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Long noncoding RNA LSINCT5 is upregulated and promotes the progression of esophageal squamous cell carcinoma

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Abstract. – OBJECTIVE: Long stress-induced noncoding transcripts 5 (LSINCT5) has been reported to be upregulated in several human cancers and related to poor prognosis. However, its involvement in esophageal squamous cell carcinoma (ESCC) remains largely unknown. We aim to evaluate the expression and putative role of LSINCT5 on the progression of ESCC.

MATERIALS AND METHODS: LSINCT5 expression was first examined in the ESCC cell lines using RT-qPCR, and the next-generation RNA-Seq technology was employed to analyze and functionally annotate the differential gene expression before and after LSINCT5 knockdown in ESCC was made. Based on the functional annotation results, the effects of LSINCT5 knockdown on cell growth, migration, invasion, and epithelial-to-mesenchymal transition (EMT) were assessed in the ESCC cell lines. Finally, the expression and clinicopathological significance of LSINCT5 in ESCC and corresponding nontumor tissues were further explored using RT-qPCR.

RESULTS: The RT-qPCR results showed that LSINCT5 expression was significantly upregulated in the ESCC cell lines. The differential gene expression analysis by next-generation RNA-Seq showed that 138 genes were up-regulated, and 227 genes were downregulated after LSINCT5 was knocked down in the ECA 109 cells. In addition, the functional annotation revealed that the differentially expressed genes were mainly functionally involved in tight junctions, ECM-receptor interactions, and MAPK signaling pathway. Further *in vitro* studies indicated that the knockdown of LSINCT5 significantly suppressed proliferation, migration, invasion, and EMT in ESCC cells. Finally, a comparative study of paired ESCC and corresponding nontumor tissues showed that LSINCT5 was upregulated in the ESCC tissues, and the increased LSINCT5 expression was related to late clinical stages, large tumor sizes, and lymph node metastasis.

CONCLUSIONS: The results indicate that LSINCT5 is upregulated in ESCC and may act as an oncogene promoting the progression of ESCC.

Key Words:
LncRNA LSINCT5, Esophageal squamous cell carcinoma (ESCC), ESCC progression, Apoptosis.

Introduction

Esophageal cancer is one of the most common malignancies worldwide and ranks as one of the top five deadliest cancers in China[1]. The esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EA) are the two main types of esophageal cancer. In China, more than 90% of esophageal cancers are ESCC[2]. The diagnosing of ESCC at early stages of disease development is difficult, and the advanced ESCC frequently presents with extensive local invasion or regional lymph node metastasis. The 5-year survival rate for ESCC patients worldwide remains below 40%[3]. Therefore, it is urgently needed to

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identify more accurate biomarkers to guide early diagnosis, therapeutic strategies, and the prognosis of ESCC.

Long noncoding RNAs (lncRNAs) are a class of noncoding RNA transcripts that are longer than 200 nt[4]. Some studies[5,6] have begun to explore the roles of lncRNAs in carcinogenesis, and have found that lncRNAs are involved in several biological functions and pathological processes, including proliferation, apoptosis, differentiation, the epithelial-mesenchymal transition (EMT), and metastasis, demonstrating their potential roles in both oncogenic and tumor-suppressive pathways. Several lncRNAs such as HOTAIR, POU3F3, and SNHG16[7-9] have been reported to participate in diverse biological processes involved in ESCC, including cell proliferation, apoptosis, metastasis, and angiogenesis.

Long stress-induced noncoding transcripts 5 (LSINCT5) is a 2.6-kb polyadenylated intergenic lncRNA located in the nucleus on the negative strand and is potentially transcribed by RNA polymerase III. LSINCT5 is substantially upregulated in breast and ovarian cancer cells[10] and predicts a negative prognosis in gastric cancer[11]. The mechanistic investigations showed that LSINCT5 could physically interact with NCYM, a *de novo* gene product from the MYCN cis-antisense RNA, and inhibit GSK3b activity, leading to enhanced Wnt/β-catenin signaling activation and EMT[12]. However, the role and mechanism of LSINCT5 in ESCC progression remains unknown.

To explore the expression and putative role of LSINCT5 in ESCC, the LSINCT5 expression was first examined in four ESCC cell lines using RT-qPCR. Then, the next-generation RNA-Seq technology was implemented to analyze and functionally annotate the differential gene expression before and after the knockdown of LSINCT5 in ESCC. Based on the functional annotation results, the effects of LSINCT5 knockdown on cell growth, cell cycle, migration, and EMT were assessed in ESCC cell lines *in vitro*. Finally, the expression and clinicopathological significance of LSINCT5 in 64-paired ESCC and corresponding nontumor tissues were further explored using RT-qPCR.

Materials and Methods

Cell Lines and Culture Conditions

Four esophageal squamous cell carcinoma cell lines (ECA109, KYSE-30, KYSE-150, and KYSE-180) and a normal esophageal epithelial cell line (Het-1A) were obtained from the Cell Culture Center, Chinese Academy of Medical Sciences (Beijing, China). All cells were cultured in RPMI-1640 medium (Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS; Gibco, Gaithersburg, MD, USA) and maintained in a humidified incubator at 37°C with 5% CO_2.

RNA Isolation, Reverse Transcription (RT), and Quantitative PCR (qPCR)

Total RNA was extracted from the tissues and cultured cells using TRIzol (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. The complementary deoxyribonucleic acid (cDNA) was obtained by reverse transcription (RT) in a 20 μL reaction system using a PrimeScript RT reagent Kit (Applied Biosystems, Carlsbad, CA, USA) according to the manufacturer's instructions. GAPDH was used as an endogenous control to normalize the data. The thermal cycle was as follows: 30 s at 95°C, 5 s for 40 cycles at 95°C, 30 s at 60°C. The relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method. The primers for LSINCT5 and GAPDH are listed in Table I.

Small Interfering RNA Transfection

Two different small interfering RNAs (siRNAs) against LSINCT5 at different sites and one negative control siRNA (si-NC) with no definite target were synthesized by Tuoran (Shanghai, China) and employed. The cells were seeded in 6-well plates for 24 h and then transfected with designed siRNA (100 nmol/L) or si-NC (100 nmol/L) with Lipofectamine RNAi MAX (Invitrogen, Carlsbad, CA, USA) in serum-free medium, according to the manufacturer's protocols. After transfection, the cells were harvested for subsequent analyses. The si-LSINCT5 and negative control (si-NC) sequences are listed in Table I.

Next-Generation RNA-Seq Data Generation

To gain a greater understanding of the molecular mechanism and signaling pathway underlying the effect of LSINCT5 in ESCC cells, the next-generation RNA-Seq was used to screen for differential gene expression before and after LSINCT5 knockdown in ESCC (Biotechnology, Shanghai, China). The Cluster analysis, Gene Ontology (GO), and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were used to annotate the differentially expressed genes.

Table I. Primers and siRNAs used in this study.

RT-qPCR primers	Sequence
LSINCT5 (forward)	5'-TTCGGCAAGCTCCTTTTCTA-3'
LSINCT5 (reverse)	5'-GCCCAAGTCCCAAAAAGTTCT-3'
GAPDH (forward)	5'-CCGGGAAACTGTGGCGTGATGG-3'
GAPDH (reverse)	5'-AGGTGGAGGAGTGGGTGTCGCTGTT-3'
siRNA	**Sequence**
siRNA1555	Sense: 5'-GCAAGGCAGGUACAGAAAUTT-3' Antisense: 5'-AUUUCUGUACCUGCCUUGCTT-3'
siRNA1887	Sense: 5'-GGUGGGAGAUUACGUCUAUTT-3' Antisense: 5'-AUAGACGUAAUCUCCCACCTT-3'
siRNA-NC	Sense: 5'-UUCUCC GAA CGU GUC ACGUTT-3' Antisense: 5'-UUGACAAAUGGGCCAGUGGTT-3'

NC: negative control.

Cell Proliferation Assay

MTS assay was performed to examine the proliferation of ESCC cells according to the manufacturer's instructions. ECA109 cells (2,000 cells per well) were seeded in 96-well plates and incubated for 24 h, 48 h, 72 h, and 96 h after transfection. Then, 15 µl of the MTS reagent was added to each well containing 100 µl of culture medium. The plate was incubated for 2 h at 37°C in a humid atmosphere containing 5% at CO_2 atmosphere. The absorbance values of each well were measured with a universal microplate reader at a wavelength of 492 nm.

Colony formation assay

After 24 h of transfection, ECA109 cells were reseeded into 6-well plates at 2,000 cells per well, and the culture medium was replaced every 4 days. After 10 days of incubation at 37°C, the colony formation was halted, and the cells were washed twice with PBS, fixed and stained with 0.1% crystal violet. The colonies were counted under an optical microscope.

Cell Cycle Analysis

The cells were harvested, washed with PBS, and fixed with 70% ethanol at 4°C overnight. After fixation, the cells were washed, resuspended in PBS, incubated with ribonuclease at 37°C for 30 minutes, and stained with propidium iodide (Beyotime, Beijing, China) in the dark at 4°C for 30 minutes. Then, the cell cycle distribution was analyzed with an Accuri C6 flow cytometer.

Migration and Invasion Assays

The cell migration assay was carried out using Transwell® Permeable Supports with 8-mm pores in 24-well tissue culture plates (Corning Incorporated, Corning, NY, USA). The cell invasion assay was performed using a modified BD BioCoat™ Matrigel™ Invasion Chamber with 8-mm pores in 24-well tissue culture plates (BD Biosciences, Franklin Lakes, NJ, USA). Then, 1 × 10^6 cells in 500 µl of serum-free RPMI-1640 medium was added to the upper chambers after the inserts of a 24-well culture plate. In contrast, culture medium containing 20% FBS in the lower chamber served as the chemoattractant. The cells that had migrated through the filters to the lower sides of the chambers were stained with crystal violet (Beyotime, Beijing, China) air-dried, photographed, and counted.

Scratch Test

The cells were seeded in 6-well plates. At approximately 48 h post-transfection when the cell confluence reached at least 80% confluence, the scratch wounds were made using a tip to scrape the cell layer across each culture plate. To ensure the documentation of the same region, the wells were marked across the wound area. The medium was replaced with serum-free medium, and the cells were incubated with medium containing 1 mM mitomycin to inhibit cell division. Phase contrast images were recorded randomly under an inverted microscope at the time of scratching and at 24 h, 36 h, and 48 h after scratching. The relative distance of cell migration to the scratched area was measured, and a healing percentage was calculated.

Western Blot Analysis

Cultured cells were harvested and lysed using RIPA buffer (Sigma-Aldrich, St. Louis, MO,

USA). The protein concentration was quantified using a BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA). The equivalent amounts of protein were separated by SDS polyacrylamide gel electrophoresis through a 10% gel and transferred to prewetted polyvinylidene difluoride (PVDF) membranes (EMD Millipore, Billerica, MA, USA). After the membrane was blocked for 2 h with 5% skim milk, it was incubated with primary antibodies against MMP9, E-cadherin, N-cadherin, vimentin, or GAPDH (Cell Signaling Technology, Danvers, MA, USA) for 2 h. Finally, the membrane was incubated with HRP-conjugated secondary antibodies (Cell Signaling Technology, Trask Lane Danvers, MA, USA) for other 2 h. The band signals were visualized using a chemiluminescence assay (ECL, Millipore, Billerica, MA, USA).

Cases

Sixty-four paired samples of fresh ESCC tissues and corresponding nontumor tissues were collected from patients who underwent resection of their primary ESCCs at Hebei Tumor Hospital from September 2015 to April 2016. After surgical resection, the tumor specimens and corresponding nontumor tissues were collected and stored in liquid nitrogen until use. The carcinoma diagnosis was histopathologically confirmed and none of the patients received local or systemic preoperative therapy.

The clinical information was obtained from medical records, pathology reports, and personal interviews with the subjects. The recorded clinicopathological features included age, sex, tumor size, and histological stage.

Statistical Analysis

The statistical comparisons between the two different groups were determined by the Student's *t*-test using GraphPad Prism 4.0 (GraphPad Software, La Jolla, CA, USA), and the Chi-square test was used for clinicopathological association analysis. The results are presented as the mean ± SEM. $p < 0.05$ was considered statistically significant (*p-values < 0.05, **p-values <0.01).

Results

LSINCT5 Is Upregulated in ESCC Cell Lines In Vitro

The RT-qPCR results in the four ESCC cell lines (ECA109, KYSE-30, KYSE-150, and KYSE-180) and a normal esophageal epithelial cell line (Het-1A) revealed that LSINCT5 expression in all the ESCC cell lines was significantly higher than that in Het-1A cells (Figure 1A) suggesting

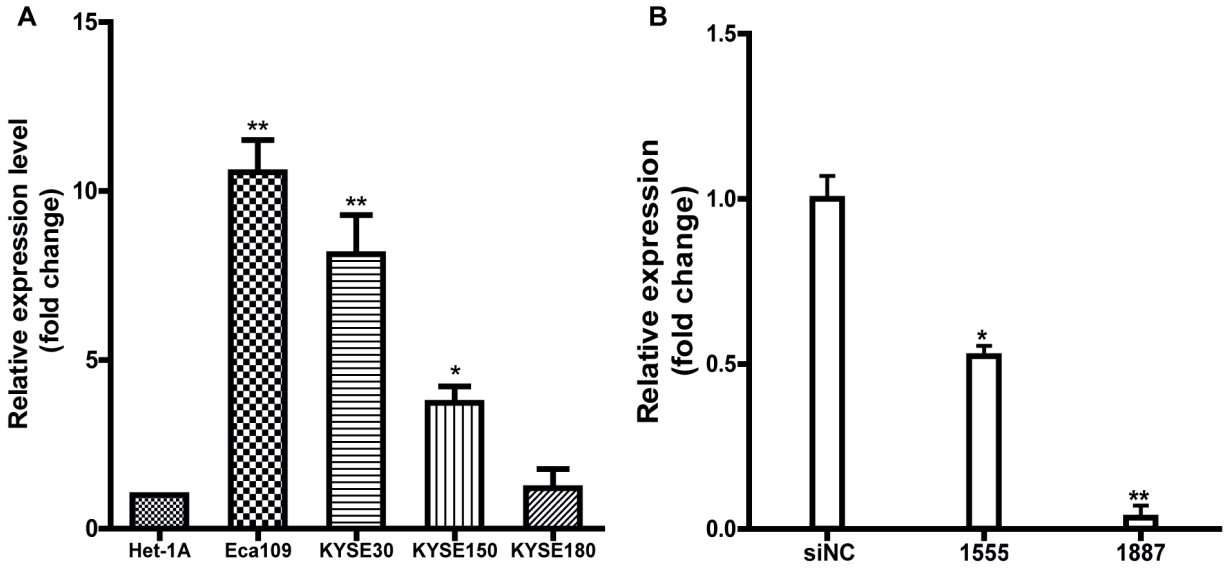

Figure 1. Relative LSINCT5 expression in esophageal squamous cell carcinoma (ESCC) cell lines. ***A,*** LSINCT5 expression levels in the ESCC cell lines (ECA109, KYSE30, KYSE150, and KYSE180) compared with those in the normal epithelial cell line Het-1A. The data are presented as fold changes in expression relative to Het-1A. ***B,*** RT-qPCR was performed to measure the knockdown efficiency of LSINCT5-specific siRNA. Significant differences between the two groups were analyzed using the unpaired Student's *t*-tests. *$p < 0.05$, **$p < 0.01$.

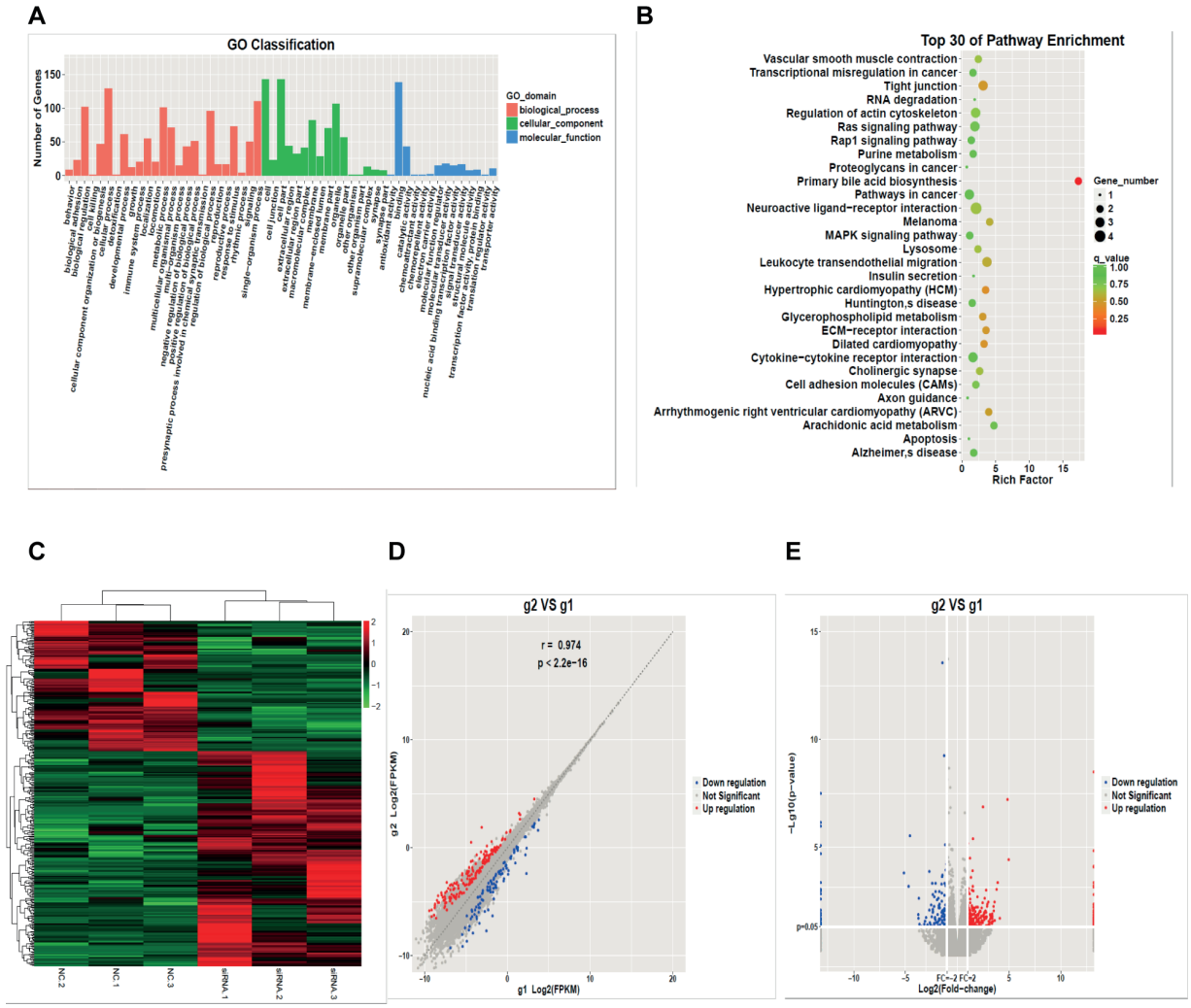

Figure 2. Function annotations of differentially expressed genes with next-generation RNA-Seq. ***A,*** Gene Ontology (GO) categories biological process (BP), molecular function (MF) and cellular component (CC) are shown. ***B,*** Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis of the pathways of the differentially expressed genes. The figure shows only the top 30 enrichment KEGG pathways. ***C,*** Hierarchical clustering of differentially expressed genes in the si-LSINCT5 group compared with the si-NC group. Each row represents a differentially expressed gene, while each column represents a sample. On the heatmap, green represents the downregulated genes and red represents the upregulated genes. ***D,*** Scatter plot of differentially expressed genes. Genes above the top red line and below the bottom blue line indicate > 2.0-fold changes between si-LSINCT5 group and si-NC group. ***E,*** Volcano plots displaying the differentially expressed gene distributions. The red and blue points represent the statistically significant differentially up- or downregulated genes, respectively.

that LSINCT5 is significantly upregulated in the ESCC cell lines.

Among the four ESCC cell lines, ECA109 cells showed the highest expression of LSINCT5 (Figure 1A), thus, this cell line was used for subsequent experiments. The knockdown efficiency results of LSINCT5-specific siRNA transfection showed that both siRNA1555 and siRNA1887 effectively inhibited LSINCT5 expression in ECA109 cells compared to the control (si-NC). The knockdown efficiency of siRNA1887 (96%) was higher than that of siRNA1555 (47%). ($p < 0.001$, Figure 1B).

Differentially Expressed Genes and Functional Annotation After LSINCT5 Knockdown In ECA109 Cells In Vitro

The differential gene expression analysis by next-generation RNA-Seq showed that 138 genes were upregulated and that 227 were downregulated in LSINCT5-knockdown ECA109 cells *in vitro* (Figure 2C, D, E).

The functional annotation and pathway enrichment analyses showed that the differentially expressed genes were mainly categorized into tight junction, ECM-receptor interactions, the MAPK

signaling pathway, the Ras signaling pathway, and the cell adhesion molecules (CAMs) pathway which were mainly related to cell proliferation, adhesion, migration, etc[13-17] (Figure 2A, B).

Knockdown of LSINCT5 Suppresses ESCC Cell Proliferation In Vitro

To investigate the biological function of LSINCT5, an MTS assay was performed to detect the proliferation ability of ESCC cells transfected with siRNA1887. The MTS results showed that the proliferation of ESCC cells transiently transfected with siRNA1887 was significantly inhibited, compared with that of the cells in the si-NC groups (Figure 3A, B).Consistent with this outcome, the colony formation assay revealed that LSINCT5 knockdown by siRNA1887 significantly inhibited the anchorage-independent growth of the ESCC cells, as indicated by the formation of fewer and smaller colonies in soft agar (Figure 3C). The flow cytometric analysis of the cell cycle progression showed that the LSINCT5 siRNA-transfected cells were significantly arrested at G0/G1 phase (Figure 3D), which suggests the role of LSINCT5 in inducing the transition from G0/G1 to the S-phase.

Knockdown of LSINCT5 Inhibits Cell Migration and Invasion In Vitro

To evaluate the effect of si-LSINCT5 on cell migration and invasion, a transwell assay was employed. As demonstrated in Figure 4A and B, compared with the control cells, the cells transfected with siRNA1887 exhibited relatively weakened migration and invasion capacities.

The scratch test showed that 48 h after wound creation, the scratches of the experimental group closed slower than the scratches of the si-NC

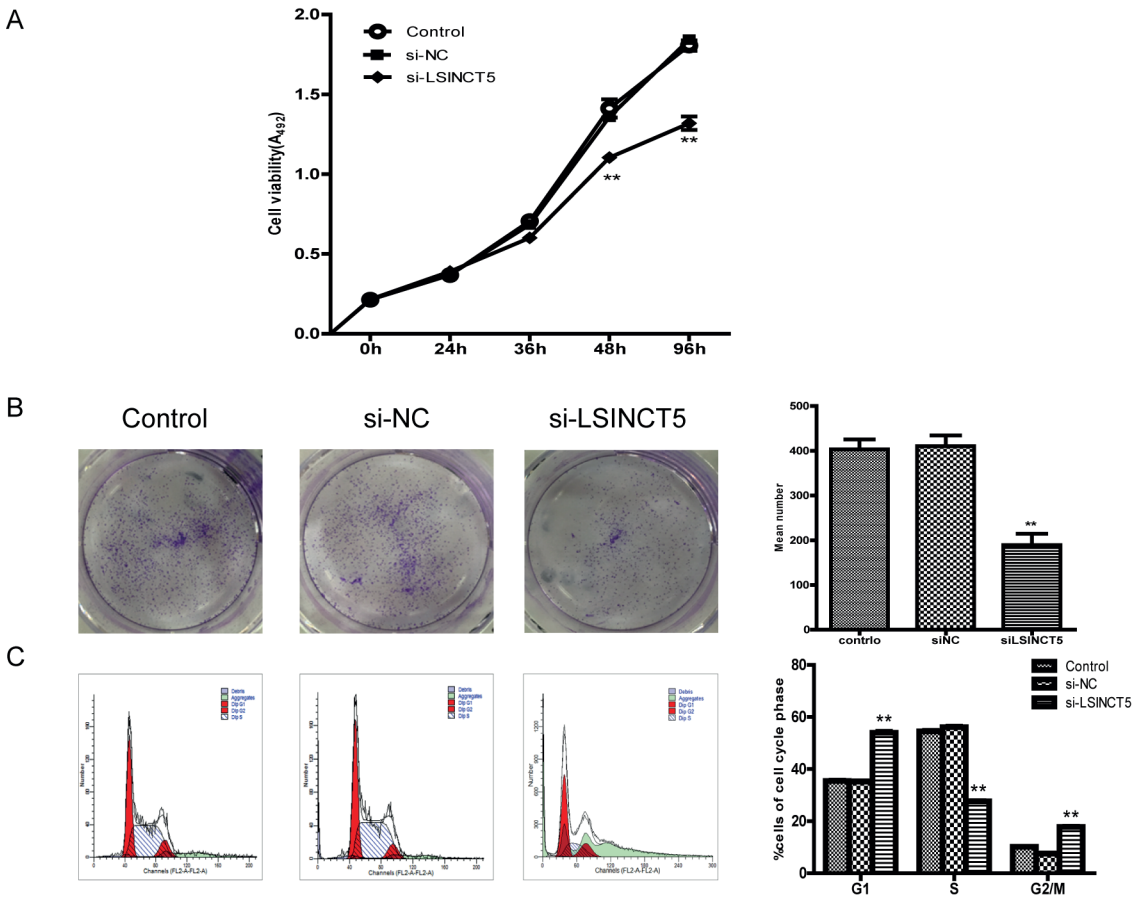

Figure 3. LSINCT5 Knockdown suppressed ESCC cell proliferation *in vitro*. ***A,*** and ***B,*** MTS assays were performed to measure the proliferation ability of ESCC cells transfected with si-LSINCT5. The values represent the mean ± SD of three independent experiments. ***C,*** The colony formation assays were conducted to measure the proliferation ability of ESCC cells transfected with si-LSINCT5. ***D,*** The flow cytometric analyses were performed to examine the cell cycle distribution of the cells transfected with si-LSINCT5. *$p < 0.05$, **$p < 0.01$.

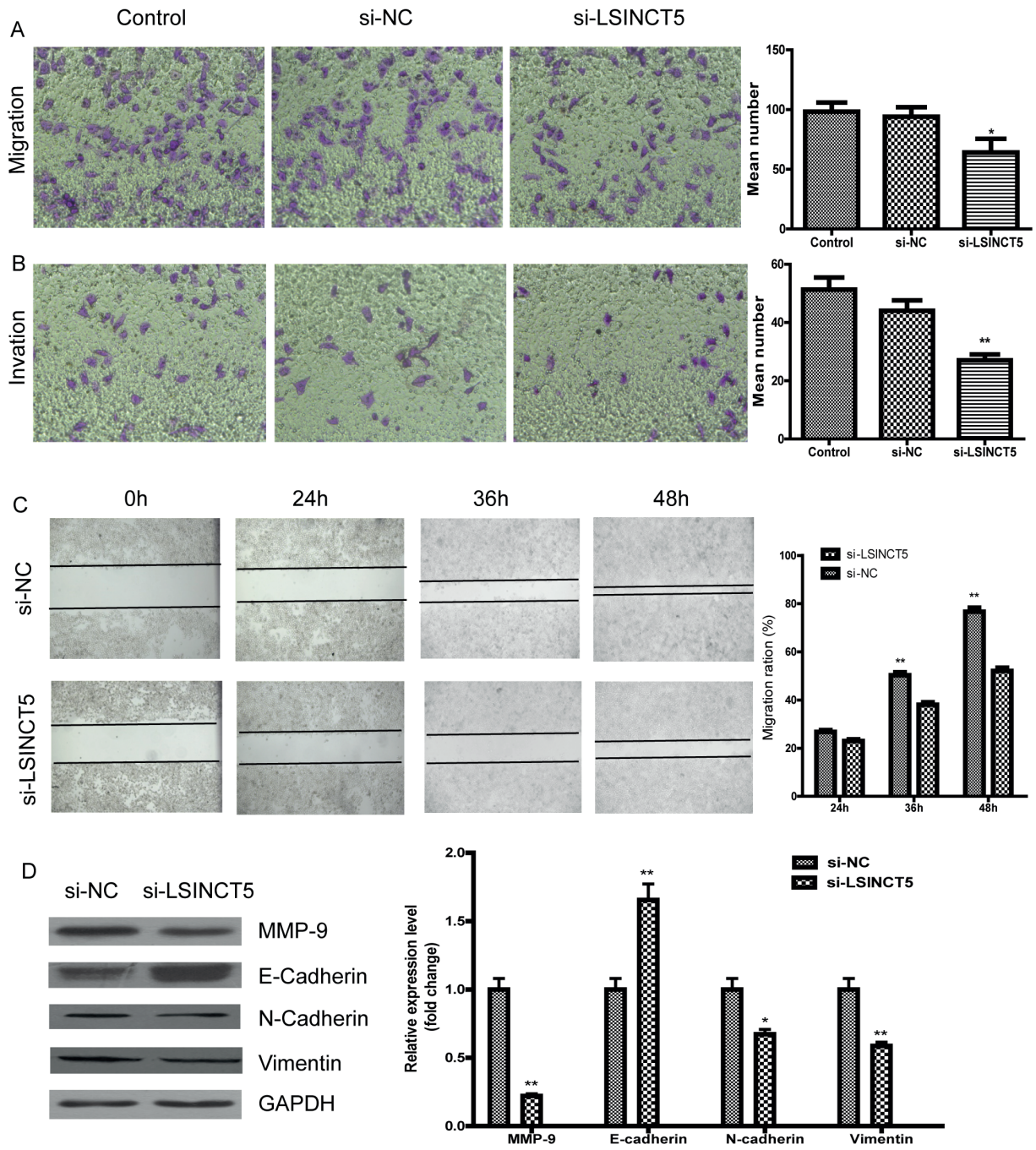

Figure 4. Knockdown of LSINCT5 inhibited cell migration, invasion, and EMT. ***A,*** The transwell assays were employed to evaluate the effect of LSINCT5 on cell migration (Magnification: 100×). ***B,*** The Matrigel assay was conducted to evaluate the effect of LSINCT5 on cell invasion (Magnification: 100×). ***C,*** The scratch test was performed to evaluate the effect of LSINCT5 on cell migration (Magnification: 40×). ***D,*** Western blot and RT-qPCR assays were used to measure the expression levels of the epithelial protein marker E-cadherin, and of the mesenchymal markers, N-cadherin, vimentin, and MMP9, in cells transfected with LSINCT5. The error bars represent the mean ± SD of at least three independent experiments. *$p < 0.05$, **$p < 0.01$ *vs.* control group.

group did, and they were almost completely covered by cells. This suggests that the inhibition of LSINCT5 expression reduces the migration ability of ECA109 cells ($p < 0.001$) (Figure 4C).

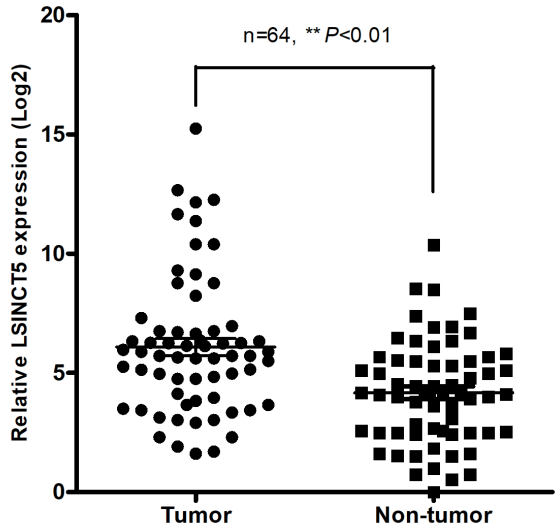

Figure 5. The relative LSINCT5 expression in paired ESCC tissues (n = 64) and the corresponding nontumor tissues (n = 64). LSINCT5 expression was examined by RT-qPCR and normalized to GAPDH expression. The significant differences between the two groups were analyzed using the paired Student's t-tests. *$p < 0.05$, **$p < 0.01$.

Knockdown of LSINCT5 inhibits EMT in ESCC cells

To determine whether LSINCT5 affects the EMT process in ESCC cells, we performed PCR and Western blotting to measure the expression level of EMT-related markers. As shown in Figure 4D, the silence of LSINCT by siRNA1887 significantly reduced the mRNA and protein expression levels of the mesenchymal cell markers N-cadherin, vimentin, and MMP9, but increased the expression levels of the epithelial cell marker, E-cadherin. These investigations indicated that LSINCT promotes EMT in ESCC cells.

LSINCT5 Is Upregulated in ESCC Tissues and Is Correlated with Tumor Size, TNM Stage, and Lymph Node Metastasis

Finally, we measured LSINCT5 expression levels in 64 pairs of ESCC and the corresponding nontumor tissues by RT-qPCR. As shown in Figure 5, LSINCT5 expression in ESCC tissues was significantly elevated in 70.31% (45 of 64, fold ≥1.0) ($p < 0.01$).

Table II. Correlation between LSINCT5 expression and clinicopathological characteristics in ESCC.

Clinical Parameter	LSINCT5		Chi-squared test p-value
	High: no. of cases	Low: no. of cases	
Age (years)			0.2071
<60	12	14	
≥60	24	14	
Gender			0.1238
Male	18	20	
Female	18	8	
Tumor size			0.0266*
<4 cm	21	24	
≥4 cm	15	4	
Tumor stage			0.0033*
I	5	11	
II	10	12	
III	21	5	
Location			
Upper	8	6	0.6260
Middle	14	14	
Lower	14	8	
Tumor differentiation			
Well, moderate	30	24	1.0
Poor	6	4	
LM			
Absence	5	13	
Presence	31	15	0.0055*

*$p < 0.05$ and was defined as statistically significant.

The expression levels of LSINCT5 were significantly associated with tumor size ($p = 0.027$), TNM stage ($p = 0.003$), and lymph node metastasis ($p = 0.006$). However, there were no statistically significant differences in LSINCT5 expression among the groups based on age, sex, tumor location, or tumor differentiation ($p > 0.05$; Table II).

Discussion

In the present study, we characterized the roles of LSINCT5 in ESCC for the first time and demonstrated that LSINCT5 expression is significantly upregulated in the ESCC cell lines. In addition, the next-generation RNA-Seq and the *in vitro* experiments showed that the LSINCT5 knockdown inhibits ESCC cell proliferation, migration, and invasion. The preliminary results revealed that LSINCT5 may exert functions in the migration and invasion of ESCC cells by modulating EMT. Moreover, we found that LSINCT5 was overexpressed in ESCC tissues and correlated with larger tumor size, advanced TNM stage, and more lymph node metastasis.

LncRNAs have emerged as key regulators in cancer initiation and progression. A recently identified lncRNA LSINCT5 has been shown to be upregulated in breast cancer, ovarian cancer, gastric cancer, non-small cell lung cancer (NSCLC) and bladder cancers[10-12,18]. The findings in this study further showed that LSINCT5 is also overexpressed in ESCC. These results are consistent with the results of other cancers, suggesting that LSINCT5 plays an oncogenic role in these cancers.

To explore the putative functional role of LSINCT5 in ESCC, we performed the differential gene expression analysis of the mRNAs from ESCC cells before and after the knockdown of siRNA-mediated LSINCT5 using next-generation RNA-Seq and functional annotation to identify pathways of interest. Differential gene expression analysis showed that 138 genes were upregulated while 227 were downregulated in the LSINCT5-knockdown ECA109 cells. KEGG pathway annotation identified a dozen signaling pathways that were enriched among the differentially expressed genes. In addition, among these pathways, tight junctions, ECM receptor interactions and MAPK signaling pathways were included. It is commonly known that the ECM-receptor interaction leads to direct or indirect control of cellular activities, such as adhesion, migration, differentiation, proliferation, and apoptosis[15]. The MAPK signaling pathway has been reported to be associated with cell proliferation, differentiation, migration, senescence, and apoptosis in ESCC, breast cancer, and other cancers[19,20]. Tight junctions are essential structures that maintain contacts between the epithelial cells and determine the epithelial cell polarity. The disruption of the intercellular adhesion structures and the loss of cell polarity are hallmarks of malignant transformation in epithelial cells, as part of EMT. The partial loss of the tight junctions also contributes to cancer cell invasion and metastasis[21-23]. Taken together, the pathway annotation data suggest that the differentially expressed genes induced by LSINCT5 knockdown play important roles in cellular proliferation, adhesion, and migration.

Recently, the function of LSINCT5 has been explored in several human cancers. Silva et al[10] have shown that LSINCT5 knockdown could reduce ovarian and breast cancer cell proliferation. *In vitro* studies indicated that in gastric and bladder cancer, the upregulation of LSINCT5 expression significantly promoted cell migration and invasion, whereas the knockdown of LSINCT5 expression impeded these biological processes[11,12]. To further explore the role of LSINCT5 in biological behaviors of ESCC, the changes in proliferation, cell cycle distribution, adhesion, and migration of ECA109 cells after siRNA mediated-knockdown LSINCT5 were analyzed *in vitro*. The results illustrated that compared with the control group, the LSINCT5 knockdown inhibited the migration and invasion capability of ECA109 cells. The findings of the *in vitro* experiments further confirmed the pathway annotation results in this study, and the results revealed that the role of LSINCT5 was basically identical to its role in other cancers reported in the literature. These results suggest that LSINCT5 acts as an oncogenic lncRNA by influencing many cellular processes, including proliferation and migration in the carcinogenesis of cancers.

EMT is the transcriptional reprogramming of epithelial cells and is characterized by decreased adhesion and enhanced migration and invasion. An increasing number of studies[24,25] have recently shown that lncRNAs function as tumor suppressors or oncogenes by affecting EMT-related signaling pathways. It is well recognized that EMT plays a critical role in ESCC cell migration and invasion[26]. The putative role of EMT in the regulatory effects of LSINCT5 on the biological behaviors of ECA109 cells *in vitro* was explored

in our study with the determination of EMT marker expression. The results revealed that the LSINCT5 knockdown significantly increased the mRNA and protein expression levels of E-cadherin and decreased the expression levels of MMP9, N-cadherin, and vimentin. Thus, the preliminary work in this study demonstrated that LSINCT5 might promote ESCC cell migration by regulating the EMT process.

Based on our *in vitro* studies, we examined the expression and significance of LSINCT5 in human ESCC tissues to confirm the results from the ESCC cell lines. We found that LSINCT5 expression was significantly upregulated in ESCC tissues. In addition, the increased LSINCT5 expression was correlated with a larger tumor size, advanced TNM stage, and more lymph node metastasis, indicating that LSINCT5 may be a promising biomarker for late-stage ESCC with metastasis. The results were in accordance with *in vitro* study with ESCC cell lines and consistent with other studies[11,12,27] in breast, ovarian, gastric and bladder cancers. The upregulation of LSINCT5 may be a common feature in cancers and may be a novel indicator of prognosis.

The precise mechanism by which LSINCT5 regulates the expression of its target genes is unclear. Some researches showed[18,27] that LSINCT5 promotes SKOV3 cell invasion and metastasis by activating the CXCR4/CXCL12 axis and might contribute to NSCLC tumorigenesis by stabilizing the oncogenic factor HMGA2. Zhu et al[12] found that LSINCT5 could physically interact with NCYM, a *de novo* gene product from the MYCN cis-antisense RNA, and inhibit GSK3b activity leading to enhanced Wnt/β-catenin signaling activation and EMT. Nevertheless, as the target genes of lncRNAs may vary among different cancer tissues and cell types, the specific target genes controlled by LSINCT5 in ESCC cancer remain unknown, and further studies are required to explore the mechanism of LSINCT5 in ESCC. The precise mechanism of the effects of LSINCT5 on ESCC was not explored which is the limitation of this study that will be examined in our future work.

Conclusions

We indicated that LSINCT5 is upregulated in ESCC and may act as an oncogene promoting the progression of ESCC. The results in this study are preliminary, and more work is required to further determine the detailed mechanisms of LSINCT5 functions in ESCC and the potential of LSINCT5 as a therapeutic target for ESCC.

Conflict of Interests

The Authors declare that they have no conflict of interests.

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