Original Article

Long non-coding RNA LINC01116 is overexpressed in lung adenocarcinoma and promotes tumor proliferation and metastasis

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Abstract: Long non-coding RNA LINC01116 is involved in the occurrence and progression of a variety of cancers. However, the specific role of LINC01116 in lung adenocarcinoma (LUAD) remains unclear. In this work, we found that LINC01116 was overexpressed in LUAD tissues and cell lines and that increased expression was significantly associated with worse prognoses in patients with LUAD. Univariate and multivariate Cox regression analyses indicated that LINC01116 was an independent risk factor for the prognosis of patients with LUAD. Downregulation of LINC01116 significantly inhibited cell proliferation and migration, promoted cell apoptosis, and prevented cell progression from G1 to S phase. In addition, downregulation of LINC01116 significantly inhibited the epithelial-mesenchymal transition, leading to an increased expression of the epithelial marker E-cadherin and decreased expression of the mesenchymal markers N-cadherin and vimentin. In summary, our results suggest that LINC01116 may act as an oncogene in LUAD and may be a valuable prognostic biomarker for patients with LUAD.

Keywords: LINC01116, lung adenocarcinoma, prognosis, proliferation, EMT

Introduction

Lung cancer is the primary cause of tumor-related deaths worldwide, making it a public health issue of global concern [1, 2]. Non-small cell lung cancer (NSCLC) is the most common subtype of lung cancer, accounting for more than 80% of primary cases [3, 4]. Lung adenocarcinoma (LUAD), the predominant subtype of NSCLC, causes over 500,000 deaths per year around the world [5]. Although patients with LUAD can sometimes be successfully treated with targeted therapy or immunotherapy, survival rates remain low, especially in patients with metastatic disease [6]. Moving forward, it will be crucial to explore the molecular mechanisms of LUAD development and metastasis to develop better therapeutic approaches and improve survival rates.

Long non-coding RNA (LncRNA) is a type of non-coding RNA that is longer than 200 nucleotides and does not encode proteins [7]. With the development of gene sequencing and chip detection technologies, an increasing number of LncRNAs have recently been identified [8]. Crucial roles for LncRNA have been identified in the development of various cancers [9, 10]. Aberrantly expressed LncRNAs are involved in many biological processes of tumors, contributing to tumorigenesis, metastasis, and worse prognosis [11, 12]. Studies have shown that LncRNAs act as epigenetic regulators in several cancers [13, 14]. Further evidence has suggested that LncRNAs can regulate tumor occurrence and development by acting both as oncogenes and tumor suppressor genes [15, 16]. As a result, it has been suggested that LncRNAs may serve well as diagnostic or prognostic biomarkers, or even as therapeutic targets [17-19]. LINC01116 is a novel LncRNA located in 2q31.1. Previous studies have demonstrated that it is overexpressed in different types of cancers, including osteosarcoma [20], oral squ-
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Table 1. Clinical information of LUAD patients in TCGA and GEO datasets

<table>
<thead>
<tr>
<th>Clinical factors</th>
<th>TCGA dataset (n = 448)</th>
<th>GEO datasets (n = 381)</th>
</tr>
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<tbody>
<tr>
<td>Vital status</td>
<td></td>
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</tr>
<tr>
<td>Alive</td>
<td>299 (66.7)</td>
<td>286 (75.1)</td>
</tr>
<tr>
<td>Dead</td>
<td>149 (33.3)</td>
<td>95 (24.9)</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤65</td>
<td>213 (47.5)</td>
<td>233 (61.2)</td>
</tr>
<tr>
<td>&gt;65</td>
<td>235 (52.5)</td>
<td>148 (38.8)</td>
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<td>Gender</td>
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</tr>
<tr>
<td>Female</td>
<td>240 (53.6)</td>
<td>190 (49.9)</td>
</tr>
<tr>
<td>Male</td>
<td>208 (46.4)</td>
<td>191 (50.1)</td>
</tr>
<tr>
<td>Stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>241 (53.8)</td>
<td>275 (72.2)</td>
</tr>
<tr>
<td>II</td>
<td>111 (24.8)</td>
<td>95 (24.9)</td>
</tr>
<tr>
<td>III/IV</td>
<td>96 (21.4)</td>
<td>8 (2.1)</td>
</tr>
<tr>
<td>T stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>151 (33.7)</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>241 (53.8)</td>
<td>-</td>
</tr>
<tr>
<td>3/4</td>
<td>56 (12.5)</td>
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<td>N stage</td>
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<td>0</td>
<td>294 (65.6)</td>
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<tr>
<td>1</td>
<td>87 (19.4)</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>67 (20.0)</td>
<td>-</td>
</tr>
</tbody>
</table>

LUAD, lung adenocarcinoma; TCGA, The Cancer Genome Atlas; GEO, Gene Expression Omnibus; TNM, tumor-node-metastasis. Data were shown as n (%).

Materials and methods

Acquisition of RNA expression data and clinical information

A dataset including RNA sequencing data from 535 LUAD tumor and 59 non-tumor tissues, along with other relevant clinical information, was downloaded from The Cancer Genome Atlas website (TCGA, https://gdc-portal.nci.nih.gov/). We performed differential expression analysis on the RNA sequencing data using the Wilcox test with R software. In subsequent analyses, a total of 448 LUAD patients were analyzed after excluding patients with incomplete clinical information or with an overall survival (OS) of less than 60 days. The clinical factors included OS, survival status, age, sex, tumor (T) stage, node (N) stage, and tumor node metastasis (TNM) stage. Three Gene Expression Omnibus (GEO) datasets were acquired from the official website (http://www.ncbi.nlm.nih.gov/geo) of GEO and included gene expression profiles, relevant clinical data, and the corresponding probe information. The R package “sva” was used to normalize the expression of different batches after merging the three GEO datasets together. A total of 381 LUAD patients were selected for subsequent analyses after excluding patients whose pathological type was not adenocarcinoma, whose overall survival was less than 60 days, or whose clinical information was incomplete. Of the 381 LUAD patients, 226 were from GSE31210, 30 were from GSE29013, and the other 125 patients were from GSE50081. The clinical factors in the GEO datasets included OS, age, sex, and TNM stage. Detailed information on the TCGA and GEO datasets is listed in Table 1.

Tissue collection

We collected tumor and matched adjacent non-tumor tissues from 21 patients with LUAD treated at the Second Affiliated Hospital of Xian Jiaotong University (Xian, China) from January 2016 to January 2019. Among them, 8 were women and 13 were men, and the median age was 62 years (range, 40-76 years). Our study protocol was approved by the Ethics Committee of the Second Affiliated Hospital of Xian Jiaotong University (Xian, China) and all 21 patients provided written informed consent.
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Table 2. Small interfering RNA sequences

<table>
<thead>
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<tr>
<td>siRNA1</td>
<td>GCAGTGTTAGAAGACATTT</td>
</tr>
<tr>
<td>siRNA2</td>
<td>GCCGCATAGTGTAACCTTTT</td>
</tr>
<tr>
<td>siRNA3</td>
<td>CCCATTAGTGTGTGCACCTT</td>
</tr>
<tr>
<td>siNC</td>
<td>TTCTCGAAGCTGTCACGTT</td>
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</table>

Table 3. Primer sequences

<table>
<thead>
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<tr>
<td>LINC01116</td>
<td>F: TTGCTCCCTTACCCCGAAG</td>
</tr>
<tr>
<td></td>
<td>R: TGGGTGATGGCAGAGTGAGACC</td>
</tr>
<tr>
<td>ACTB</td>
<td>F: CCGGCCCAAGCACAAT</td>
</tr>
<tr>
<td></td>
<td>R: GGGCGGACTCGTCATAC</td>
</tr>
</tbody>
</table>

Cell culture and transfection

Three human LUAD cell lines A549, pc-9, and H1299 and one normal bronchial epithelial cell line BEAS-2B were used in this study. All four cell lines were obtained from the Cellular Institute of Chinese Academy of Science (Shanghai, China) and were grown in RPMI-1640 (Hyclone, USA) medium supplemented with 10% fetal bovine serum (FBS; Biological Industries). All cell lines were cultured in a humidified atmosphere containing 5% CO2 at 37°C. Three short interfering RNAs (siRNAs) against LINC01116 and one negative control were produced by GenePharma (Shanghai, China). Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) was used to transfect siRNAs when the cell density reached around 70-80%. The sequences of those siRNAs are listed in Table 2.

Quantitative real-time polymerase chain reaction (qRT-PCR)

Fast1000 (Xfyangbio) and Fast200 (Xfyangbio) were used to extract total RNA from lung tissues and cell lines, respectively. The total RNA was reverse transcribed to cDNA using the PrimerScript™ RT kit (TAKARA BIO INC). TB Green® Premix Ex Taq™ II (TAKARA BIO INC) kit was used for real-time PCR and all reactions were performed in triplicate. In this study, ACTB was used as a control. The primers used in real-time PCR were designed and produced by Sangon Biotech Co. Ltd. (Shanghai, China) and the sequences of primers are shown in Table 3. All operations were performed according to the manufacturer’s instructions. The 2ΔΔCq method [30] was used to evaluate relative gene expression.

Cell counting assay

The cell counting kit-8 (CCK-8) assay was used to assess cell proliferation. First, 2 × 10^3 transfected cells were seeded per well of a 96-well plate. After incubation for 24, 48, or 72 h, 100 μl medium containing 10 μl CCK-8 solution (Keygen Biotech, China) were added to each well and incubated for 2 hours. The absorbance at 450 nm was measured on a spectrophotometer (Bio Tek, USA).

EdU assay

Cell growth was measured using the EdU (5-ethynyl-2’-deoxyuridine) assay kit (RiboBio Co., Ltd, China). First, 4 × 10^3 transfected cells were seeded in each well of 96-well plates and incubated for 24 h. Then, the medium was replaced with one containing 50 μM EdU and cultured for another 2 h. Next, we fixed the cells with 4% paraformaldehyde, stained the EdU-positive cells with Apollo solution and stained the DNA using Hoechst reagents. All assay steps were carried out in sequence according to the kit’s instructions. Finally, a fluorescence microscope (Nikon, Tokyo, Japan) was used to acquire images. The extent of cell proliferation was calculated as the EdU positivity rate = (the number of EdU-positive cells/the number of DNA-stained cells) × 100%.

Cell apoptosis assay

Cells in 6-well plates were incubated for another 24 h after transfection with siRNA. Next, the transfected cells were digested and incubated at room temperature for 15 minutes in the dark after the addition of a solution containing 500 μl of binding buffer, 5 μl of Annexin V-APC and 5 μl of 7-amino-actinomycin D (7-AAD). Finally, cell apoptosis was detected using a flow cytometer (Becton-Dickinson; BD Biosciences) within 1 h.

Cell cycle assay

A549 cells were incubated in 6-well plates for another 24 h after transfection with siRNA. Next, the transfected cells were digested and fixed in pre-cooled 70% ethanol at -20°C overnight. On the second day, fixed cells were incu-
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Figure 1. LINC01116 was significantly overexpressed in LUAD tissues and cell lines. A. Differential expression analysis showed that LINC01116 was overexpressed in 535 lung adenocarcinoma tumor tissues compared with 59 non-tumor lung tissues. ***P < 0.001. B. qRT-PCR showed that the expression level of LINC01116 in 21 lung adenocarcinoma tissues was higher than that in corresponding adjacent non-tumor lung tissues. *P < 0.05. C. qRT-PCR found that the expression level of LINC01116 were higher in H1299, PC-9, and A549 than that in BEAS-2B. *P < 0.05, **P < 0.01, ***P < 0.001.

bated with 500 μl working solution (RNase A: Propidium Iodide = 9:1) for 30 min at room temperature in the dark. Finally, cell cycle status was detected using a flow cytometer (Becton-Dickinson; BD Biosciences).

Transwell migration assay

Cells were grown on 24-well Transwell plates (Corning, USA) for the Transwell migration assay. First, 4 × 10⁴ transfected cells were seeded in the upper chamber in 200 μl FBS-free medium, and 600 μl of medium containing 10% FBS was added to the lower chamber. After incubation for 24 h, the cells were fixed with paraformaldehyde and stained with crystal violet. The cells in the upper chamber were wiped off, and the cells in the lower chamber were counted under the microscope.

Wound healing assay

Transfected cells were grown to 100% confluence in 6-well plates, and then scratches were made using 10-μl pipette tips. Then, the complete medium was replaced with low serum medium (1% FBS). The cells migrated into the scraped areas and images were captured at 0, 24, and 48 h. The wound healing area was calculated using ImageJ software (version 1.52t).

Western blotting

Total cellular proteins were extracted using the radio immunoprecipitation assay lysate (Xi’an HEART Biotech). Proteins were separated using 10% sodium deodecyl sulfate-polyacrylamide gel electrophoresis and were transferred to polyvinylidene difluoride membranes. The membranes were blocked in 5% (w/v) nonfat dry milk powder in 0.1% Tris buffered saline/Tween 20 (TBST) for 2 h at room temperature and incubated with various primary antibodies overnight at 4°C. The primary antibodies against β-actin, E-cadherin, N-cadherin, and vimentin were acquired from Cell Signaling Technology (Danvers, MA, USA). Next, membranes were washed and then incubated with horseradish peroxidase-conjugated secondary antibodies, acquired from Cell Signaling Technology, for 1 h at room temperature. Enhanced chemiluminescence (Thermo Company, USA) was used to quantify protein bands. ImageJ software (version 1.52t) was used to analyze relative protein expression.

Statistical analysis

Kaplan-Meier survival analyses were conducted using the R package “survival”, and the two-sided log-rank test was used to compare differences. GraphPad Prism 6.0 Software (GraphPad Inc) was used to analyze experimental data. Student’s t-test and one-way ANOVA were used to analyze differences in means across groups and P < 0.05 was considered statistically significant.

Results

LINC01116 was overexpressed in LUAD tissues and cell lines

We analyzed the differential expression of RNA in the 535 LUAD tissues and 59 non-tumor tissues from RNA-sequencing data obtained from the TCGA database and found that LINC01116 was significantly overexpressed in tumor tissues relative to non-tumor tissues (log FC = 2.06, P = 9.78 × 10⁻⁶, Figure 1A). In order to
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Figure 2. LINC01116 was significantly associated with the prognosis of LUAD patients. A. Kaplan-Meier survival curves of overall survival in 448 LUAD patients from the TCGA dataset, based on LINC01116 expression. The LUAD patients with high expression of LINC01116 tended to have worse prognosis. B. Univariate Cox regression analysis of LINC01116 and other clinical factors in 448 LUAD patients in the TCGA dataset. T stage, N stage, TNM stage, and LINC01116 expression were closely related to overall survival and acted as risk factors of prognosis. C. Multivariate Cox regression analysis of LINC01116 and other clinical factors in 448 LUAD patients in TCGA dataset. LINC01116 was still significantly associated with prognosis after adjusting for clinical factors. D. The expression of LINC01116 among different TNM stages in the TCGA dataset. Patients in the advanced TNM stages were more likely to have higher expression of LINC01116. E. The expression of
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LINC01116 was significantly associated with worse outcomes (P = 0.012, Figure 2A). Using the median expression value as a cut-off, the low-expression group had a higher median survival (4.38 years) compared with that in high-expression group (3.73 years). The 3- and 5-year survival rates were 63.9% and 39.1% in the low-expression group, and 53.9% and 30.2% in high-expression group, respectively. To further explore the prognostic value of LINC01116 in LUAD, univariate Cox regression analysis was performed. Here, LINC01116 was a prognostic risk factor of LUAD patients (hazard ratio (HR) = 1.109, P < 0.0001, Figure 2B). Next, we performed multivariate Cox regression analysis to evaluate whether LINC01116 expression remained an independent prognostic risk factor in the presence of other clinical factors such as age, sex, T stage, N stage, and TNM stage. Indeed, LINC01116 was still an independent prognostic risk factor after adjusting for the other clinical factors (HR = 1.082, P < 0.0001, Figure 2C). Further, we analyzed the correlation between LINC01116 and T stage, N stage, and TNM stage among samples in the TCGA dataset. The expression of LINC01116 was higher in patients with advanced TNM stage (P < 0.0052; Figure 2D) and T stage (P = 0.0014, Figure 2E). However, although no significant difference was found between the expression of LINC01116 and N stage (P = 0.0998, Figure 2F), LINC01116 tended to be more highly expressed in patients with advanced N stage.

To further verify the prognostic value of LINC01116, we analyzed LINC01116 in a dataset consisting of three GEO datasets (GSE31210, GSE50081, and GSE29013). Using the median expression of LINC01116 as the cut-off, the 3- and 5-year survival rates were 90.2% and 77.3% in the low-expression group and 79.2% and 69.2% in the high-expression group.

### Table 4. Average expression level of LINC01116 in different clinical stages

<table>
<thead>
<tr>
<th>Clinical stages</th>
<th>Average expression level</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TCGA dataset</strong></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>0.987 ± 0.1583</td>
</tr>
<tr>
<td>T2</td>
<td>1.222 ± 0.1408</td>
</tr>
<tr>
<td>T3+4</td>
<td>2.657 ± 0.8565</td>
</tr>
<tr>
<td>stage I</td>
<td>1.003 ± 0.1389</td>
</tr>
<tr>
<td>Stage II</td>
<td>1.274 ± 0.1851</td>
</tr>
<tr>
<td>Stage III/IV</td>
<td>2.181 ± 0.5197</td>
</tr>
<tr>
<td>N0</td>
<td>1.126 ± 0.1375</td>
</tr>
<tr>
<td>N1</td>
<td>1.510 ± 0.3524</td>
</tr>
<tr>
<td>N2</td>
<td>1.979 ± 0.5988</td>
</tr>
<tr>
<td><strong>GEO datasets</strong></td>
<td></td>
</tr>
<tr>
<td>Stage I</td>
<td>5.334 ± 0.0843</td>
</tr>
<tr>
<td>Stage II/III</td>
<td>5.914 ± 0.1327</td>
</tr>
</tbody>
</table>

TCGA, The Cancer Genome Atlas; GEO, Gene Expression Omnibus Data were shown as mean ± SD.

verify this result, we used qRT-PCR to measure the expression of LINC01116 in 21 paired LUAD tumor tissues and adjacent non-tumor tissues and found a consistent result: LINC01116 expression was significantly higher in LUAD tissues (P = 0.0102, Figure 1B). Next, we detected the expression of LINC01116 in LUAD cell lines (A549, H1299, and PC-9) and normal bronchial epithelial cells (BEAS-2B) by qRT-PCR. Again, we found that the expression of LINC01116 in the three LUAD cell lines was significantly higher than in BEAS-2B (P < 0.0001 for H1299, P = 0.023 for PC-9, and P < 0.0001 for A549, Figure 1C). A549 cells, which showed the highest expression of LINC01116 compared with the others, were chosen for the subsequent experiments.

**LINC01116 was a significant independent prognostic biomarker in LUAD**

Kaplan-Meier survival analysis was performed on the TCGA dataset to evaluate the relationship between overall survival (OS) and the expression of LINC01116. High expression of LINC01116 among different T stages in the TCGA dataset. Patients with advanced T stage were more likely to have higher expression of LINC01116. F. The expression of LINC01116 among different N stages in the TCGA dataset. Patients in advanced N stage were more likely to highly express LINC01116. G. Univariate Cox regression analysis of LINC01116 and other clinical factors in 381 LUAD patients in the GEO datasets. LINC01116 and TNM stage were highly related to overall survival and acted as risk factors for prognosis. H. Multivariate Cox regression analysis of LINC01116 and other clinical factors in 381 LUAD patients in the GEO datasets. LINC01116 still was significantly associated with overall survival after adjusting for other clinical factors. I. The expression level of LINC01116 between TNM stage I and stage II/III in GEO datasets. The expression of LINC01116 in TNM stage I was significantly lower than that in stage II/III. **P < 0.001.**
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Univariate Cox regression analysis showed that LINC01116 was closely related to prognosis (HR = 1.331, P < 0.001, Figure 2G). Further, multivariate Cox regression analysis demonstrated that LINC01116 was an independent prognostic biomarker after adjusting for age, sex, and TNM stage (HR = 1.276, P = 0.002, Figure 2H). The relationship between LINC01116 and TNM stage was further investigated. We found that LINC01116 was significantly correlated with the TNM stage: the expression of LINC01116 in LUAD patients with TNM stage I was significantly lower than in LUAD patients with TNM stage II/III (P = 0.0003, Figure 2I). The average expression of LINC01116 in each clinical subgroup is listed in Table 4.

Downregulation of LINC01116 suppressed cell proliferation

To better understand the role of LINC01116 in the development of LUAD, we transfected A549 cells with siRNAs to downregulate the expression of LINC01116. As expected, qRT-PCR showed that siRNA1 and siRNA2 significantly reduced the expression of LINC01116 compared with the negative control (Figure 3A). The knockdown rates were 96.1% for siRNA1, 84.0% for siRNA2, and 58.7% for siRNA3. Thus, siRNA1 and siRNA2 were chosen for further experiments and named the group transfected with negative control siRNA as siNC group. The results of the CCK-8 assay indicated that absorbance at 450 nm (indicating cell proliferation)
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Figure 4. Downregulation of LINCO1116 inhibited cell apoptosis and prevented G1 to S phase transition. A. After transfecting for 24 h, cells were digested, treated with 500 μl of binding buffer, 5 μl Annexin V-APC, and 5 μl 7-AAD at room temperature for 15 minutes in the dark. Finally, cell apoptosis was analyzed using a flow cytometer. Cell apoptosis in the siRNA1 and siRNA2 groups was significantly higher than the siNC group. **P < 0.01. B. After transfection for 24 h, cells were digested and fixed in pre-cooled 70% ethanol at -20°C overnight. On the second day, fixed cells were incubated with 500 μl working solution (RNase A: propidium iodide = 9:1) for 30 min at room temperature in the dark. Finally, cell cycle status was detected using a flow cytometer. The proportion of cells in G1 phase in the siRNA1 group was significantly higher than that in the siNC group. **P < 0.01.

was significantly reduced in the siRNA1 and siRNA2 groups at 24, 48, and 72 h compared with the siNC group (Figure 3B). The absorbance at 450 nm was 0.28 ± 0.01 for the siRNA1 group, 0.28 ± 0.01 for the siRNA2 group, and 0.31 ± 0.02 for the siNC group at 24 h. At 48 h, it was 0.49 ± 0.04 for the siRNA1 group, 0.54 ± 0.06 for the siRNA2 group, and 0.82 ± 0.04 for the siNC group. At 72 h, it was 0.75 ± 0.03 for the siRNA1 group, 0.82 ± 0.10 for the siRNA2 group, and 1.15 ± 0.08 for the siNC group. An EdU assay showed that the EdU-positive rates in LINCO1116-knockdown groups were markedly lower than in the negative control group (Figure 3C). The EdU-positive rates were 20.95 ± 1.45% for the siRNA1 group, 26.00 ± 0.20% for the siRNA2 group, and 41.75 ± 3.65% for the siNC group. These results suggested that downregulation LINCO1116 significantly suppressed cell proliferation.

**Downregulation of LINCO1116 promoted cell apoptosis and prevented cell cycle progression**

Flow cytometry was used to detect changes in cell apoptosis and cell cycle status after decreasing the expression of LINCO1116. Consistent with our prior experiments, downregulation of LINCO1116 significantly promoted cell apoptosis (Figure 4A). The cell apoptosis rates were 11.70 ± 0.92% for the siRNA1 group, 10.19 ± 0.55% for the siRNA2 group, and 6.34 ± 0.23% for the siNC group. The cell cycle stage was also affected by the downregulation of LINCO1116. Downregulation of LINCO1116 prevented cells from transitioning from G1 to S phase (P = 0.0077, Figure 4B). The proportion of cells in G1 phase was 89.24 ± 0.92% in the siRNA1 group and 79.77 ± 1.67% in the siNC group.

**Downregulation of LINCO1116 suppressed cell migration**

A Transwell migration assay and wound healing assay were used to detect changes in cell migration ability after downregulation of LINCO1116. The number of cells migrating from the upper chamber to the lower chamber in the siRNA1 and siRNA2 groups was significantly lower than that in the siNC group (Figure 5A). The number of migrating cells in each 10x field
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Figure 5. Downregulation of LINC01116 significantly suppressed LUAD cell migration. A. Scratches were made when transfected cells reached 100% confluence in 6-well plates and then, cells were cultured in low serum medium (1% FBS). Images were acquired at 0, 24, and 48 h after making scratches. The wound healing area in LINC01116-downregulated groups was significantly decreased after 24 h and 48 h. *P < 0.05, **P < 0.01. B. Transfected cells were seeded in upper chambers with 200 μl FBS-free medium, and 600 μl medium with 10% FBS was added to the lower chambers. After incubating for 24 h, the cells were fixed with paraformaldehyde and stained with crystal violet. We acquired images and calculated the number of cells that had migrated to the lower chamber. The number of migrated cells was significantly lower in the LINC01116-downregulated groups relative to the negative control group. **P < 0.01.

of view under the microscope was 23.00 ± 1.00 for the siRNA1 group, 27.00 ± 1.53 for the siRNA2 group, and 76.33 ± 8.45 for the siNC group. In the wound healing assay, the cell migration area in the LINC01116-knockdown groups was significantly less than in the negative control group (Figure 5B). The area of wound healing was 25.52 ± 1.95% for the siRNA1 group, 21.76 ± 2.41% for the siRNA2 group, and 36.54 ± 0.94% for the siNC group at 24 h. At 48 h, the area of wound healing was 46.95 ± 3.13% for the siRNA1 group, 43.31 ± 5.66% for the siRNA2 group, and 67.51 ± 1.99% for the siNC group.

Downregulation of LINC01116 suppressed epithelial-mesenchymal transition

Epithelial-mesenchymal transition (EMT) is a crucial mechanism in tumor metastasis. The-
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Therefore, we detected the relative expression of EMT-related proteins, such as the epithelial marker E-cadherin and the mesenchymal markers N-cadherin and vimentin. E-cadherin was significantly upregulated, but N-cadherin and vimentin were both downregulated in LINC01116-knockdown groups compared to the negative control group (Figure 6).

Discussion

Lung adenocarcinoma is a subtype of lung cancer with high morbidity and mortality [31]. The survival rate of patients with LUAD remains low because of a lack of effective early diagnostic markers and a high rate of metastasis [4]. In recent years, an increasing number of abnormally expressed genes have been found to play crucial roles in the occurrence and development of various cancers [11]. Thus, exploring the function and mechanisms of action of differentially expressed LncRNAs in LUAD may provide novel insights to set the foundation for new methods of diagnosis and treatment.

LncRNA LINC01116 is abnormally expressed in many kinds of human cancers [21, 23, 24]. As a result, the function of LINC01116 in various tumors has become a topic of increasing scientific interest. It has been suggested that LINC01116 is involved in biological processes such as cell growth, invasion, migration, and others. Previous studies have indicated that LINC01116 regulates the expression of target genes by acting as a ceRNA. For example, LINC01116 competes with miR-145 to regulate the expression of ESR1 in breast cancer [26]. Several reports have shown that LINC01116 affects tumor occurrence and progression via regulating known oncogenes or tumor suppressor genes such as MYC [22], P53 [20], etc. One recent study suggested that a novel isoform of LINC01116 may act on sulforaphane to suppress prostate cancer [32]. Clearly, these reports confirm that LINC01116 plays an important role in the progression of several cancers. However, to date, only a few studies have assessed the relationship between LINC01116 and lung cancer, and the specific functions and mechanisms of LINC01116 in LUAD remain poorly understood.

This study provides the first analysis of the expression of LINC01116 in LUAD tissues and cell lines. LINC01116 was significantly overexpressed in LUAD tissues and cell lines relative to normal cells, and high LINC01116 expression was correlated with worse outcomes. Univariate and multivariate Cox regression analyses indicated that LINC01116 was an independent prognostic risk factor for LUAD. LUAD patients with advanced T stage, N stage, and TNM stage were more likely to have high expression of LINC01116. The expression of LINC01116 was positively correlated with other prognostic factors for LUAD, further confirming that LINC01116 may serve well as a prognostic risk factor. These studies indicated that LINC01116 is
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an independent predictor of prognosis in patients with LUAD.

Downregulation of the expression of LINC01116 in A549 cells affected many biological processes. The results of the CCK-8 and EdU assays indicated that cell proliferation was significantly suppressed after siRNA knockdown of LINC01116. To further explore the changes in apparent cell proliferation, we measured changes in cell apoptosis and cell cycle using flow cytometry. Downregulation of LINC01116 promoted cell apoptosis and prevented G1-S phase transition. These results suggested that LINC01116 may promote cell proliferation via inhibiting cell apoptosis and promoting cell cycle progression. In addition, Transwell and wound healing assays were used to measure changes in cell migration ability after knockdown of LINC01116. Cell migration was significantly suppressed in downregulated groups. Thus, we hypothesize that LUAD patients with high expression of LINC01116 are more likely to have metastases.

The EMT is a crucial stage of the progression toward metastasis, contributing to the local infiltration of cancer cells [33, 34]. Therefore, we detected the expression of EMT-related protein markers to determine whether downregulation of LINC01116 had an impact on EMT. Indeed, in LINC01116-knockdown cells, the expression of the epithelial marker E-cadherin was increased, but the expression of the mesenchymal markers, N-cadherin and vimentin, were both decreased. These results suggest that LINC01116 may promote the metastasis of LUAD by regulating EMT.

In conclusion, these experiments found that LINC01116 was overexpressed in LUAD tissues and cell lines and that increased expression was significantly associated with worse prognosis. Cox regression analysis demonstrated that LINC01116 was an independent prognostic risk factor for worse outcomes among patients with LUAD. Downregulation of LINC01116 markedly inhibited cell proliferation and migration, promoted cell apoptosis, and slowed progression through the cell cycle. Further, LINC01116 promoted the expression of EMT markers. Together, these results suggest that LINC01116 may be involved in the development and metastasis of LUAD and may have value as a prognostic indicator or therapeutic target.

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Disclosure of conflict of interest

None.

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