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# LINC00511 can promote the proliferation, migration and invasion of esophageal cancer cells through regulating microRNA-150-5p

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**Abstract. – OBJECTIVE: The aim of this study was to investigate the potential role of LINC00511 in esophageal cancer (ECa), and to explore its underlying mechanism through *in vitro* cell experiments.**

**PATIENTS AND METHODS: LINC00511 expression in ECa was analyzed by GEPIA database and verified by real-time fluorescence quantitative polymerase chain reaction (qPCR). The bioinformatics website was used to analyze the miRNAs that can bind to LINC00511, and the regulatory relationship between them was verified through Luciferase assay, qPCR as well as Western blotting analysis. Then, the impacts of LINC00511 and microRNA-150-5p on the proliferation or invasiveness of ECa cell lines Kyse30 and ECA109 were investigated by cell counting kit-8 (CCK-8) test and transwell experiment, respectively. Meanwhile, cell cycle and apoptosis were detected by flow cytometry.**

**RESULTS: Analysis results of the GEPIA database revealed that LINC00511 had a significant high expression in ECa tissue samples in comparison with normal control ones, which is consistent with qPCR results. Meanwhile, a significant negative correlation was found between LINC00511 and microRNA-150-5p. In brief, LINC00511 was able to bind to microRNA-150-5p and inhibited its expression. Besides, overexpression of LINC00511 enhanced ECa cell proliferation and migration, accelerated cell cycle, and suppressed cell apoptosis, while transfection with microRNA-150-5p mimics caused the opposite effects.**

**CONCLUSIONS: This study shows for the first time that LINC00511 modulates the progression of ECa by binding to microRNA-150-5p.**

*Key Words:*
ECa, LINC00511, MicroRNA-150-5p, Cell proliferation, Cell invasion.

## Introduction

As one of the most common malignant tumors of the digestive system, esophageal cancer (ECa) is characterized by its high morbidity and mortality. More than 300,000 people die of this cancer every year, ranking sixth among malignant tumors worldwide[1,2]. According to data obtained from 2004 to 2010, the five-year survival rate of ECa patients has reached to about 17.5%[3]. In the early stage, ECa patients only have mild discomfort when swallowing solid food without typical features, which therefore leads to a high misdiagnosis rate. The disease is often diagnosed in the late stage, resulting in difficulty in treatment, poor prognosis, and terrible life quality of patients[4]. As a result, it is essential to further study the pathogenesis of ECa to improve the therapeutic effect and prognosis of ECa patients.

Long non-coding RNAs (lncRNAs) are defined as transcripts larger than 200 nucleotides that cannot be translated into proteins[5] and can be engaged in a variety of biological functions *via* modulating downstream genes, especially in tumor progression[6]. LncRNAs exert critical impacts on biological processes such as embryonic development, cell differentiation, transcription, and post-transcriptional gene regulation[7,8], and in tumors. Among these, lncRNA tp73-as1 can elevate the proliferation rate of non-small-cell lung cancer (NSCLC) cells through the modulation of microRNA141-3p[9]. LncRNA HGBC prompts the development of gallbladder cancer *via* regulating microRNA502-3p /SET/AKT axis[10]. LINC00994 enhances gastric cancer cell invasive ability *via*



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inhibiting microRNA765-3p[11]. In addition, various lncRNAs, such as lncRNA UCA1[12], PEG10[13], tp73-as1[14], and MEG3[15] have been proved to affect ECa progression. However, the potential mechanism remains elusive.

MicroRNAs (miRNAs), a kind of short non-protein coding RNAs of about 22 nucleotides in length, have been verified to play a significant part in cellular processes through gene regulation[16]. Currently, miRNAs are recognized as key post-transcriptional regulators of gene expression in cancer cells[17]. MiRNAs were first identified in 1993, and nearly 2,000 miRNAs have been identified since then, which play a pivotal role in cell proliferation, cell cycle, and cell apoptosis[18]. MiRNAs can both serve as cancer-promoting and cancer-inhibiting genes in different tumors. In particular, low expression of microRNA342-3p can suppress the growth and invasion of nasopharyngeal carcinoma cells by inhibiting FOXQ1 expression[19]. MicroRNA-744 can act as an oncogene to prompt the development of prostate cancer through targeted binding to LKB1[20]. MicroRNA885-5p inhibits the migration and proliferation of osteosarcoma cells by regulating β-catenin[21]. However, the study of miRNAs in ECa is still very limited and requires further study.

Therefore, the possible implication of LINC00511 in ECa progression was tested in this study, and it was found that LINC00511 was abnormally highly expressed in ECa tissues and cell lines. Further, this work described a novel mechanism for LINC00511 action in the development of ECa through *in vitro* cell experiments.

## Patients and Methods

### GEPIA Database

GEPIA database (http://GEPIA.cancer-pku.cn/index.html) was used to evaluate LINC00511 expression and the survival prognosis of patients, as well as to mine and analyze The Cancer Genome Atlas (TCGA) and GTEx gene sequencing data.

### Sample Collection

60 pairs of tissue specimens were collected from our hospital and stored in -80°C refrigerator. This investigation complied with the Declaration of Helsinki and was approved by the Ethics Committee of Shanxian Central Hospital of Shandong Province. Signed written informed consents were obtained from all participants before the study.

### Cell Culture

Human normal esophageal epithelial cell line (HEEC) and ECa cell lines (Kyse30, Kyse70, Kyse150, and ECA109) were obtained from the American Biological Resource Center (American Type Culture Collection (ATCC; Manassas, VA, USA). All cells were cultured in Roswell Park Memorial Institute-1640 (RPMI-1640) medium (HyClone, South Logan, UT, USA) supplemented with 10% fetal bovine serum (FBS; Gibco, Rockville, MD, USA) and 1% penicillin/streptomycin in a cell incubator at 37°C with 5% $CO_2$.

### Cell Transfection

For transient transfection, Lipofectamine 3000 reagent was mixed with si-LINC00511 or microRNA-150-5p mimics (GenePharma, Shanghai, China) when cell density reached to more than 60%.

### Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)

Total RNA was extracted from cultured cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Quantitative PCR was carried out using the SYBR Green Real Time PCR kit (TaKaRa, Otsu, Shiga, Japan), with glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and U6 used as internal references. The primer sequences are shown as follows: LINC00511: forward: 5′-CGCAAGGACCCTCTGTTAGG-3′, reverse: 5′-GAAGGCGGATCGTCTCTCAG-3′, microRNA-150-5p: forward: 5′-TCGGCGTCTCCCAACCCTCTTGTAC-3′, reverse: 5′-GTCGTATCCAGTGCAGGGTCCGAGGT-3′, GAPDH: forward: 5′-CGGAGTCAACGGATTTGGTCGTAT-3′, reverse: 5′-AGCCTTCTCCATGGTGGTGAAGAC-3′, and U6: forward: 5′-GCTGAGGTGACGGTCTCAAA-3′, reverse: 5′-GCCTCCCAGTTTCATGGACA-3′.

### Cell Counting Kit-8 (CCK-8) Test

Cells were plated in 96-well plates ($3\times10^3$ cells/well) in 100 uL culture medium. CCK-8 assay (Dojindo, Molecular Technologies, Kumamoto, Japan) was performed according to the manufacturer's protocol.

### Transwell Assay

24-well plate (Corning Incorporated, Corning, NY, USA) with an 8 mm chamber was used. The invasion experiment was performed by diluting a matrix gel matrix gel with Dulbecco's Modified Eagle's Medium (DMEM; Gibco, Rockville, MD, USA) containing no FBS overnight at a ratio of 1:

6. At 24 h after transfection, cells were prepared into cell suspensions and seeded in the upper chamber. The migrated cells were counted after washing with crystal violet and observed. Finally, migration experiments were performed without the addition of a matrix gel, and the remaining steps were the same as above.

### Cell Cycle Detection

Cells were collected and treated with 70% ethanol at 4°C overnight. Cell cycle detection was carried out using a cell cycle kit (Beyotime Biotechnology, Shanghai, China) based on the product instructions and then analyzed by a FACSCalibur flow cytometer (BD Bioscience, Franklin Lakes, NJ, USA).

### Apoptosis Detection

Cell apoptosis analysis was conducted using Annexin V-FITC (fluorescein isothiocyanate) apoptosis kit (Sigma-Aldrich, St. Louis, MO, USA), and the apoptosis rate was then assessed by a flow cytometer.

### Dual-Luciferase Assay

ECA109 and Kyse30 cells were co-transfected with reporter vector and microRNA-150-5p using lipofectamine2000 reagent (Invitrogen, Carlsbad, CA, USA). Then, the Luciferase activity of each group was measured after 48 h of transfection.

### Statistical Analysis

Statistical analyses were performed with Statistical Product and Service Solutions (SPSS) 19.0 (IBM, Armonk, NY, USA). Student's *t*-test was used to compare differences between samples analyzed. $p<0.05$ suggested that the difference was statistically significant.

## Results

### Linc00511 Shows a High Level In ECa Tissues

GEPIA database revealed that ECa tumor tissues contained a relative high expression of LINC00511 as compared with the normal ones (Figure 1A). However, no significant association was detectable between LINC00511 expression and the prognosis of ECa patients (Figure 1B). To further verify LINC00511 expression in ECa tissues, LINC00511 expression was detected in 60 ECa tissues and corresponding normal control tissues by qPCR, and the results were consistent with those shown in the GEPIA database (Figure 2A).

### Reduced Expression of MicroRNA-150-5p In ECa Tissues and Cells

It is well known that LncRNA can regulate the expression of downstream genes by binding certain miRNAs to exert biological effects. There-

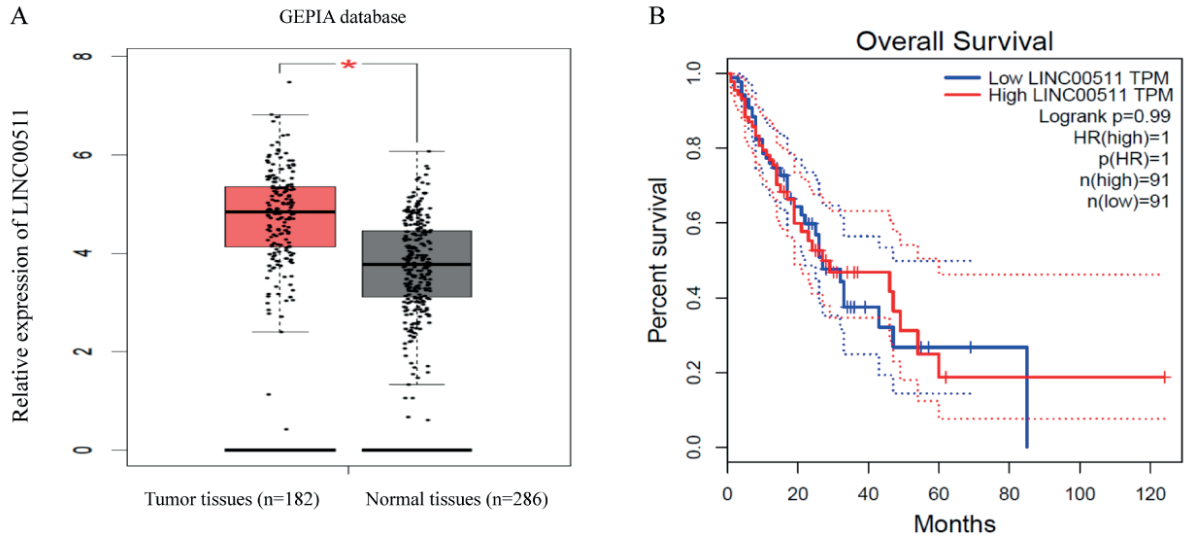


**Figure 1.** LINC00511 has a high expression in ECa. **A,** GEPIA database reveals the expression of LINC00511 in ECa tissues and corresponding normal tissues. **B,** Analysis of the correlation between the expression level of LINC00511 and the prognosis of patients with ECa by GEPIA database. Data are presented as mean ± S.D., *$p<0.05$.

fore, the possible miRNAs that bind to LINC00511 were predicted through StarBase website (http://starbase.sysu.edu.cn), among which microRNA-150-5p was found to get the highest binding score. Therefore, microRNA-150-5p expression was detected, and it was found that in ECa tissue samples, it was remarkably lower than that in normal control (Figure 2B), suggesting that microRNA-150-5p may affect ECa progression. *In vitro* cell experiments also revealed the same tendency in the expression of LINC00511 and microRNA-150-5p (Figure 2C, 2D). In addition, a negative correlation between LINC00511 and microRNA-150-5p in esophageal tissues was uncovered ($r$=-0.535, $p$<0.001) (Figure 2E).

### LINC00511 Binds to MicroRNA-150-5p and Inhibit Its Expression

LINC00511 and microRNA-150-5p have been confirmed to be abnormally expressed in ECa tissue specimens and negatively correlated, but the specific regulatory mechanism remains elusive. The binding site of LINC00511 to microRNA-150-5p was predicted (Figure 3A), and the LINC00511 wild-type overexpression plasmid (LINC00511 WT) and mutant overexpression plasmid (LINC00511 MUT) were constructed. Figure 3B shows that overexpression of microRNA-150-5p markedly suppressed the luciferase activity of LINC00511 WT group, while no significant effect on the LINC00511 MUT group was detectable, indicating that LINC00511 can bind to microRNA-150-5p (Figure 3B). To test whether microRNA-150-5 mediates LINC00511 action on ECa progression, LINC00511 overexpression and knockdown were performed *in vitro*. As a result, overexpression of LINC00511 led to an increase in microRNA-150-5p expression while its knockdown oppositely caused a reduction. The above results suggest that LINC00511 can be engaged in the progression of ECa *via* suppressing microRNA-150-5p expression.

### Effects of LINC00511 and MicroRNA-150-5p on Cell Proliferation and Invasiveness

To demonstrate the implication of LINC00511 and microRNA-150-5p in ECa cell functions *in vitro*, LINC00511 and microRNA-150-5p expressions in ECa cells Kyse30 and ECA109 were simultaneously changed, and then cell proliferation rate and migration abilities were detected by CCK-8 and transwell tests. Figure 4A-4C in-

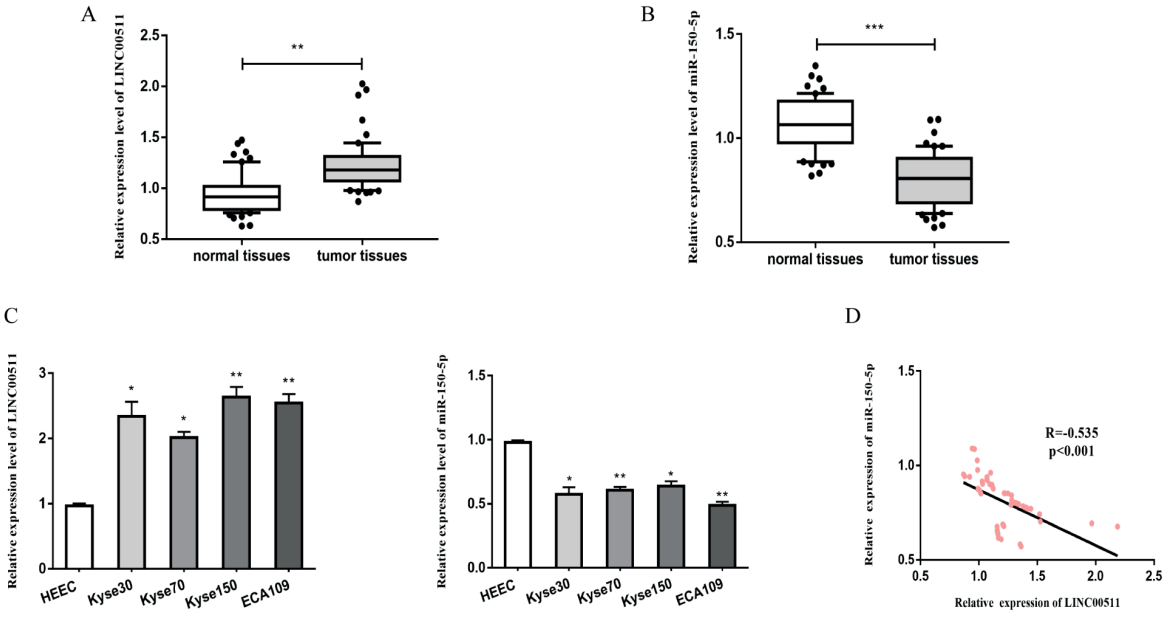


**Figure 2.** MiR-150-5p expression is significantly reduced in ECa. **A,** Expression of LINC00511 in 60 cases of ECa tissues and corresponding normal tissues. **B,** Expression of miR-150-5p in 60 cases of ECa tissues and corresponding normal tissues. **C,** LINC00511 expression levels in normal cell lines and ECa cell lines. **D,** miR-150-5p expression levels in normal cell lines and ECa cell lines. **E,** Correlation between the expression level of LINC00511 and the expression level of miR-150-5p in ECa tissues. Data are presented as mean ± S.D., *$p$<0.05, **$p$<0.01, ***$p$<0.001.

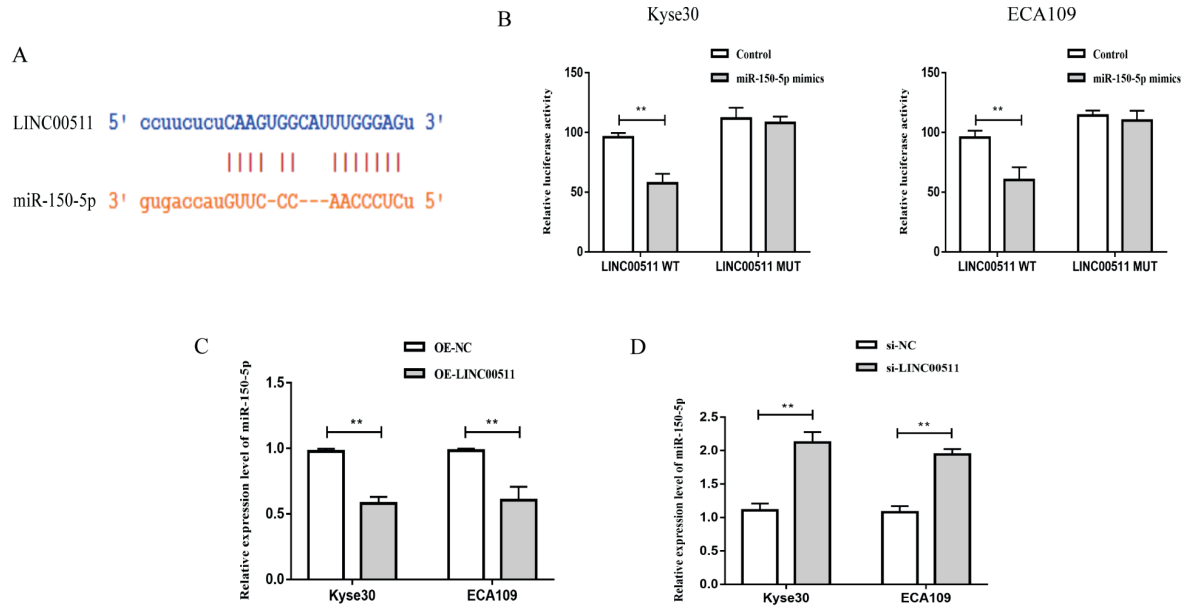


**Figure 3.** LINC00511 is able to bind miR-150-5p and inhibit miR-150-5p expression. **A,** Potential binding site for LINC00511 and miR-150-5p. **B,** Dual-Luciferase reporter gene experiment verifies the binding relationship between LINC00511 and miR-150-5p. **C,** Expression level of miR-150-5p was detected after overexpressing LINC00511 in ECa cells. **D,** Expression level of miR-150-5p was detected after suppressing the expression of LINC00511 in ECa cells. Data are presented as mean ± S.D., $^{**}p<0.01$

dicates that overexpression of LINC00511 significantly enhanced cell abilities in replication as well as invasion, but simultaneous transfection of cells with microRNA-150-5p mimics blunted those cell functions. At the same time, flow cytometry revealed that LINC00511 gain of function markedly accelerates the cell cycle while suppressing cell apoptosis, which could also be reversed by microRNA-150-5p overexpression (Figure 4D, 4E). Taken together, these results indicate that LINC00511 can enhance cell invasion ability and accelerate cell replication, while microRNA-150-5p shows an opposite effect.

## Discussion

As one of the common primary malignant tumors of the digestive tract, the incidence of ECa is three times higher in men than in women, mostly occurring in rural areas[22,23]. The etiology of ECa is relatively complex. In addition to genetic and environmental factors, diet and lifestyle also contribute to the generation of ECa. This complicated pathogenesis leads to the lack of internationally recognized standard treatment program for ECa[24].

LncRNAs can participate in the process of cell biology to regulate the stability of mRNA, which can be selectively spliced through "RNA adsorption" or as ceRNAs[25]. LINC00511 is located on chromosome 17q24.3 and is mainly expressed in the cytoplasm, which implies its important role in post-transcriptional gene regulation[26]. Currently, LINC00511 has been considered as an oncogene in tongue squamous cell carcinoma, ductal adenocarcinoma of the pancreas and NSCLC[27,28]. Nevertheless, the specific molecular mechanism of LINC00511 in the development of ECa remains unclear.

We revealed that LINC00511 was abnormally expressed in ECa tissues by analyzing the database and confirmed the results *in vivo* and *in vitro* experiments by qPCR detection. As predicted by the bioinformatics website, Luciferase assay verified that LINC00511 could bind to microRNA-150-5p and negatively regulate its expression. It was speculated that LINC00511 may play a cancer-promoting role by binding microRNA-150-5p.

MicroRNAs have received increasing attention in researches[29-31] on cell biological processes such as cell proliferation, migration, differentiation, and apoptosis. In tumor research field, increasing studies have been conducted on microRNA-150-5p, a member of the microRNA150

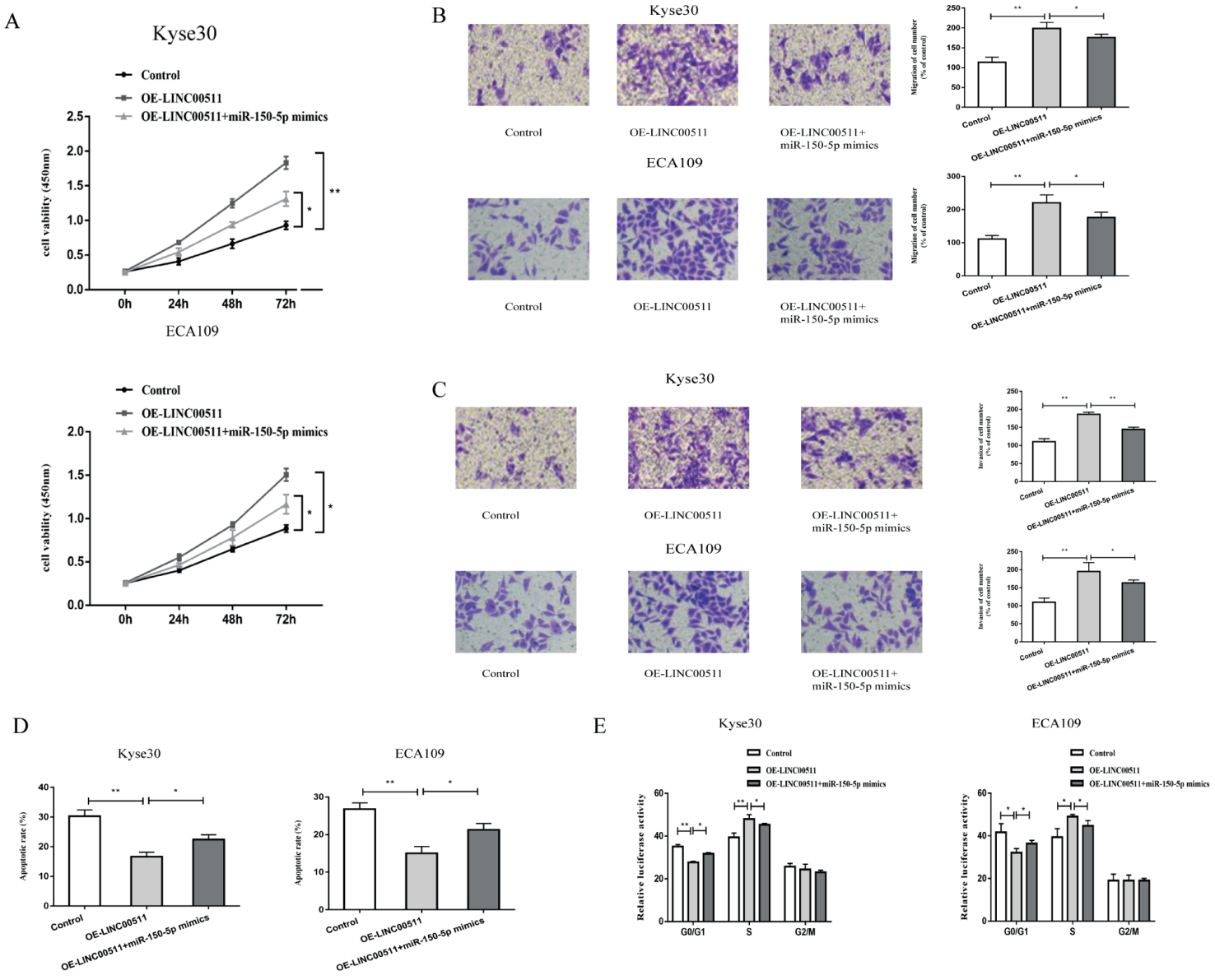


**Figure 4.** Effects of LINC00511 and miR-150-5p on cell proliferation, migration, and invasion capabilities. **A,** CCK8 test is used to detect the effects of LINC00511 and miR-150-5p on cell proliferation. **B,** Effects of LINC00511 and miR-150-5p on cell migration ability are detected by transwell migration experiments (magnification: 20x). **C,** Effects of LINC00511 and miR-150-5p on cell invasion are tested by transwell invasion experiments (magnification: 20x). **D,** Effects of LINC00511 and miR-150-5p on apoptosis are detected by flow cytometry. **E,** Effects of LINC00511 and miR-150-5p on the cell cycle are detected by flow cytometry. Data are presented as mean ± S.D., *$p<0.05$, **$p<0.01$

family. In thyroid papillary cancer cells, microRNA-150-5p is confirmed to accelerate epithelial mesenchymal transformation *via* regulating BRAFV600E[32], while in colorectal adenocarcinoma, it can be recruited to the promoter region of tp53 to promote the proliferation of colorectal cancer cells[33]. In addition, it is considered as a novel biomarker for predicting the prognosis of lung cancer[34]. These studies suggest a crucial role of microRNA-150-5p in the occurrence of tumors, but the molecular mechanism of how microRNA-150-5p exerts its effect on ECa so far is not fully understood so far.

The findings of this study show that overexpression of LINC00511 remarkably promoted the migration, as well as replication of ECa cells *in vitro*, which could be partially reversed by simultaneous upregulation of microRNA-150-5p. These results suggest that LINC00511 can promote the progression of ECa by binding microRNA-150-5p. However, which genes regulated by LINC00511 by binding to microRNA-150-5p and their potential biological functions remain to be further investigated. Furthermore, in this study there are some limitations. The follow-up period of the included patients was too short to analyze the association between LINC0051 and age, gender, OS, TNM stage, or other clinical pathological features. In our future research, we plan to conduct the analysis based on data of 5 years follow-up and also expand the clinical sample.

## Conclusions

Briefly, we preliminarily explore the expression and potential molecular mechanism of LINC00511 in ECa, and thus provide a new strategy for the treatment of ECa.

### Conflict of Interests

The Authors declare that they have no conflict of interests.

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