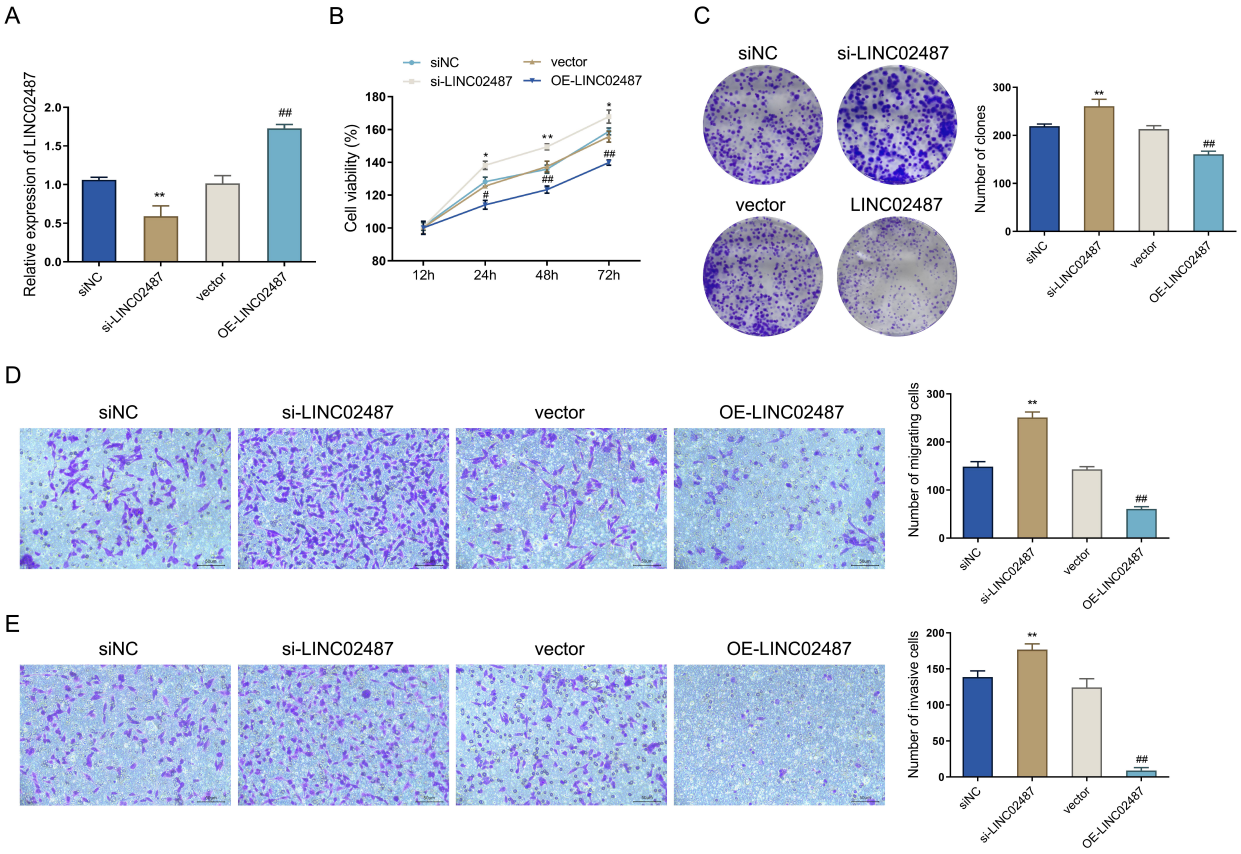

Fig. 2. The expression level of *LINC02487* affects the malignant biological behavior of cervical cancer cells. (A) RT-qPCR for detection of the *LINC02487* expression in cells of the siNC group, si-*LINC02487* group, vector group and *LINC02487* group. (B) Cell Counting Kit-8 (CCK-8) for measurement of the cell proliferation level in each group. (C) The colony formation ability of cells in each group was detected by cell colony formation assay. Magnification: 10×, petri dish: 6-well plate with 34.8 mm diameter. (D,E) The migration (D) and invasion (E) abilities of cells in each group were checked by Transwell, scale bar = 50 µm. n = 3 per group, $^{*}p < 0.05$, $^{**}p < 0.01$ vs. siNC group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ vs. vector group.

sults of CCK-8 and colony formation experiments showed that, compared to the vector+siNC group, cell proliferation was reduced in the *LINC02487*+siNC group ($p < 0.01$) and notably increased in the vector+si-*PTEN* group ($p < 0.01$). Moreover, compared to the *LINC02487*+siNC group, cell proliferation was significantly upregulated in the *LINC02487*+si-*PTEN* group ($p < 0.01$), while it was markedly decreased in the vector+si-*PTEN* group ($p < 0.01$) (Fig. 4C,D). In the Transwell assay, relative to the vector+siNC group, the invasion and migration levels of cells were significantly decreased in the *LINC02487*+siNC group ($p < 0.01$) and notably increased in the vector+si-*PTEN* group ($p < 0.01$). Furthermore, the invasion and migration abilities of cells were remarkably elevated in the *LINC02487*+si-*PTEN* group compared to the *LINC02487*+siNC group ($p < 0.01$), while they were significantly reduced in the *LINC02487*+si-*PTEN* group compared to the vector+si-*PTEN* group ($p < 0.01$) (Fig. 4E,F). These findings suggest that *PTEN* knockdown can counteract the effect of *LINC02487* overexpression on the malignant biological behaviors of cervical cancer cells.

LINC02487 Inhibits the Akt/mTOR Signaling Pathway through Upregulating the Expression of PTEN

Furthermore, we investigated the activation of the PTEN/Akt/mTOR signaling pathway in SiHa cells after PTEN knockdown. Western blot results revealed that in the *LINC02487*+siNC group, the PTEN protein level increased significantly compared to the vector+siNC group ($p < 0.01$), while the protein levels of p-mTOR and p-Akt, as well as the p-mTOR/mTOR and p-Akt/Akt ratios, markedly decreased ($p < 0.01$). Conversely, the vector+si-PTEN group exhibited a significant decrease in PTEN protein ($p < 0.01$), along with notable increases in p-mTOR and p-Akt proteins, and marked elevations in p-Akt/Akt and p-mTOR/mTOR ratios compared to the vector+siNC group ($p < 0.01$). Additionally, in the *LINC02487*+si-PTEN group,

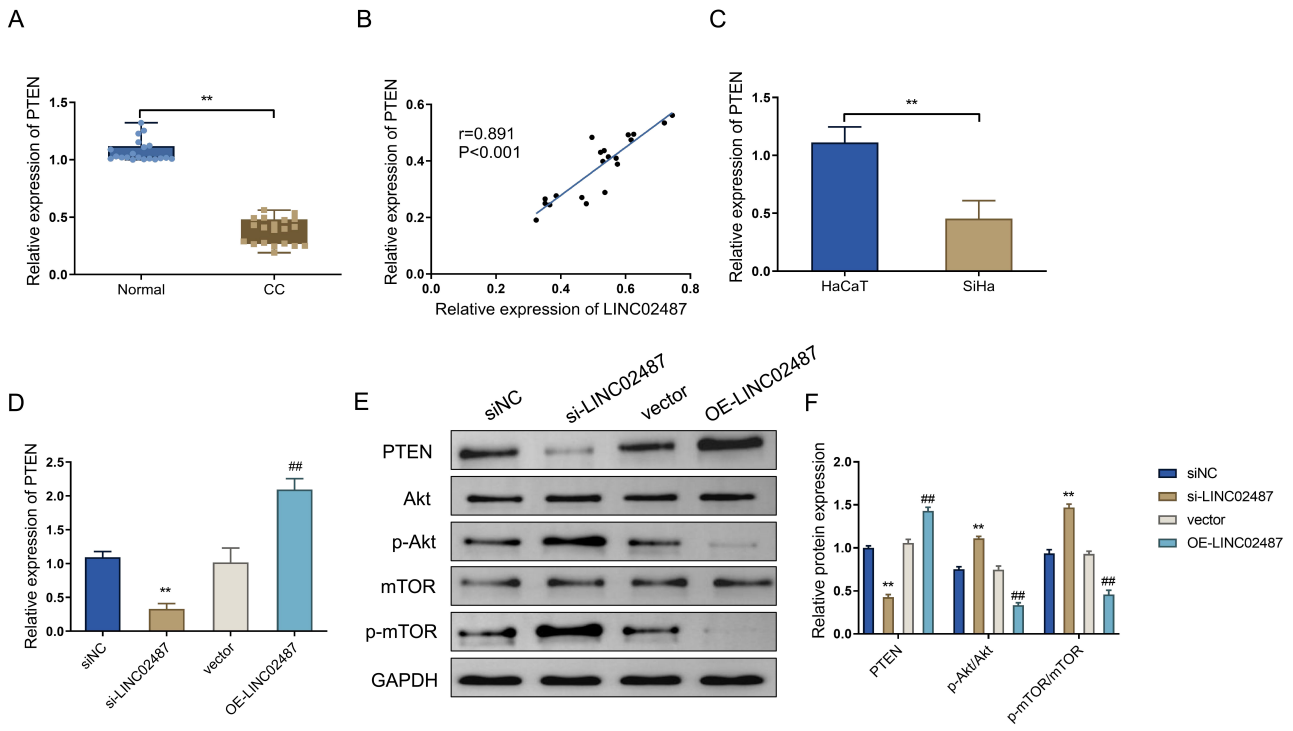

Fig. 3. *LINC02487* upregulates *PTEN* expression and inhibits the Akt/mechanistic target of the rapamycin (mTOR) signaling pathway. (A) The PTEN expression level was tested using RT-qPCR in the CC and Normal groups, $^{**}p < 0.01$. (B) *LINC02487* and PTEN expression levels measured by correlation analysis. (C) The *PTEN* expression level was checked by RT-qPCR in the HaCaT and SiHa groups. (D) The *PTEN* expression level was determined by RT-qPCR in cells of each group. (E) The measurement of the protein levels of Akt, p-Akt, PTEN, p-mTOR and mTOR was performed using western blot in cells of each group; the representative strip was shown above. (F) Quantitative statistical analysis results of PTEN protein level, p-mTOR/mTOR ratio and p-Akt/Akt ratio. n = 3 per group, $^{\#\#}p < 0.01$ vs. vector group; $^{**}p < 0.01$ vs. siNC group.

the PTEN protein level decreased considerably compared to the *LINC02487*+siNC group ($p < 0.01$), while the levels of p-Akt and p-mTOR proteins increased significantly ($p < 0.01$), along with remarkable elevations in p-Akt/Akt and p-mTOR/mTOR ratios ($p < 0.01$). Moreover, in the *LINC02487*+si-PTEN group, the PTEN protein level increased markedly relative to the vector+si-PTEN group ($p < 0.01$), while the levels of p-Akt and p-mTOR proteins decreased notably ($p < 0.01$), and the p-mTOR/mTOR and p-Akt/Akt ratios dropped significantly ($p < 0.01$) (Fig. 5A,B).

Based on the above outcomes, it is evident that *LINC02487* can inhibit the Akt/mTOR signaling pathway in CC cells by upregulating the expression of PTEN, consequently restraining the malignant biological behavior of CC cells.

Discussion

As elucidated in this study, both *LINC02487* and *PTEN* expression levels were found to be decreased in clinical tissue samples and cell models of CC, with *LINC02487* demonstrating a positive regulatory effect on *PTEN* expression. In *in vitro* experiments, overexpression of *LINC02487* significantly inhibited the malignant biological behavior of CC cells, whereas knocking down *PTEN* reversed the anticancer effect of *LINC02487* overexpression. This mechanism is likely associated with the modulation of the PTEN/Akt/mTOR signaling pathway. Therefore, we propose that targeted regulation of *PTEN* expression by *LINC02487* inhibits the progression of CC. This study not only unveils the anticancer effect of *LINC02487* in the pathogenesis of CC for the first time but also establishes a positive correlation between *LINC02487* and *PTEN*. These findings provide a theoretical foundation for the clinical development of anti-CC therapeutic interventions targeting *LINC02487*.

The intricate relationship between lncRNA molecules and cancer is well-established. While some lncRNAs may exert tumor-suppressive effects, others can promote cancer development. The abnormal expression levels of lncRNAs in tumor tissues serve as crucial indicators of their potential functions. Generally, high expression levels of lncRNAs in tumor tissue suggest a cancer-promoting role, whereas low expression levels may indicate a tumor-suppressive function [21]. Building upon this premise, we initially investi-

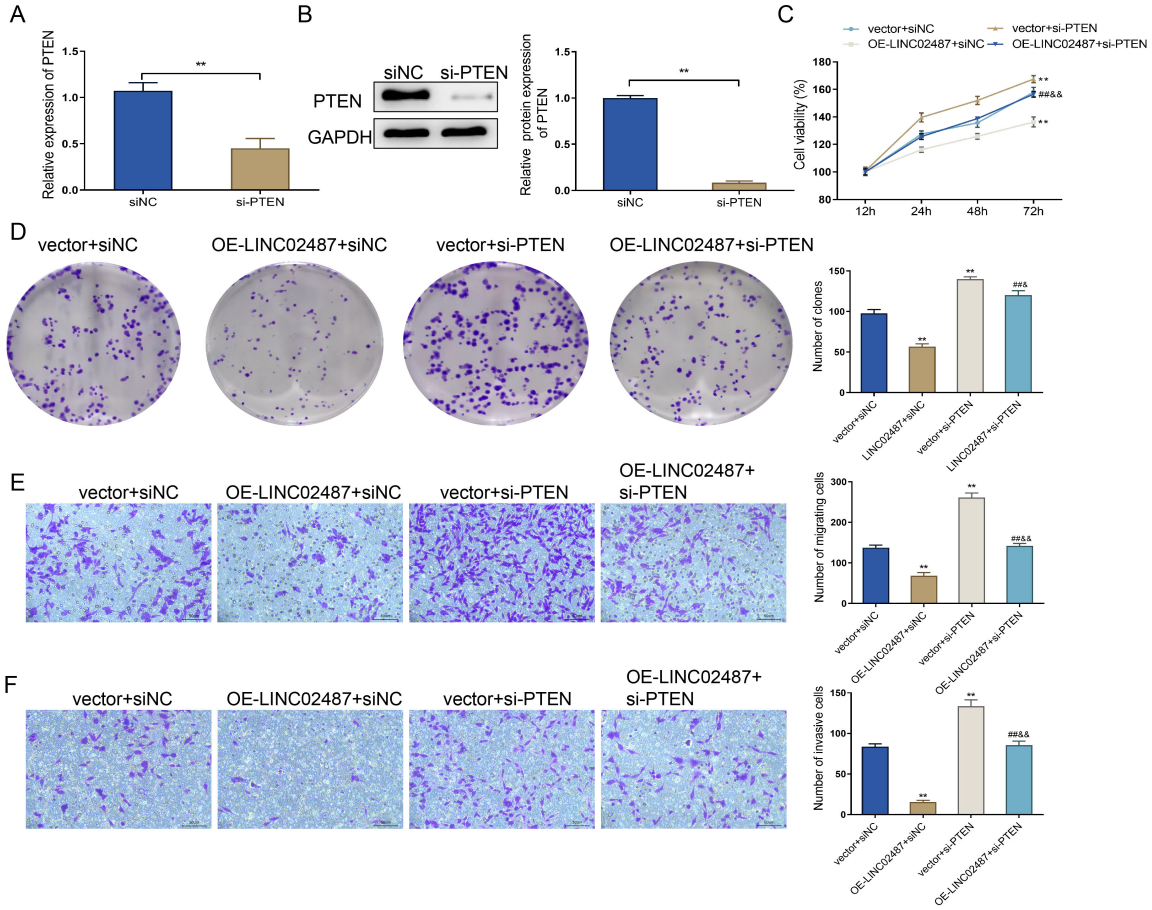

Fig. 4. Knocking down *PTEN* counteracts the anticancer effect of *LINC02487*. (A) The *PTEN* expression level detected by RT-qPCR in the si-*PTEN* group and siNC group, $^{**}p < 0.01$. (B) The expression level of PTEN protein was assessed using western blot in the si-*PTEN* group and siNC group, $^{**}p < 0.01$. (C–F) The colony formation, invasion, proliferation, and migration levels of cells in each group were investigated by CCK-8 (C), colony formation assay (magnification: 10×, petri dish: 6-well plate with 34.8 mm diameter) (D) and Transwell (E,F), respectively, scale bar = 50 µm. n = 3 per group, $^{\#\#}p < 0.01$ vs. *LINC02487*+siNC group; $^{**}p < 0.01$ vs. vector+siNC group; $^{\&}p < 0.05$, $^{\&\&}p < 0.01$ vs. vector+si-PTEN group.

gated the expression of *LINC02487* in CC. Our research revealed that *LINC02487* expression was significantly downregulated in clinical tissue samples and cell models of CC. Although previous studies have scarcely examined the role of *LINC02487* in CC, clinical investigations focusing on OSCC have reported a significant decrease in *LINC02487* levels in tumor tissues compared to adjacent normal tissues or healthy subjects' oral mucosa [10–12].

Considering the potential similarities in the pathogenesis of malignant tumors across different anatomical sites, we find that the conclusions drawn from previous studies align closely with the findings of our investigation. Subsequently, the results of differential expression level analysis were further corroborated through *in vitro* experiments involving the knockdown and overexpression of *LINC02487*. These experiments revealed that knocking down *LINC02487* significantly promoted the malignant biological behavior of CC cells, whereas overexpressing *LINC02487* led to significant inhibition of various malignant characteristics of CC cells. Feng *et al.* [13] demonstrated that *in vitro*, *LINC02487* directly inhibited the invasion and migration of OSCC cells, which is largely consistent with our findings. However, their study solely focused on the effect of *LINC02487* overexpression. In contrast, our research explored the impact of *LINC02487* from two perspectives—knockdown and overexpression—adding strength to our conclusions. Consequently, the findings of this study robustly support the conclusion that *LINC02487* acts as an anticancer factor in CC.

Additionally, we conducted a comparison between SiHa cells in the vector+siNC group and vector+si-*PTEN* group to elucidate the role of *PTEN* knockdown on the malignant biological behavior of CC cell models. In summary, *PTEN* knockdown notably promoted the migration, proliferation, invasion, and colony formation of CC cells, indicating that *PTEN* also exerts a similar inhibitory effect on the progression of CC as *LINC02487*. It is noteworthy that mutations or deletions of *PTEN* are prevalent in CC patients,

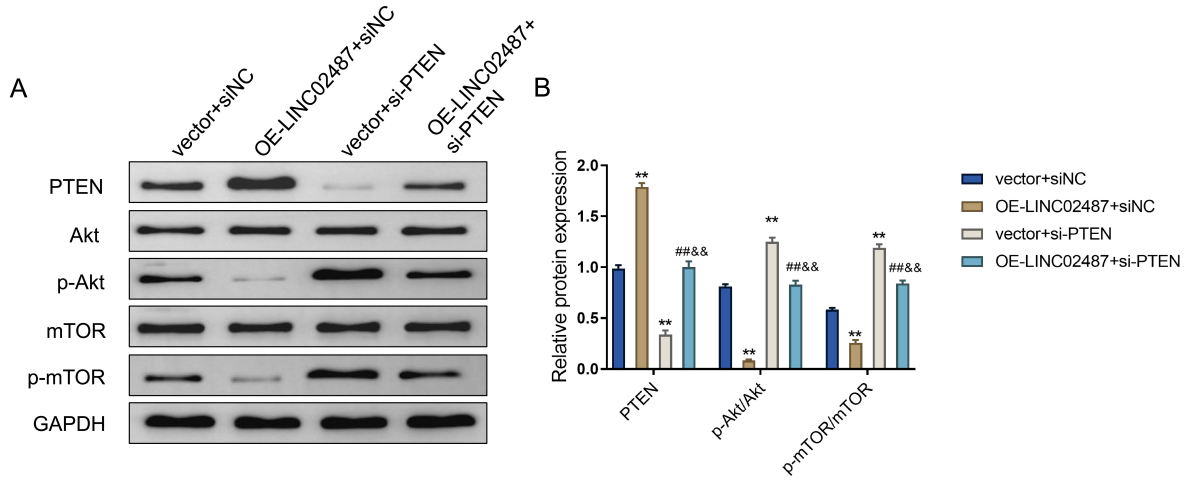

Fig. 5. *LINC02487* inhibits the Akt/mTOR signaling pathway by upregulating the expression of PTEN. (A) Western blot for the measurement of the levels of PTEN, p-Akt, Akt, p-mTOR and mTOR protein in cells of each group (the representative strip was shown above). (B) Quantitative statistical analysis for the PTEN protein level, p-mTOR/mTOR ratio and p-Akt/Akt ratio. n = 3 per group, $^{\#\#}p < 0.01$ vs. *LINC02487*+siNC group; $^{**}p < 0.01$ vs. vector+siNC group; $^{\&\&}p < 0.01$ vs. vector+si-PTEN group.

affecting approximately 40% of females with CC [22]. Previous studies have proposed that alterations in *PTEN* may lead to the inactivation of its bispecific phosphatase protein, thereby weakening its anticancer functions such as cell cycle regulation, inhibition of angiogenesis, and promotion of apoptosis, ultimately facilitating the occurrence and progression of CC [23,24]. These hypotheses align well with the results of this study.

There have been previous studies indicating that several lncRNAs can participate in the pathological process of CC by regulating *PTEN* expression. For instance, lncRNA ILF3-AS1 can elevate *PTEN* expression levels, thereby promoting apoptosis and inhibiting the proliferation of CC cells [25]. Additionally, lncRNA HOTAIR can downregulate *PTEN* expression levels, induce EMT in CC cells, and consequently lead to drug resistance in CC cells [26]. Moreover, lncRNA *LINC00673* has been found to enhance the invasion and migration abilities of CC cells by downregulating *PTEN* expression levels [27].

Following the confirmation that the malignant biological behavior of CC cells can be inhibited by both *LINC02487* and *PTEN*, we further investigated whether *LINC02487* could serve as an upstream regulator of *PTEN*. Firstly, a positive correlation between *LINC02487* and *PTEN* expression levels was observed in both CC tissue samples and cell models through correlation analysis. Additionally, *PTEN* knockdown could essentially counteract the anticancer effect of *LINC02487* overexpression (no significant difference was found in SiHa cells between the vector+siNC group and *LINC02487*+si-PTEN group). Therefore, the anti-CC effect of *LINC02487* is predominantly achieved by upregulating *PTEN* expression.

As we are aware, one of the crucial pathways through which PTEN protein exerts its anticancer role is by activating the PTEN/Akt/mTOR signaling pathway [28]. In this signaling cascade, upon activation by external signals, the kinase PI3K phosphorylates phosphatidylinositol 4,5-bisphosphate (PIP2) on the cell membrane, converting it into phosphatidylinositol 3,4,5-trisphosphate (PIP3), a second messenger. Subsequently, PIP3 binds to Akt, leading to its phosphorylation and activation into p-Akt. Activated p-Akt then phosphorylates its substrate mTOR, converting it into p-mTOR. This activation of the Akt/mTOR pathway accelerates cancer progression by enhancing cell migration, proliferation, and adjacent angiogenesis [29]. However, when there is an adequate amount of PTEN protein present, it exhibits its intrinsic phosphatase activity by dephosphorylating PIP3 into PIP2, thereby reducing the cellular levels of PIP3 and inhibiting the activation of downstream molecules [30]. This mechanism underscores the potent anticancer effect of the PTEN protein.

In this study, both knocking down *LINC02487* and *PTEN* led to a significant increase in the p-mTOR/mTOR and p-Akt/Akt ratios in CC cells. This observation indicates that *LINC02487* inhibits the Akt/mTOR signaling pathway by upregulating *PTEN* expression. Conversely, this finding also suggests that *LINC02487* may exert its anticancer effect by inhibiting the Akt/mTOR signaling pathway through the elevation of *PTEN* expression in CC cells.

As demonstrated in this study, the clinical significance lies in the potential of *LINC02487* as a promising target for treating CC. With the advancements in lipid nanoparticle (LNP) technology, LNPs can serve as effective carriers for delivering small RNA molecules. By encapsulating small

RNA molecules within LNPs and adjusting their surface ligands, LNPs can accurately release these molecules into the cytoplasm of target cells [31]. Therefore, targeted delivery of *LINC02487* to CC cells using this method holds promise as a novel treatment approach.

Compared to the complex and invasive radical surgery that often entails significant patient discomfort, targeted delivery therapy with *LINC02487* offers a simpler and more effective alternative. Relative to radiotherapy or chemotherapy, targeted delivery therapy with *LINC02487* is also anticipated to be safer and result in fewer systemic adverse reactions.

However, it's important to acknowledge some limitations of this study. Firstly, the regulatory relationship between *LINC02487* and *PTEN* warrants further validation and it remains unclear whether *LINC02487* can play a role in other pathways. Additionally, while *LINC02487* has been shown to inhibit the progression of OSCC by directly binding to the USP17 protein [13], it's uncertain whether *LINC02487* exerts a similar effect in CC. Furthermore, although this study primarily validated the anticancer effect of *LINC02487 in vitro*, the lack of *in vivo* tumorigenesis experiments may somewhat diminish the persuasiveness of the findings. Therefore, further *in vitro* and *in vivo* experiments are warranted to comprehensively investigate the therapeutic potential of *LINC02487* in CC.

Conclusion

In summary, our study findings elucidate that the expression levels of *LINC02487* and *PTEN* are downregulated in both clinical tissue samples and cell models of CC. Through *in vitro* experiments, we demonstrated that overexpression of *LINC02487* inhibits the Akt/mTOR signaling pathway by elevating *PTEN* expression levels, consequently suppressing the invasion, migration, and proliferation of CC cells. This study unveils the anticancer role of *LINC02487* in the pathogenesis of CC and establishes its potential correlation with *PTEN* for the first time.

Availability of Data and Materials

Data involved in the present work are available from the corresponding author upon request.

Author Contributions

RS and SHL designed the research study. RS and SHL performed the research. SLH, HYL and XWW provided help and advice on experiments. SLH, HYL and XWW analyzed the data. All authors were involved in drafting and critical revision of the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

This study was in line with the Declaration of Helsinki. The review and approval were performed by the Ethics Committee of The Affiliated Hospital of Yangzhou University, Yangzhou University (F2022-83). Additionally, informed consent was obtained from all participants prior to surgery.

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Conflict of Interest

The authors declare no conflict of interest.

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