

Silencing of LIM and SH3 Protein 1 (LASP-1) Inhibits Thyroid Cancer Cell Proliferation and Invasion

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LIM and SH3 protein 1 (LASP-1) is a specific focal adhesion protein that was first identified in breast cancer and then reported to be involved in cell proliferation and migration. Many studies have demonstrated the essential role of LASP-1 in cancer progression. However, there have been no studies on the association of LASP-1 with thyroid cancer. In this study, we investigated the expression pattern and biological function of LASP-1 in thyroid cancer. We found that LASP-1 was highly expressed in thyroid cancer tissues and cell lines. LASP-1 silencing had antiproliferative and anti-invasive effects on thyroid cancer cells. Moreover, tumor xenograft experiments showed that LASP-1 silencing suppressed thyroid cancer cell growth *in vivo*. We also demonstrated that LASP-1 silencing decreased the protein expression of p-PI3K and p-Akt. In conclusion, these findings suggest LASP-1 to be an oncogene and a potential therapeutic target in thyroid cancer.

Key words: LIM and SH3 protein 1 (LASP-1); Proliferation; Migration; Invasion; Thyroid cancer

INTRODUCTION

Thyroid cancer, a common endocrine malignancy, originates from follicular epithelial cells^{1,2}. According to histological types, thyroid cancer is classified into papillary thyroid cancer, follicular thyroid cancer, poorly differentiated thyroid cancer, anaplastic thyroid cancer, and medullary thyroid cancer³. Considerable advancements have been made in the treatment of thyroid cancer, and patients with the disease can be treated via multiple approaches such as thyroidectomy, radioiodine, external beam radiation, and chronic thyroid-stimulating hormone suppression^{4,5}. Unfortunately, a significant proportion of thyroid cancer patients succumb to the disease due to recurrence, aggressiveness, or metastasis⁶. Thus, we need to explore new molecular markers and better understand the molecular mechanisms underlying thyroid cancer progression so as to improve the long-term survival of thyroid cancer patients.

LIM and SH3 protein 1 (LASP-1), a kind of scaffolding protein-binding actin and zyxin, is ubiquitously expressed and has a special domain structure containing both LIM and SH3 domains^{7–10}. Localization of LASP-1 has been suggested in multiple sites of actin assembly, such as focal adhesions, focal contacts, lamellipodia, pseudopodia, and membrane ruffles^{9,11–14}. LASP-1 was first identified from a cDNA library of breast cancer metastases and

found to be highly expressed in 8%–12% of human breast cancers, indicating a nonnegligible role for LASP-1 in the development of breast cancer¹¹. Furthermore, increasing evidence has demonstrated overexpression of LASP-1 in different kinds of cancers and the promoting effect of LASP-1 on tumor growth^{15–18}. However, the role of LASP-1 in thyroid cancer has not yet been elucidated.

Here we investigated the biological functions of LASP-1 in thyroid cancer. We found that LASP-1 was highly expressed in thyroid cancer tissues and cell lines. LASP-1 silencing had antiproliferative and anti-invasive effects on thyroid cancer cells. Moreover, tumor xenograft experiments showed that LASP-1 silencing suppressed thyroid cancer cell growth *in vivo*. We also demonstrated that LASP-1 silencing inhibited the activation of the PI3K/Akt signaling pathway. These results suggest LASP-1 to be an oncogene in thyroid cancer.

MATERIALS AND METHODS

Patients and Tissue Samples

Human thyroid cancer tissues and adjacent normal tissues were obtained from 28 patients who received surgery in the Huaihe Hospital of Henan University (Kaifeng, P.R. China). Tissue samples were frozen in liquid nitrogen immediately after collection and then stored at -80°C until use. Each patient provided written consent.

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All experiments in the study were approved by the ethics committee of the Huaihe Hospital of Henan University.

Cell Lines and Cell Culture

Human thyroid cancer cell lines (TPC1 and FTC133) and a normal human thyroid epithelial cell line (Nthy-ori 3-1) were purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA). All cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco, Rockville, MD, USA) containing 10% fetal bovine serum (FBS; Gibco), 100 U/ml penicillin, and 100 mg/ml streptomycin (Sigma-Aldrich, St. Louis, MO, USA) and maintained in a humidified atmosphere at 37°C with 5% CO₂.

RNA Extraction and Quantitative RT-PCR

Total RNA was extracted from tissues and cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and reversely transcribed using PrimeScript RT Reagent Kit (Takara, Dalian, P.R. China). Amplification of cDNA was conducted using SYBR Green Premix Ex Taq (Takara) under the following conditions: 95°C for 1 min, 35 cycles of 95°C for 15 s, 56°C for 30 s, and 72°C for 30 s. The following primers were used: LASP-1, 5'-CAGCCCCA GTCTCCATACAG-3' (forward) and 5'-ATACTGATGT CGCGGCGG-3' (reverse); GAPDH, 5'-CATCTCTGCC CCCTCTGCTGA-3' (forward) and 5'-GGATGACCTG CCCACAGCCT-3' (reverse). The mRNA expression level of GAPDH was used for normalization. Fold changes were calculated according to the 2^{-ΔΔCT} method as previously described¹⁹.

Western Blot Analysis

Tissues and cells were lysed in RIPA buffer (Sigma-Aldrich). Supernatants were gathered, subjected to 12% SDS-PAGE, and then transferred onto PVDF membranes (Bio-Rad, Hercules, CA, USA). Subsequent to blocking in 5% skim milk, the membranes were probed overnight at 4°C with primary antibodies against LASP-1, p-PI3K, PI3K, p-Akt, Akt, or GAPDH followed by incubation for 2 h at room temperature with specific secondary antibodies. All antibodies were purchased from Cell Signaling Technology (Danvers, MA, USA). Protein bands were visualized using an ECL detection system (Bio-Rad). Band intensities were quantified using ImageQuant software (GE Healthcare, Waukesha, WI, USA).

Small Interfering RNA (siRNA) and Transfection

Oligonucleotides of siRNA targeting LASP-1 (si-LASP-1) and control siRNA (si-NC) were purchased from GenePharma (Shanghai, P.R. China). The sequences of si-LASP-1 and si-NC were, respectively, 5'-CUUA UCCAGACAGUUCACCCdTdT-3' and 5'-AGAGAUG

UAGUCGCUCGCUdTdT-3'. Cells of 70% confluence were transfected with si-LASP-1 or si-NC using Lipofectamine 3000 (Invitrogen). LASP-1 silencing was confirmed via Western blot.

CCK-8 Assay

The proliferative ability of thyroid cancer cells was assessed using the CCK-8 assay. In brief, cells were planted into 96-well plates at a density of 5×10³ cells/well. CCK-8 (10 μl) was added to each well at different time points and incubated at 37°C for 4 h. The absorbance was measured at 450 nm using a spectrophotometer.

Wound Healing Assay

The migratory capacity was evaluated via wound healing assay. Cells (2×10⁵)/well were seeded into six-well plates and cultured to full confluence. After scratching with a sterile pipette tip (200 μl), cells were washed with PBS three times and then cultured in serum-free medium for 48 h. Cell migration was observed under an inverted microscope at 0 and 48 h. The distance between wound edges was calculated using ImageJ software.

Transwell Assay

The ability of thyroid cancer cells to invade was measured using Transwell chambers with Matrigel-coated inserts (BD Biosciences, San Jose, CA, USA). Cells in serum-free medium were added to the upper chambers at a density of 2×10⁵ cells/well. RPMI-1640 (Sigma-Aldrich) containing 10% FBS was added to the lower chambers. Following incubation at 37°C for 48 h, noninvading cells were wiped off with a cotton swab, and invading cells were stained with 0.1% crystal violet and photographed under a microscope. Five fields were randomly selected to count the number of invading cells.

Xenograft Experiments

All animal experiments were carried out in accordance with the procedure approved by the Institutional Animal Care and Use Committee of Henan University. Male nude mice (BALB/c, 5 weeks old) were obtained from Henan Laboratory Animal Center (Zhengzhou, P.R. China) and bred under specific pathogen-free condition. After suspending in 100 μl of serum-free RPMI-1640, 2×10⁵ TPC1 cells with stable transfection of si-LASP-1 or si-NC were subcutaneously injected into the flank of nude mice (n=6). Thirty-five days later, mice were sacrificed to measure tumor size and weight.

Statistical Analysis

Data were obtained from at least three independent experiments and expressed as means ± standard deviation (SD). Statistical analysis was performed using SPSS 19.0.

Group differences were compared via Student's *t*-tests. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Expression of LASP-1 in Thyroid Cancer Tissues and Cell Lines

We determined the expression level of LASP-1 in thyroid cancer tissues and corresponding normal tissues. RT-PCR and Western blot analysis showed that LASP-1 was expressed at a high level in thyroid cancer tissues

in comparison with the normal tissues (Fig. 1A and B). We next investigated LASP-1 expression in thyroid cancer cell lines and a normal thyroid epithelial cell line. LASP-1 was expressed at a higher level in TPC1 and FTC133 cells than in Nthy-ori 3-1 cells (Fig. 1C and D).

The Effect of LASP-1 Silencing on Thyroid Cancer Cell Proliferation In Vitro

Prior to investigation of the role of LASP-1 in the proliferation of thyroid cancer cells, TPC1 and FTC133 cells

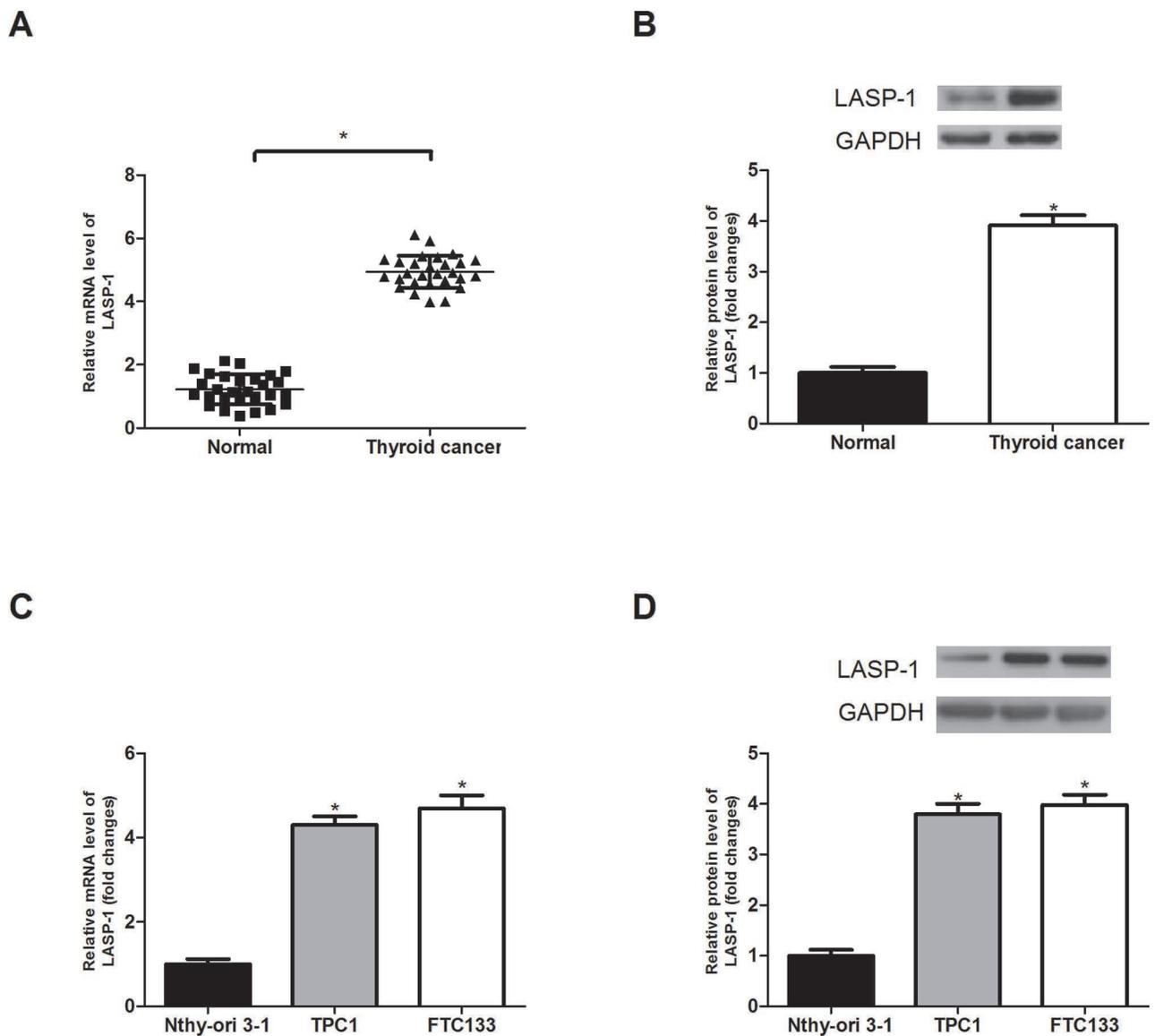


Figure 1. The expression of LASP-1 in thyroid cancer tissues and cell lines. (A, B) The relative expression of LASP-1 in thyroid cancer tissues and matching normal tissues was determined by RT-PCR and Western blot analysis ($n=28$). (C, D) The relative expression of LASP-1 in thyroid cancer cell lines (TPC1 and FTC133) and a normal thyroid epithelial cell line (Nthy-ori 3-1) was determined by RT-PCR and Western blot analysis. $*p < 0.05$.

were transfected with si-LASP-1 for LASP-1 silencing. Western blot analysis demonstrated that the expression of LASP-1 was greatly decreased in TPC1 and FTC133 cells after transfection with si-LASP-1 compared to the si-NC group (Fig. 2A and B). The CCK-8 assay was performed to test the proliferative ability of thyroid cancer cells. LASP-1 silencing significantly inhibited the proliferation of TPC1 and FTC133 cells (Fig. 2C and D).

The Effect of LASP-1 Silencing on Thyroid Cancer Cell Migration and Invasion In Vitro

Wound healing and Transwell assays were used to test the effect of LASP-1 silencing on thyroid cancer cell migration and invasion, respectively. The wound healing assay showed that LASP-1 silencing slowed down the

rate of wound closure in comparison with that of control cells (Fig. 3A and B). The Transwell assay indicated that decreased LASP-1 expression inhibited TPC1 and FTC133 cell invasion in comparison with the control cells (Fig. 3C and D).

The Effect of LASP-1 Silencing on the Activation of the PI3K/Akt Signaling Pathway

The PI3K/Akt signaling pathway is known to be an important player in tumor development²⁰, so we investigated the effect of LASP-1 silencing on the expression of some crucial molecules involved in the pathway. LASP-1 silencing in TPC1 cells significantly reduced the levels of PI3K and Akt phosphorylation without affecting the protein expression of PI3K and Akt (Fig. 4).

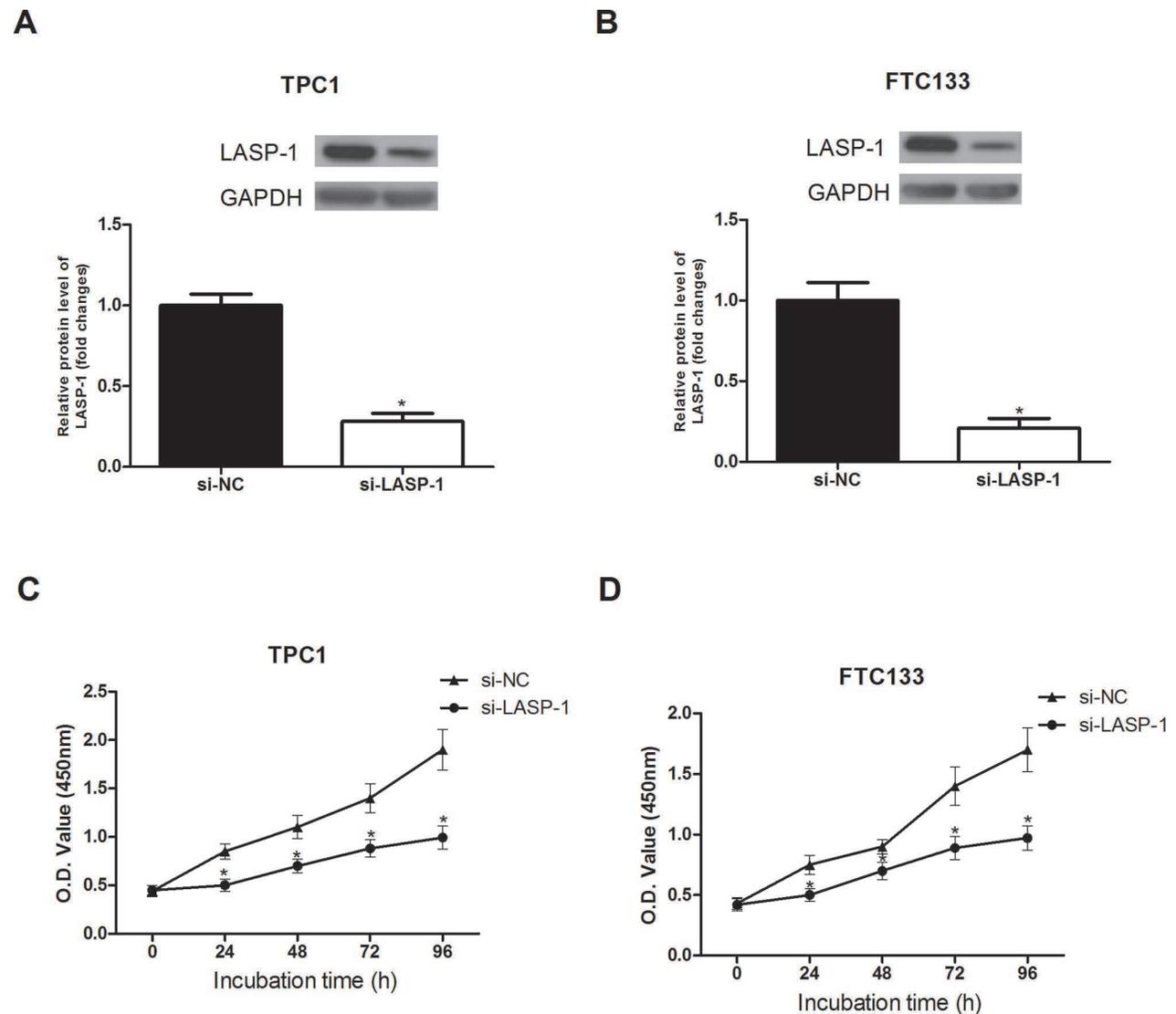


Figure 2. The effect of LASP-1 silencing on thyroid cancer cell proliferation in vitro. (A, B) The relative expression of LASP-1 was detected by Western blot analysis after transfection of TPC1 and FTC133 cells with si-LASP-1 or si-NC. (C, D) The proliferation rate of TPC1 and FTC133 cells was remarkably reduced after LASP-1 silencing. * $p < 0.05$.

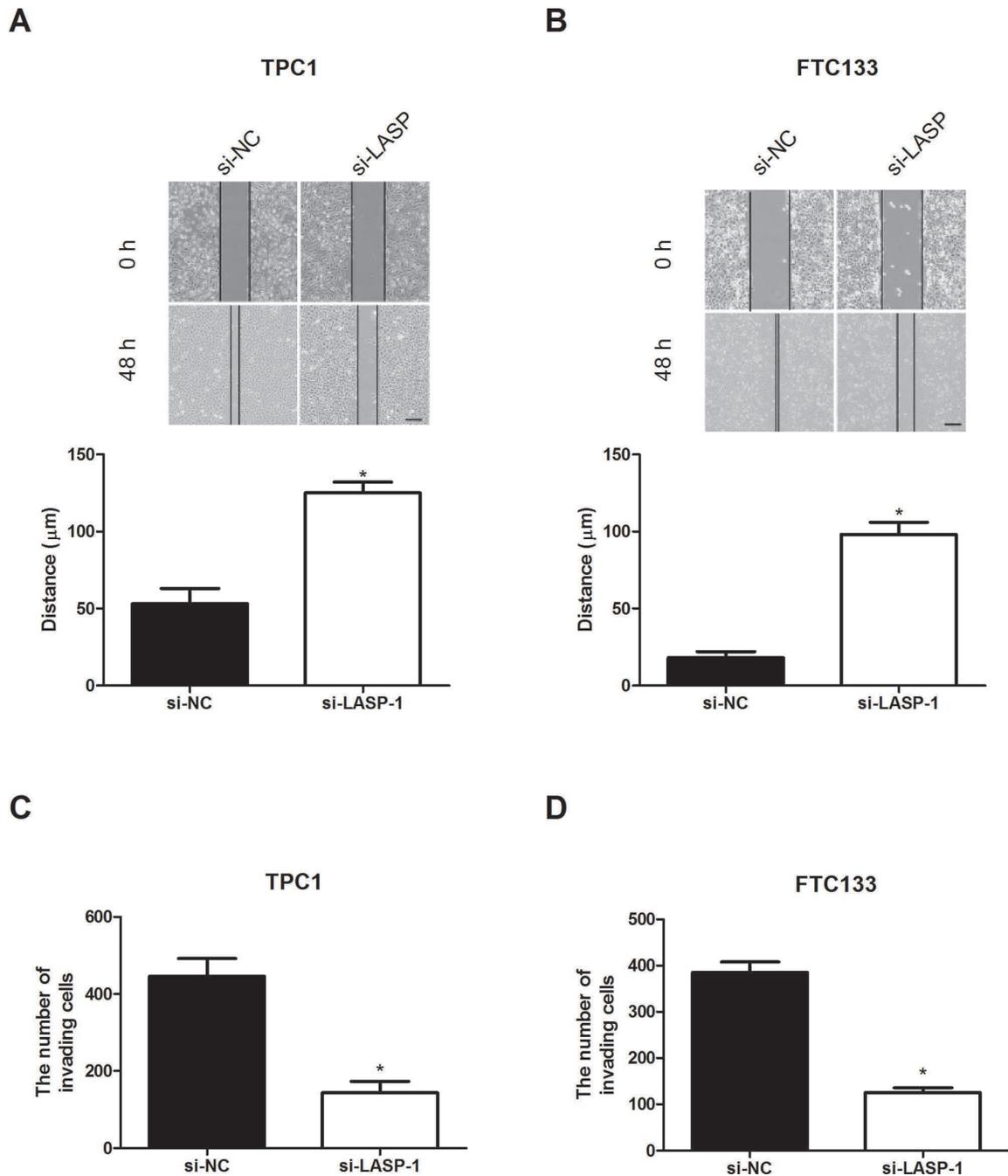


Figure 3. The effect of LASP-1 silencing on thyroid cancer cell migration and invasion in vitro. (A, B) The wound healing assay showed that the migratory capacities of TPC1 and FTC133 cells were reduced after LASP-1 silencing, in comparison with the control cells. (C, D) The Transwell assay revealed that the invasive abilities of TPC1 and FTC133 cells were weakened after LASP-1 silencing, in comparison with the control cells. * $p < 0.05$.

The Effect of LASP-1 Silencing on Thyroid Cancer Cell Growth In Vivo

To further examine the in vivo tumorigenic capability of LASP-1, we subcutaneously injected si-LASP-1-TPC1 or si-NC-TPC1 cells into nude mice ($n=6$). The results showed that tumors induced by the si-LASP-1-TPC1 cells

exhibited a smaller volume (Fig. 5A) and a lighter weight (Fig. 5B) than those induced by si-NC-TPC1 cells.

DISCUSSION

Statistically, thyroid cancer has an increasing worldwide incidence that has reached a new peak in 2016²¹⁻²³.

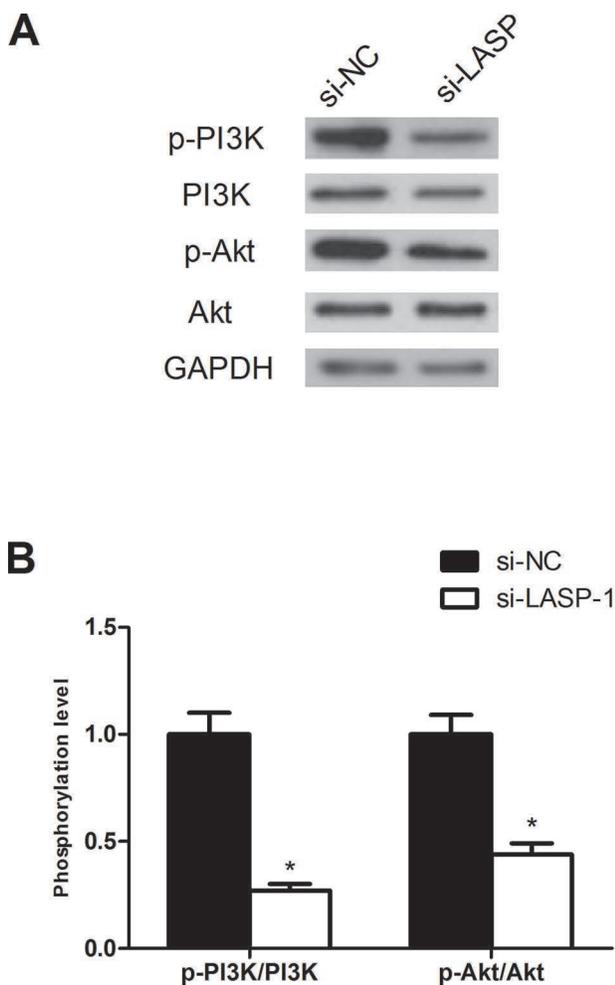


Figure 4. The effect of LASP-1 silencing on the activation of PI3K/Akt signaling pathway. (A) The expression levels of p-PI3K, PI3K, p-Akt, and Akt in si-LASP-1-TPC1 and si-NC-TPC1 cells were detected by Western blot analysis. (B) Quantification of p-PI3K/PI3K and p-Akt/Akt. * $p < 0.05$.

Exploring a novel therapeutic target may be an effective way to solve this thorny problem.

LASP-1 is a specific focal adhesion protein that was first identified from breast cancer and then found to be involved in cell proliferation and migration. Many studies have demonstrated overexpression of LASP-1 in various cancers and showed an essential role for LASP-1 in cancer progression. For example, Grunewald et al. reported a high expression of LASP-1 in ovarian cancer tissues and cell lines²⁴. They also suggested that down-regulation of LASP-1 inhibited ovarian cancer cell proliferation and migration²⁴. These observations expanded the role of LASP-1 in cellular processes. Similarly, Shimizu et al. found a significant upregulation of LASP-1 in oral cancer and an inhibitory effect of LASP-1 knockdown on oral cancer cell growth in vitro and in vivo²⁵. In our

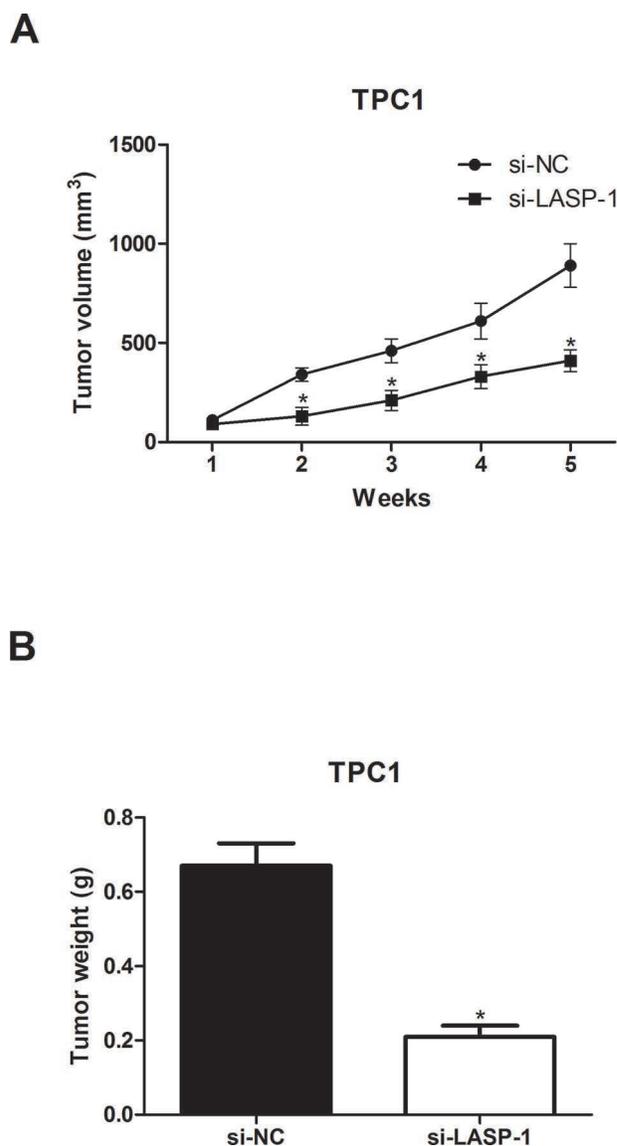


Figure 5. The effect of LASP-1 silencing on thyroid cancer cell growth in vivo. (A) The volume of tumors was measured every week after subcutaneous injection of si-LASP-1-TPC1 or si-NC-TPC1 cells into nude mice. (B) The histogram presented the mean weight of tumors from the si-LASP-1 and si-NC groups. * $p < 0.05$.

study, we evaluated the expression pattern of LASP-1 in thyroid cancer tissues and cell lines. The results showed that LASP-1 had a high expression level in thyroid cancer tissues and cell lines, indicating its implication in the carcinogenesis of thyroid cancer. We performed in vitro experiments to investigate the effect of LASP-1 silencing on thyroid cancer cell proliferation, migration, and invasion. Consistent with the aforementioned findings, our study results revealed that LASP-1 silencing markedly inhibited these cellular processes. In addition, we

carried out in vivo tumorigenesis assays and found that LASP-1 silencing strongly suppressed thyroid cancer cell growth in nude mice, which further verified the in vitro results. Taken together, our study provided evidence demonstrating the important role of LASP-1 in thyroid cancer progression.

The PI3K/Akt signaling pathway has been found to play a significant role in cell proliferation, migration, and invasion of various cancers²⁶⁻²⁸. Oncogenes activate PI3K and subsequently promote cancer cell growth and survival²⁹. Akt is a key molecule of the PI3K pathway, and its activation will cause phosphorylation and impact downstream targets³⁰. Furthermore, Akt activation is frequently observed in cancer³¹. More importantly, numerous studies have demonstrated a close relationship between the PI3K/Akt signaling pathway and thyroid cancer development. For example, Fu et al. reported that targeting the PI3K/Akt signaling pathway could remarkably inhibit cell growth in thyroid cancer³². Another study demonstrated that the PI3K/Akt signaling pathway was activated in thyroid cancer cells to induce epithelial–mesenchymal transition and ultimately promote cell migration and invasion³³. In this study, we found that LASP-1 silencing suppressed the activation of PI3K/Akt signaling pathway by reducing the protein expression of p-PI3K and p-Akt. Based on all the evidence, we suggest that LASP-1 silencing inhibits the proliferation, migration, and invasion of thyroid cancer cells via regulating the PI3K/Akt signaling pathway. Considering that the development of thyroid cancer is a complex process and involves multiple pathways, more studies are required for further investigation on the mechanisms underlying the inhibitory effect of LASP-1 silencing on thyroid cancer cells.

In conclusion, we reported overexpression of LASP-1 in thyroid cancer tissues and cell lines. LASP-1 silencing inhibited thyroid cancer cell proliferation, migration, and invasion in vitro. In addition, xenograft experiments showed that LASP-1 silencing suppressed thyroid cancer cell growth in vivo. We also found that LASP-1 silencing decreased the protein expression of p-PI3K and p-Akt, inhibiting activation of the PI3K/Akt signaling pathway. These findings provide an important implication that LASP-1 may be a promising therapeutic target for thyroid cancer.

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