

Original Research

LINC01572 is a Novel Prognostic Biomarker and Therapeutic Target in Lung Adenocarcinoma

Yaolin Zheng^{1,†}, Zhenshan Zhang^{2,3,†}, Xueyan Li¹, Leilei Wu⁴, Xinliang Liu⁵, Liang Liu⁴, Jiayan Chen^{2,*}, Dongping Wei^{1,*}

¹Department of Oncology, Nanjing First Hospital, Nanjing Medical University, 210006 Nanjing, Jiangsu, China

²Department of Radiation Oncology, Fudan University Shanghai Cancer Center, 200000 Shanghai, China

³Department of Radiation Oncology, Shanghai Proton and Heavy Ion Center, Fudan University Cancer Hospital, 200120 Shanghai, China

⁴Department of Radiation Oncology, Shanghai Pulmonary Hospital, Tongji University School of Medicine, 200433 Shanghai, China

⁵Department of Endocrinology, People's Hospital of Xuyi, 211700 Xuyi, Jiangsu, China

*Correspondence: chenjiayan2008@126.com (Jiayan Chen); weidongping2001@hotmail.com (Dongping Wei)

†These authors contributed equally.

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Abstract

Background: Lung adenocarcinoma (LUAD) is one of the most common and lethal cancer types worldwide. LINC01572 is a long non-coding RNA (lncRNA) that has been associated with the clinical characteristics of several types of malignancy. However, the biological mechanism of LINC01572 in LUAD is still unclear and remains to be elucidated. **Methods:** R packages and online bioinformatic tools were used to investigate the biological characteristics of LINC01572, including its abnormal expression, oncogenic role, and clinical prognostic value. *In vitro* and *in vivo* experiments were conducted to investigate the biological functions of LINC01572 in tumorigenesis and development. These included colony formation assays, cell migration assays, flow cytometry, cell counting kit-8 (CCK-8) cell proliferation and tumor transplant growth experiments. **Results:** Bioinformatics results showed that LINC01572 was overexpressed in both LUAD and lung squamous cell carcinoma (LUSC) patients. LINC01572 overexpression was associated with shorter overall survival (OS) in LUAD. Further study of clinical specimens confirmed that LINC01572 was highly expressed in the tumor tissue of non-small cell lung cancer (NSCLC) patients. *In vitro* experiments also confirmed that LINC01572 was overexpressed in tumor cell lines. Inhibition of LINC01572 expression significantly impaired cell proliferation, cell migration, and clone formation. Experiments in nude mouse revealed that transplanted tumors with low expression of LINC01572 had significantly slower rates of growth in terms of volume and weight compared to the control group ($p < 0.05$). In addition, gene set enrichment analysis (GSEA) and immune landscape profiling showed that LINC01572 can promote tumor initiation and progression by deregulating the cell cycle and immunocyte infiltration. **Conclusions:** LINC01572 is overexpressed in tumor tissue relative to adjacent normal tissue. Moreover, LUAD patients with high expression of LINC01572 showed a worse survival prognosis. LINC01572 is associated with tumor initiation, progression and immune dysregulation. It therefore has potential value as a novel biomarker and therapeutic target in LUAD.

Keywords: NSCLC; lung adenocarcinoma; TCGA; lncRNA; experiments

1. Introduction

The GLOBOCAN 2018 estimates of cancer incidence and mortality show that lung cancer is the most commonly diagnosed and lethal human cancer type, being responsible for >1.4 million deaths globally per year. Approximately 733,000 newly diagnosed cases of lung cancer and >610,000 lung cancer-related mortalities were reported in China in 2015, ranking first in males and second in females in terms of mortality [1,2]. More than 80% of lung cancer cases can be categorized as non-small cell lung cancer (NSCLC). These are further subdivided into lung squamous cell carcinoma (LUSC) and lung adenocarcinoma (LUAD) [3,4]. Although significant progress has been made in the diagnosis and therapy of NSCLC over the past few decades, the prognosis of such patients remains poor, with an overall five-year survival rate of <5% [5]. Due to the lack

of effective early detection biomarkers, the majority of NSCLC patients are diagnosed with an advanced stage of disease [6,7]. Distant metastasis is considered to be the primary cause of treatment failure and mortality amongst patients with NSCLC. Several biomarkers have been studied in lung cancer, including clinical factors such as tumor stage, performance status, and age, as well as molecular factors such as Epidermal Growth Factor Receptor (EGFR) mutation [8], Anaplastic Lymphoma Kinase (ALK) rearrangement [9], PD-L1 expression [10,11], and long non-coding RNA (lncRNA) expression [11]. EGFR mutations are predictive of the response to targeted therapies, while PD-L1 expression has been associated with the response to immunotherapy. Circulating tumor DNA (ctDNA) [12] is a promising biomarker for monitoring disease progression and for predicting treatment response. LncRNAs such as



SNHG15 [13], *MIR4435-2* [14] and *LINC00958* [15] have also been investigated for their potential prognostic value in lung cancer. Moreover, the LC NetWork was recently established to investigate interactions between known mRNAs, lncRNAs and miRNAs. This showed that several mRNA/lncRNA/miRNA axes play a crucial role in NSCLC development [16]. However, further research is needed to validate these findings and to identify new markers that can improve prognostication in lung cancer patients. The aim of the present study was to identify novel biomarkers with high sensitivity and specificity for NSCLC diagnosis and prognosis, as well as to investigate the underlying molecular mechanisms of NSCLC. Current biomarkers for prognosis and treatment targets remain insufficient and unsatisfactory, and hence it is essential to identify novel prognostic and/or treatment factors in NSCLC.

Current biomarkers used for cancer diagnosis are proteins. It is well known that >70% of the human genome is actively transcribed, but only 1–2% of the entire genome codes for proteins [17]. Noncoding RNAs (ncRNAs) are widely expressed in various body fluids and are quite stable, suggesting they may be valuable for diagnosing cancer [18]. lncRNA of >200 nucleotides in length are the main subtype of ncRNA. Research has shown that lncRNAs are essential in multiple biological processes including the imprinting, modification and maintenance of chromatin. Due to the high stability and tissue specificity of lncRNAs, these molecules may be valuable tumor diagnosis biomarkers in various human cancer types [19]. lncRNAs are also known to participate in the tumorigenesis of NSCLC [20]. However, the functions of most of the lncRNAs found in NSCLC to date remain unknown.

The lncRNA *LINC0572* has been associated with various clinical features of LUSC [21] and of gastric cancer [22]. So far however, few studies have been published on *LINC0572* in LUAD. In the present work we analyzed the prognostic value of *LINC0572* in LUAD patients using multiple bioinformatics tools and experiments. The results showed that *LINC0572* is a tumor-promoting factor, and that blocking its signaling pathway could restore tumor development. This preliminary data suggests that *LINC0572* could be a novel biomarker and therapeutic target in LUAD.

2. Materials and Methods

2.1 Public Database

RNA-seq data and clinical information for 594 LUAD (including 107 paired samples) and 551 LUSC (including 49 paired samples) were downloaded from the TCGA database (<https://cancergenome.nih.gov/>) (USA) in order to evaluate the characteristics of *LINC0572* expression and its prognostic value.

2.2 lncRNA Subcellular Localization

The Lncipedia portal (<https://lncipedia.org/>) is a public database used to obtain lncRNA sequences and annotations [23].

The LncLocator portal (<http://www.csbio.sjtu.edu.cn/bioinf/lncLocator/>) (China) is an online tool used to predict the subcellular localization of lncRNA [24]. Both these portals were used in the present study of *LINC0572*.

2.3 Gene Expression Analysis

The differential expression of *LINC0572* between tumor and normal samples in LUAD and LUSC was analyzed by R based on the TCGA data. InCAR (<https://lncar.renlab.org/>) (China), an online database for lncRNA analysis, was also used to evaluate *LINC0572* expression levels in LUAD and LUSC from the GSE75037 and GSE88862 databases. The associations of *LINC0572* expression with different clinical variables were also analyzed by R (3.6.3, R Foundation for Statistical Computing, Vienna, Austria) (R is the language and operating environment for statistical analysis, plotting, and operation, and it is an excellent tool for statistical calculations and statistical mapping).

2.4 Diagnostic Value

The pROC package in R was used to perform diagnostic ROC analysis with clinical data obtained from a published study [25]. The results were then visualized using the ggplot2 package (3.3.6, Springer-Verlag New York, 2016).

2.5 Tissue Acquisition and Processing

Pairs of cancerous and adjacent tissue (n = 20) were obtained from patients diagnosed with NSCLC who underwent resection at the Affiliated Nanjing Hospital, Nanjing Medical University, between 2015 and 2018. The tissues were stored at –80 °C until analysis. The use of human tissues was in accordance with guidelines from the Declaration of Helsinki.

2.6 Mice and Cell Lines

Mice were obtained from Hangzhou Ziyuan Laboratory Animal Technology Co., Ltd. The animal ethics committee of Nanjing Medical University approved the use of mice for experiments. A human bronchial epithelial cell line (16HBE) and several tumor cell lines (H1299, A549, SKMES1, H460, and SBC3) were purchased from the Cell Bank, Chinese Academy of Sciences. We confirm that mycoplasma testing has been done for the cell lines used. In our study, STR genotyping was used for cell cross-contamination and characterization. These were used to confirm abnormal expression of *LINC0572* and to examine cell phenotypes. All cell lines were cultured at 37 °C in an atmosphere containing 5% CO₂ and 95% air in RPMI-1640 medium (HyClone Cytiva; Logan, UT, USA) supplemented with 1% penicillin/streptomycin and 10% FBS (Cytiva, Logan, UT, USA).

2.7 siRNAs and shRNAs

To achieve knockdown of *LINC0572* expression in A549 and H1299 cells, two small interfering RNAs

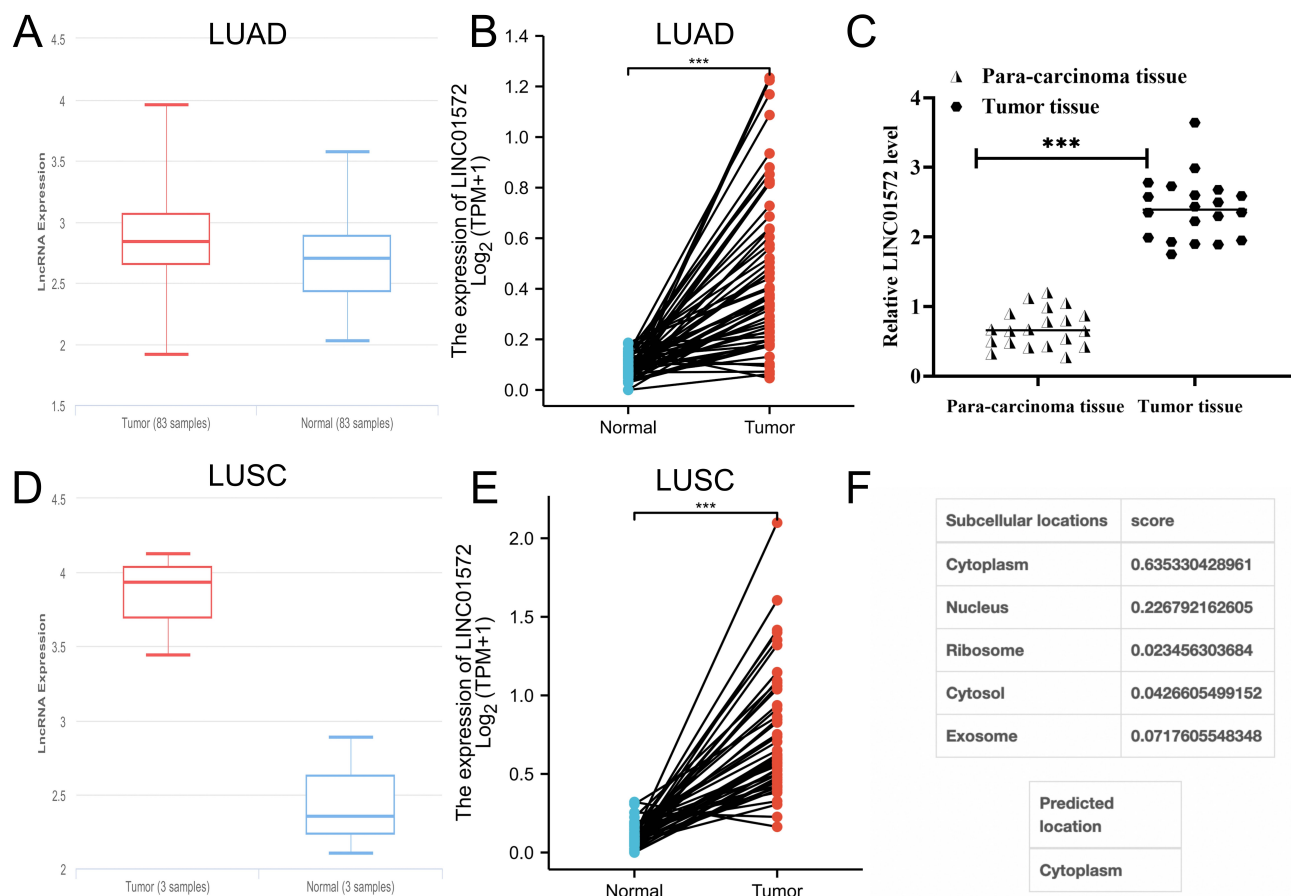


Fig. 1. Abnormal expression of *LINC01572*. (A,D) *LINC01572* was overexpressed in tumor samples from GSE75037 (LUAD) and GSE88862 (LUSC), respectively. (B,E) Upregulation of *LINC01572* was also observed in LUAD and LUSC from the TCGA database, respectively. (C) *LINC01572* expression in tumor and adjacent normal tissue from 20 paired LUAD samples. (F) Prediction of the subcellular localization of *LINC01572*. ***, $p < 0.001$. LUAD, Lung adenocarcinoma; LUSC, Lung squamous cell carcinoma.

(*siLINC01572-1*, *siLINC01572-2*) that were specific against four *LINC01572* transcript variants (NR_159372.1, NR_126330.2, NR_159371.1, NR_159370.1) were designed and synthesized by GenePharma, together with the negative control (siNC). The cells were seeded at an initial concentration of 1×10^5 cells/well in 6-well plates and grown at 37 °C until they reached 80% confluence. Transfection was then carried out using Lipofectamine® 3000 (Invitrogen; Thermo Fisher Scientific, Inc, Waltham, MA, USA) with either the siRNAs or siNC as recommended by the manufacturer. The knockdown efficiency of the siRNAs was evaluated using RT-qPCR technique.

A stable, low-expression plasmid of *LINC01572* was designed with the *si-LINC01572* and vector (pGPU6) sequences (shRNA, GenePharma, China), the information of siRNAs was listed in Table 1.

2.8 RT-qPCR

The relative expression of *LINC01572* in NSCLC tissues and cells was evaluated by RT-qPCR. Total RNA in the NSCLC tissues and cell lines was extracted using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific,

Inc., Waltham, MA, USA). cDNA for analysis by RT-qPCR was produced from 2 µg of total RNA using the Bestar™ qPCR RT kit (cat. no. 2220; DBI Bioscience, Shanghai, China) as recommended by the manufacturer. qPCR was conducted using Bestar™ qPCR MasterMix (cat. no. 2043; DBI Bioscience, China) on an ABI7500 system using the following primers: *LINC01572* forward (F), 5'-AATTTGTGGCGCTTGGTGTC-3' and reverse (R), 5'-CTGAGATCCCTGGTTCCACTG-3'; GAPDH F, 5'-ACAACCTTTGGTATCGTGGAAGG-3' and R, 5'-GCCATCACGCCACAGTTTC-3'. *LINC01572* expression was normalized to that of GAPDH. Pre-denaturation was at 95 °C for 5 minutes, followed by 30 cycles of denaturation at 95 °C, annealing at 60 °C for 30 seconds, and extension at 72 °C for 30 seconds. The reactants were finally kept at 72 °C for 7 minutes.

2.9 Cell Counting kit-8 (CCK-8) Assay

A CCK-8 kit (CK04; Dojindo, Kumamoto, Japan) was used according to the manufacturer's instructions. Transfected A549 and H1299 cells were grown in 96-well culture plates and then incubated for 0, 24, 48, and 72 h with

Table 1. The siRNA sequences are shown below.

	Sense	Antisense
<i>siLINC01572-1</i>	GAATGAAGCTGAGCGTCTCAGTGGA	TCCACTGAGACGCTCAGCTTCATTC
<i>siLINC01572-2</i>	TACACGTGGAAATATGCCAACTGAA	TTCAGTTGGCATATTTCCACGTGUA
siNC	GAACGAAGAGTCTGCGACTTGTGGA	TCCACAAGTCGCAGACTCTTCGTTC

100 μ L of CCK-8 solution. The cells were then incubated with CCK-8 solution for an additional 4 hours, after which the Optical Density (OD) at 450 nm was evaluated using a spectrophotometer. Experiments were conducted in triplicate.

2.10 Colony Formation Assay

Colony formation assays were performed with A549 and H1299 cells to assess the impact of *LINC01572* knock-down on NSCLC cell proliferation. Following transfection with *shLINC01572-1* or shNC for 48 h, cells were seeded at an initial density of 250 cells/well in 6-well plates and incubated for 2 weeks at 37 °C with 5% CO₂. Colonies were then fixed with 70% methanol for 1 h and stained with Giemsa solution for 20 minutes. The number of visible colonies was counted manually under a microscope, with triplicate wells evaluated for each group.

2.11 Cell Cycle Analysis

The effect of *LINC01572* knockdown on the cell cycle was evaluated by flow cytometry. After 48 h transfection with *shLINC01572-1*, *shLINC01572-2* or shNC, the cells were trypsinized, centrifuged at 2000 rpm for 10 minutes, and washed three times with ice-cold PBS. They were then fixed with 70% ethanol at –20 °C overnight, and the cell cycle distribution analyzed using a cell cycle kit (BestBio, China) and a Beckman Coulter FACSCalibur flow cytometer (Beckman Coulter, Inc., USA).

2.12 Transwell Assays

Transwell assays were carried out using chambers with 8 μ m pores (Corning, NY, USA) and Matrigel matrix. A549 and H1299 cells transfected with *shLINC01572-1*, *shLINC01572-2* or shNC were collected after 48 h growth and resuspended in serum-free culture medium at a concentration of 2×10^5 cells/mL. A 200 μ L cell suspension was added to the upper chamber, and 500 μ L of 10% FBS-supplemented culture medium to the lower chamber. After culturing for 24 h, migrating cells were fixed with 4% paraformaldehyde and stained with 0.5% crystal violet. Cell numbers were counted under a microscope, and this was repeated three times for each of the *shLINC01572-1*, *shLINC01572-2* and shNC groups.

2.13 Gene Set Enrichment Analysis (GSEA)

The potential mechanisms for *LINC01572* involvement in the development of LUAD were investigated using the GSEA method. LUAD samples were divided into

“high” and “low” groups based on the median expression level of *LINC01572*. Subsequently, the two groups were compared using GSEA (Broad Institute, Cambridge, Mass. USA) software and the fold-change value was used to rank differences. Enrichment analysis was conducted based on the Hallmark gene set, which was previously identified using biological knowledge. The normalized enrichment score (NES) was used as the primary statistic for assessing gene set enrichment results, while the false discovery rate (FDR) was used to estimate the probability of false positive findings in a given gene set with a particular NES value. Enrichment significance was considered to be present when the |NES| value was >1 and the FDR was <0.25.

2.14 In vivo Tumor Growth Assay

Male BALB/c mice aged 8 weeks were obtained from the Shanghai SLAC Laboratory Animal Company Ltd., Shanghai, China. These were used to evaluate the impact of *LINC01572* knockdown on NSCLC progression by establishing *in vivo* tumor growth assays. The department of Laboratory Animal Science, Fudan University, approved all animal experiments. A549 cells that were stably transfected with either *shLINC01572-1* or shNC were suspended in culture medium at a concentration of 5×10^6 cells/mL. A 200 μ L cell suspension was then subcutaneously injected into the right flank of nude mice. Tumor growth was monitored on days 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 following injection. The volume of tumors was measured using the formula: length $\times$ width² $\times$ 0.5. Euthanasia was carried out by exposing the mice to carbon dioxide. The percentage of chamber volume replaced by carbon dioxide flow was approximately 50% vol/min.

2.15 LINC01572 and the Tumor Microenvironment

The immune, stromal and ESTIMATE scores are valuable and proven indicators for predicting cancer prognosis [26,27]. The R software package ESTIMATE (<https://bioinformatics.mdanderson.org/estimate/index.html>) (Houston, TX, USA) was used to calculate these scores based on TGGA LUAD data. The relationship between *LINC01572* and immune, stromal and ESTIMATE scores was explored using the psych R package (version 2.1.6). Pearson correlation analysis was used to assess the associations between these factors.

The GSVA package (1.34.0) (Barcelona, Catalonia, Spain) [28] was used to analyze immune cell infiltration in the tumor microenvironment (TME) of LUAD patients by using expression data from TCGA and immunocyte

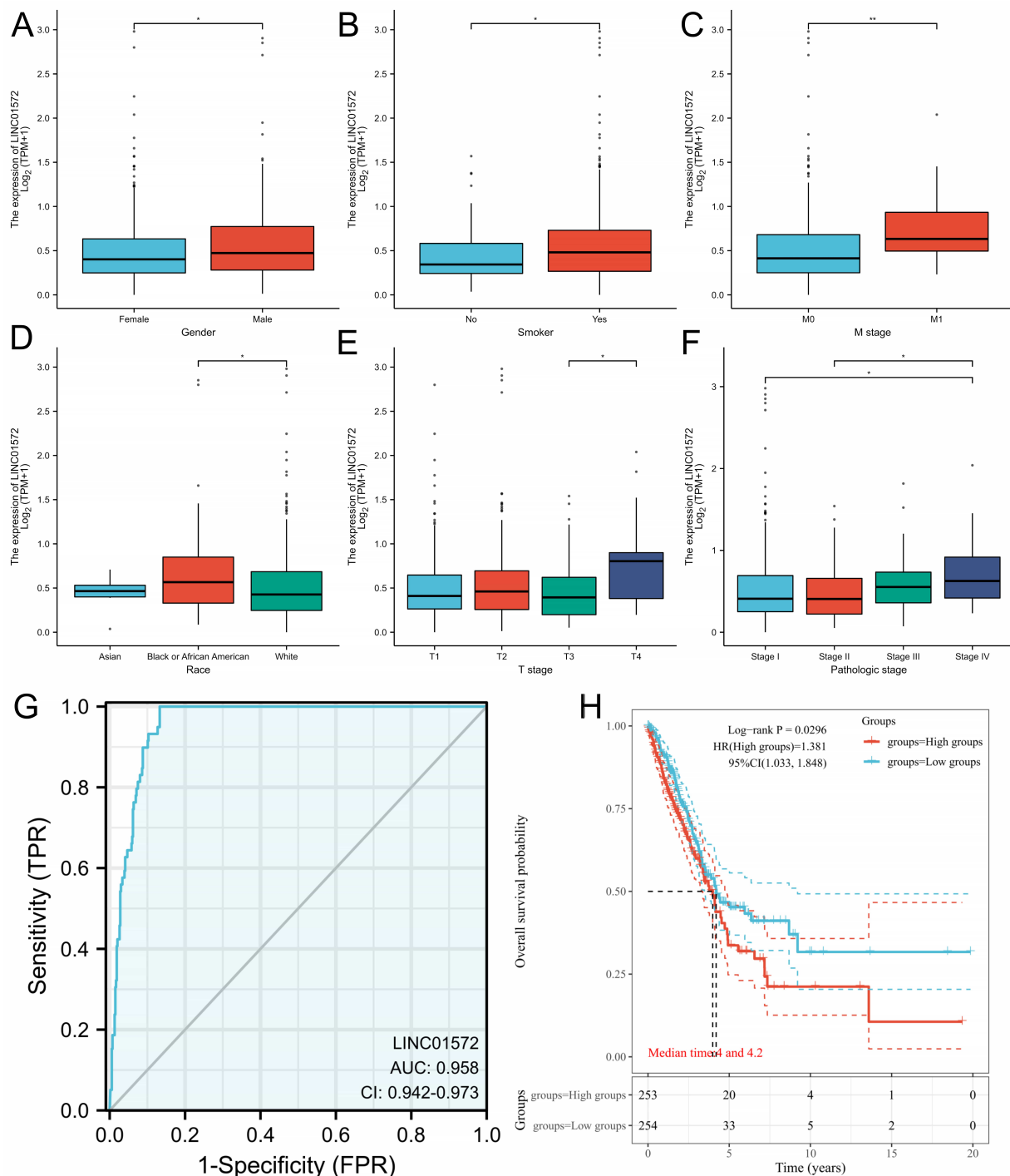


Fig. 2. Correlation analysis between clinical characteristics and *LINC01572* expression. Significantly higher *LINC01572* expression was detected in males (A), smokers (B), patients with metastasis (C), black/African-American patients (D), T4 stage (E), and pathological stage IV (F). The AUC for diagnostic ROC was calculated (G) and Kaplan-Meier survival analysis was conducted using TCGA LUAD data (H). *, $p < 0.05$; **, $p < 0.01$. LINC, Large intergenic non-coding; AUC, Areas under ROC curves; ROC, Receiver operating characteristic.

markers from previous studies [29]. Correlations between *LINC01572* and co-stimulator and co-inhibitor molecules were also investigated using markers obtained from previous studies [30]. The ggplot2 package provides visualization of significant results only.

2.16 Statistical Analysis

R packages were used to construct and analyze survival curves. Cox regression analysis was performed using the “survival” package, and the resulting data was visualized with the “survminer” package. Appropriate sta-

tistical tests including the chi-squared test, Student's *t*-test, Wilcoxon test, and Kruskal–Wallis tests were used to assess the significance of differences between groups. Statistical significance was confirmed with a *p*-value < 0.05.

3. Results

3.1 Overexpression of *LINC01572* and Analysis of Subcellular Localization

The expression of *LINC01572* in NSCLC was investigated using the GSE75037 and GSE88862 datasets. *LINC01572* expression was higher in tumor samples than in matched normal tissues in both LUAD (Fig. 1A) and LUSC (Fig. 1D). In addition, the TCGA data showed significant upregulation of *LINC01572* in LUAD (Fig. 1B) and LUSC (Fig. 1E). Analysis of *LINC01572* expression in the 20 pairs of human tumor-adjacent normal tissue of NSCLC further confirmed the above findings (Fig. 1C).

The subcellular localization of *LINC01572* was predicted by LncLocator based on the characteristics of the *LINC01572* RNA sequence. This showed that *LINC01572* was located in the cytoplasm with a score of 0.6353 (Fig. 1F).

The above findings suggest that *LINC01572* is likely to promote the initiation and development of NSCLC, although further investigation is needed.

3.2 Correlation between *LINC01572* and Clinicopathologic Features, and Prognostic Value

Correlation analyses were performed between the *LINC01572* expression level and various clinicopathological features in order to assess the clinical value of *LINC01572* in LUAD. These showed clear associations between *LINC01572* expression and male gender, smoking, T4 stage, and pathological stage IV (Fig. 2A–F; *p* < 0.05 for each).

Analysis of the diagnostic value of *LINC01572* expression in LUAD revealed the area under the curve (AUC) was 0.958 (95% CI: 0.942–0.973) (Fig. 2G), thereby indicating strong potential as a diagnostic biomarker. Using median expression as the cutoff value, survival analysis showed that LUAD patients with higher expression of *LINC01572* had significantly shorter overall survival (OS) (HR: 1.381; 95% CI: 1.033–1.8481) (Fig. 2H).

In summary, *LINC01572* appears to be a promising and novel biomarker for the diagnosis of LUAD, as well as having prognostic value and being a potential therapeutic target. We next investigated the underlying biological mechanisms of *LINC01572* in LUAD.

3.3 Activated Signaling Pathways and *LINC01572* Expression

The results of GSEA showed that G2M checkpoint and DNA repair signaling pathways were significantly activated in patients with high *LINC01572* expression (Fig. 3A,B). This finding suggests that enhanced tumor cell

proliferation and resistance to DNA damage are the main mechanisms by which *LINC01572* promotes tumor development.

3.4 Correlation between *LINC01572* Expression and Immune Function

The immune response plays an essential role in both tumor progression and elimination. Many different molecules can influence the tumor microenvironment (TME), leading to considerable diversity. Tumor cells can escape or fade depending on whether an immune suppressive TME or an immune-activated TME is dominant.

According to ESTIMATE results, *LINC01572* expression was negatively correlated to stromal (Fig. 3C) and immune (Fig. 3D) scores. This result indicates that upregulation of *LINC01572* leads to an immune suppressive TME. The relationship between *LINC01572* expression and specific immune cell infiltration was further analyzed in the TCGA LUAD cohort using the GSVA method. This showed that high *LINC01572* expression was associated with lower levels of infiltrating T cells (*p* < 0.05), CD8+ T cells (*p* < 0.001), cytotoxic T cells (*p* < 0.001) and DC (*p* < 0.001) (Fig. 3E). These results confirm those of the ESTIMATE analysis and suggest that *LINC01572* could impair the immune response, leading to tumor progression and shorter OS.

Based on the current analysis, *LINC01572* was found to be overexpressed in LUAD, as well as being a prognostic factor. Next, *in vitro* and *in vivo* experiments were conducted to further investigate the role of *LINC01572* in tumor progression.

3.5 *LINC01572* Knockdown and Analysis of Efficacy

The *LINC01572* expression level in NSCLC cell lines was first evaluated under baseline conditions. As shown in Fig. 4A, tumor cells have significantly higher expression of *LINC01572* compared to 16HBE, which is a benign human bronchial epithelial cell line. Among the tumor cell lines investigated here, H1299 and A549 exhibited relatively high overexpression of *LINC01572* and were thus selected for subsequent experiments.

Two small interfering RNAs, *siLINC01572-1* and *siLINC01572-2*, were designed to inhibit the expression of *LINC01572* in A549 and H1299 cells. The knockdown efficiency was determined using RT-qPCR. *LINC01572* expression was found to be significantly downregulated in the intervention groups (Fig. 4B,C). In addition, the two corresponding shRNAs also caused stable and significant reduction of *LINC01572* expression (Fig. 4D).

3.6 *LINC01572* Knockdown Inhibited Cell Proliferation

The effect of *LINC01572* knockdown on cell proliferation was evaluated using CCK-8 assays. Clear reductions in cell proliferation activity were observed in the *LINC01572* knockdown groups compared with the

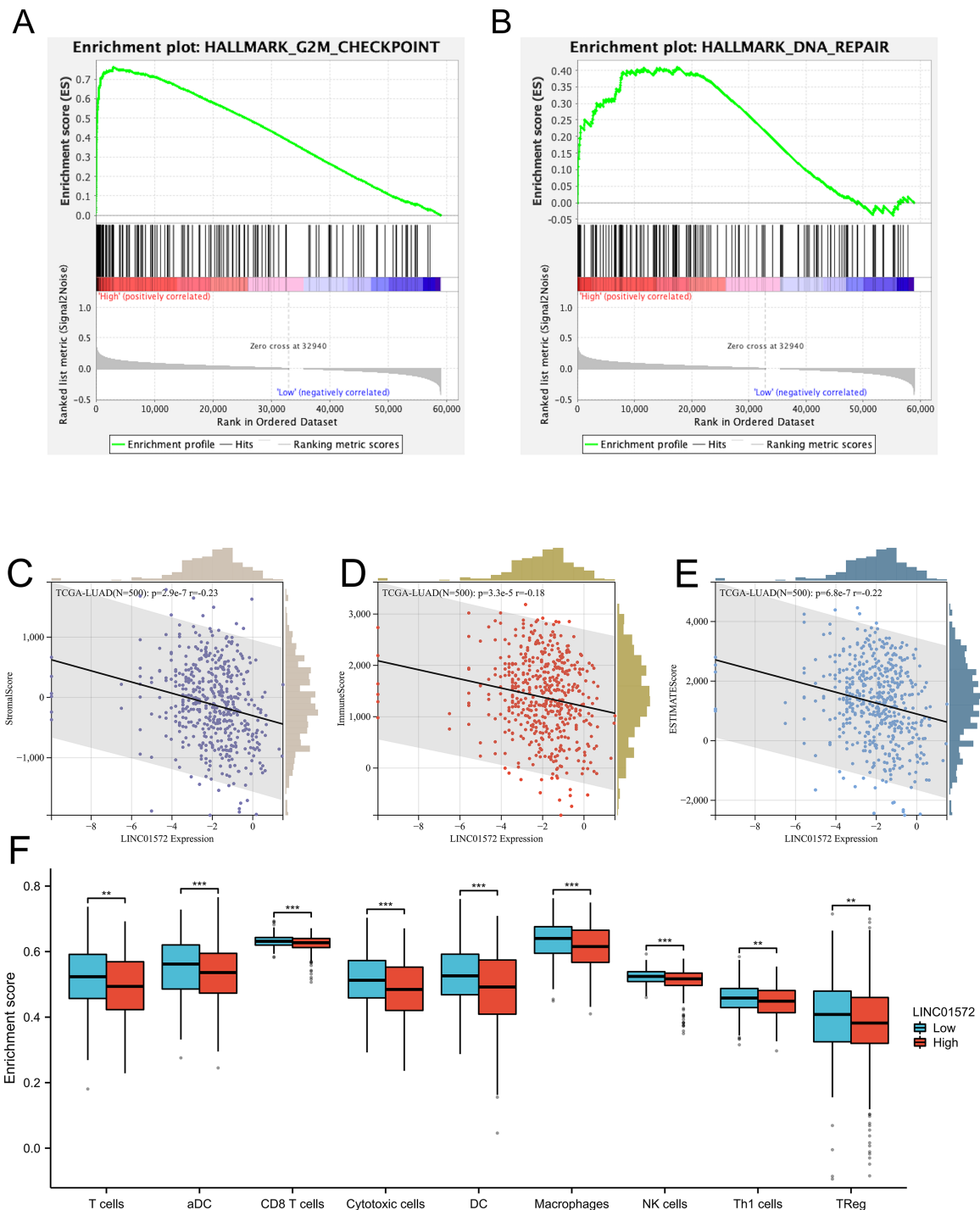


Fig. 3. Analysis of signaling pathway enrichment and of the immune microenvironment. GSEA results showed that G2M checkpoint (A) and DNA repair (B) signaling pathways were significantly activated in samples with high *LINC01572* expression. ESTIMATE analysis indicated that *LINC01572* expression was negatively correlated with local immune cell infiltration (C–E). Lymphocyte infiltration analysis further indicated that fewer cytotoxic and DC cells were present in samples with high *LINC01572* expression (F). ns, no significance. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. aDC, antigen Dendritic Cells; DC, Dendritic Cells; NK cells, Natural killer cells; Th 1, helper T lymphocyte1.

shNC group (Fig. 4E,F). Colony formation assay showed that the number of colonies following transfection with *shLINC01572-1* or *shLINC01572-2* was significantly less

than with shNC for A549 (Fig. 4G,H; $p < 0.05$) and H1299 (Fig. 4I,J; $p < 0.05$) cells.

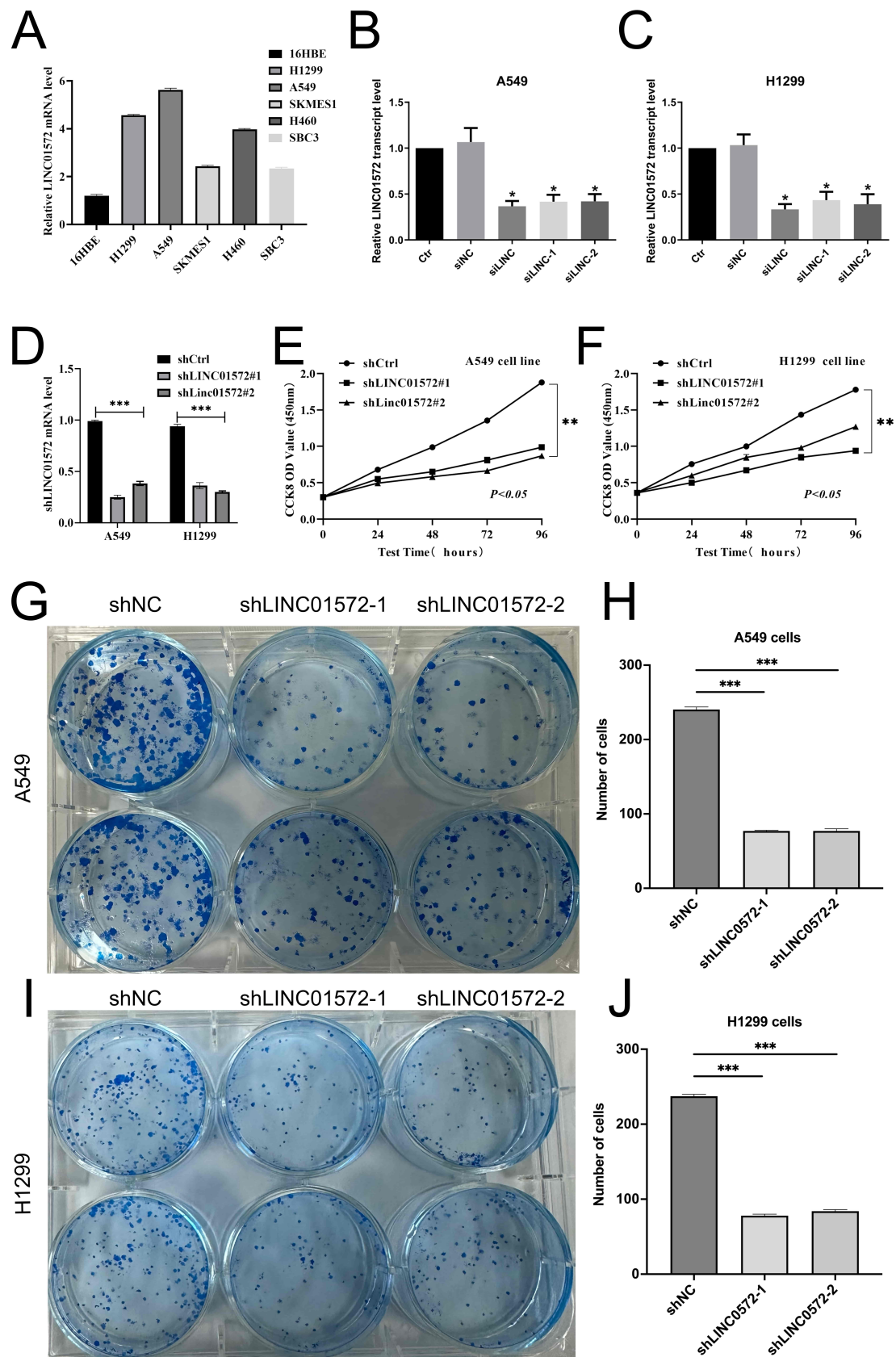


Fig. 4. Abnormal *LINC01572* expression, knockdown efficacy, and colony forming assay results. qPCR analysis of *LINC01572* expression in cell lines (A), and *LINC01572* knockdown efficacy in A549 and H1299 cells (B–D). 96 h CCK-8 assays (E,F) and 2-week colony formation assays (G–J) were also performed using A549 and H1299 cells. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

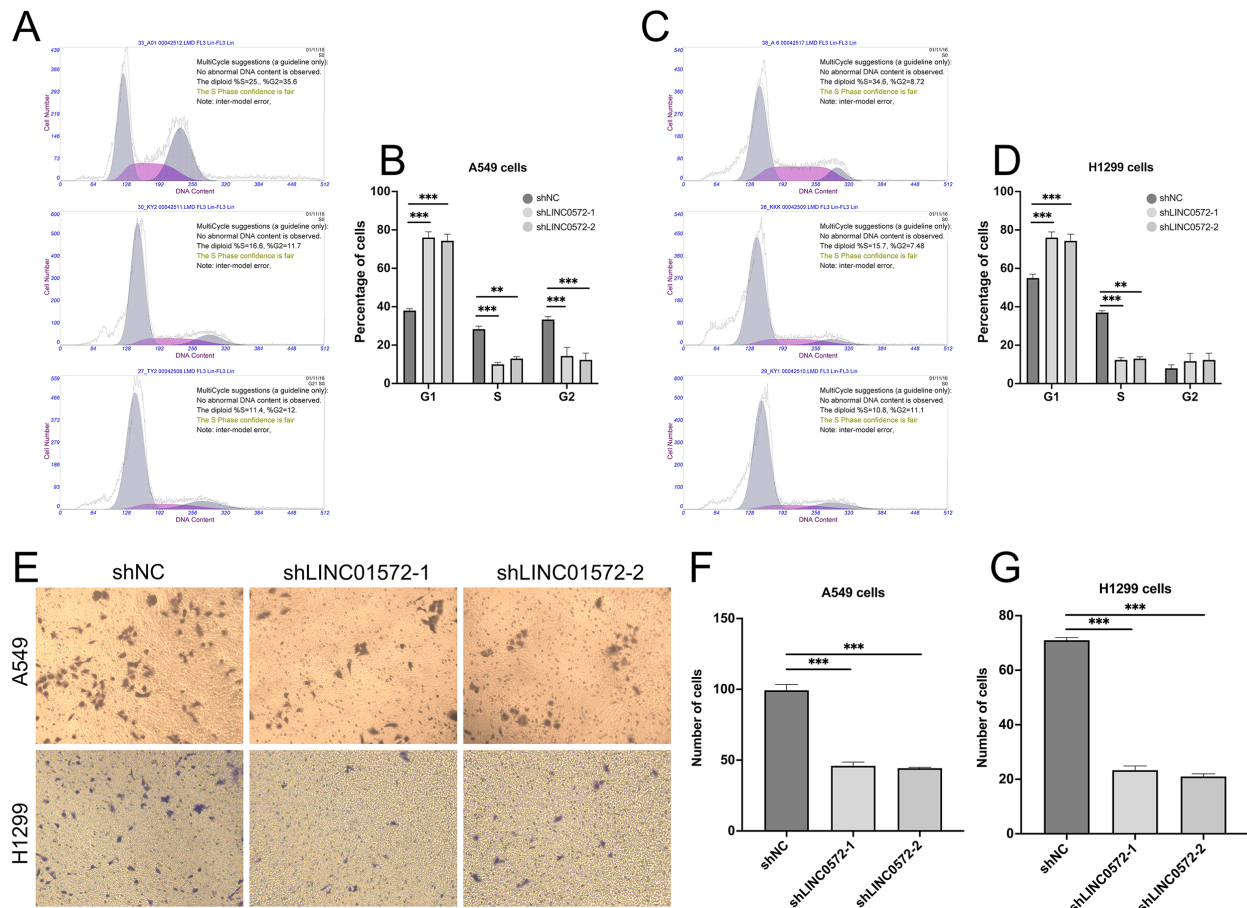


Fig. 5. Assessment of cell cycle distribution and cell migration ability. The proportion of cells in G1 phase was increased, and the proportion in G2 and M phases correspondingly decreased following knockdown of *LINC01572* in A549 (A,B) and H1299 (C,D) cells. Impaired migration ability was observed in the *shLINC01572-1* groups compared to the blank and shNC groups in A549 and H1299 cells (10 $\times$) (E). Cellular quantitative changes in migration ability in A549 cells and H1299 cells (F,G). **, $p < 0.01$; ***, $p < 0.001$.

3.7 *LINC01572* Knockdown Inhibited the Cell Cycle and Cell Migration

Flow cytometry was conducted to evaluate the effect of *LINC01572* knockdown on the cell cycle. Compared to the shNC group, a significant increase was observed in the number of cells in the G1 phase and a corresponding decrease in the number of cells in the S and G2 phases. Knockdown of *LINC01572* resulted in significant changes in the cell cycle distribution in A549 (Fig. 5A,B, $p < 0.05$) and H1299 (Fig. 5C,D, $p < 0.05$) cells.

In addition, the transwell assay showed that the migratory ability of A549 and H1299 cells was significantly reduced in the *LINC01572* knockdown groups compared to both the blank control and shNC-treated groups (Fig. 5E–G; $p < 0.05$).

3.8 *LINC01572* Knockdown Suppressed Tumor Growth in Vivo

To investigate the effects of *LINC01572* knockdown on tumor growth *in vivo*, A549 cells with stable knockdown of *LINC01572* were subcutaneously injected into the right

flank of nude mice (Fig. 6A). Tumor growth was reduced compared to tumors formed after the injection of control cells (Fig. 6B). The final tumor volume and weight were significantly smaller in the *LINC01572* knockdown group compared to the shNC group (Fig. 6C,D; $p < 0.05$). These results confirm that *LINC01572* knockdown significantly inhibited the progression of LUAD *in vivo*.

4. Discussion

Lung cancer is an aggressive tumor type with a high global incidence and mortality rate, especially in the form of NSCLC. Despite recent progress in treatment strategies, the prognosis of advanced lung cancer patients remains unsatisfactory [31]. Therefore, the identification of accurate prognostic biomarkers and novel therapeutic targets is necessary to improve the clinical outcomes of NSCLC patients.

lncRNA is a potentially useful biomarker for cancer diagnosis and treatment and may be a worthwhile avenue for the investigation of tumor growth mechanisms [32]. lncRNA-mediated gene regulation has been implicated in numerous diseases, particularly tumors, and is believed to

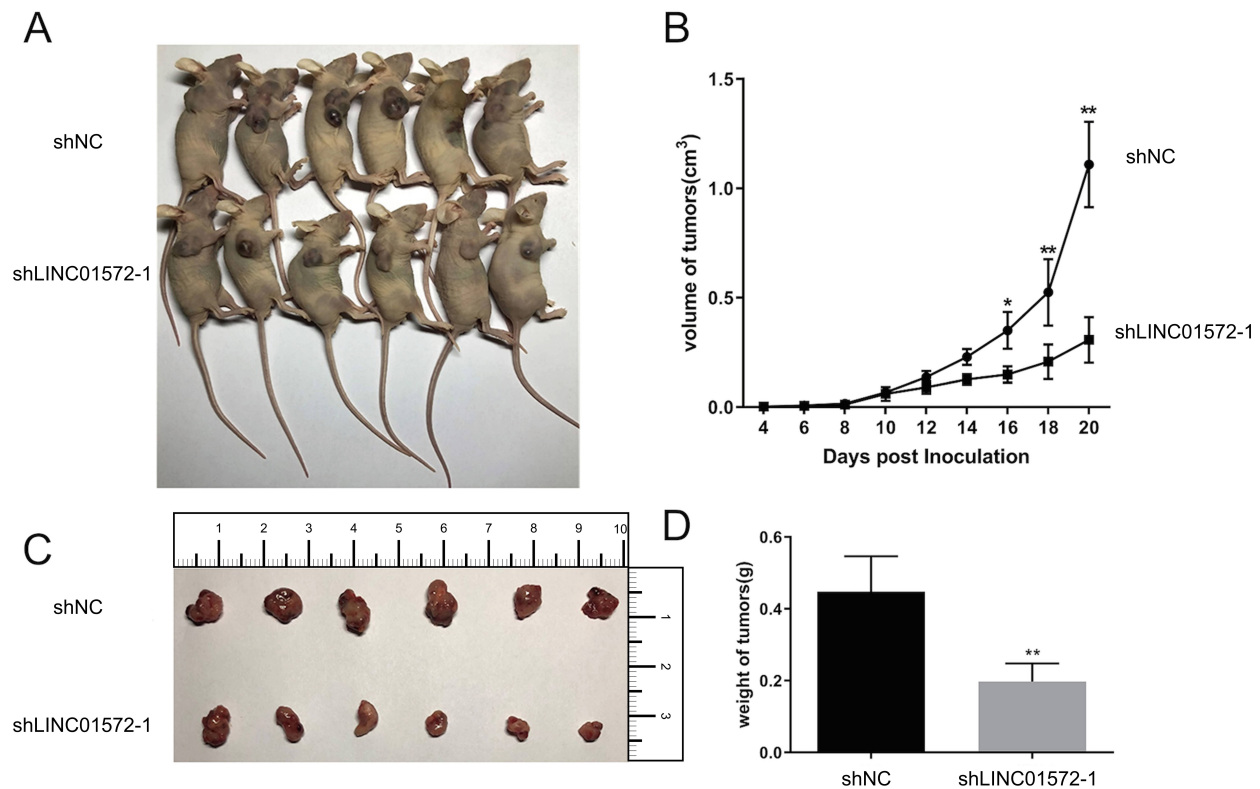


Fig. 6. *In vivo* tumor growth experiments. (A) Representative photographs of subcutaneous tumors formed after injection of A549 cells with *LINC01572* knockdown. (B) Tumor growth in mice over time. (C) Harvested transplant tumors from *LINC01572* knockdown and control mice groups. (D) Weight of the harvested transplant tumors. *, $p < 0.05$; **, $p < 0.01$.

involve several mechanisms. These likely include the interaction of lncRNAs with DNA, RNA or protein at the three different stages of transcriptional, post-transcriptional, and epigenetic regulation. The extensive capacity of lncRNAs to engage with diverse biomolecules has been amply demonstrated and may be important in multiple aspects of tumor development, including cell proliferation, differentiation, metabolism, apoptosis, migration, and drug resistance. Current evidence indicates that lncRNA could participate in many facets of tumor development, such as interaction with cancer-associated-fibroblasts [33]

The lncRNA *LINC01572* has been reported to promote the progression of hepatocellular carcinoma by sponging *miR-195-5p* [34]. It can also induce ferroptosis of LUAD cells, thus suggesting another potential therapeutic use for *LINC01572* [35]. However, there are still very few studies on *LINC01572* in LUAD. Data obtained from a public database and from the tissues of NSCLC patients show upregulation of *LINC01572* in tumor samples. Subsequent analysis in combination with clinical characteristics also showed that *LINC01572* expression was associated with higher tumor stage and shorter OS. These results indicate that *LINC01572* is likely to play an essential role in the initiation and development of LUAD.

We also used a GSEA algorithm to investigate downstream signaling pathways that are activated by

LINC01572. This showed that G2M checkpoint and DNA repair signaling pathways were activated in tumor cells with high expression of *LINC01572*. The purpose of the G2M checkpoint is to ensure the DNA is correctly replicated before the cell enters mitosis. In the event of errors, the G2M checkpoint prevents the cell from progressing to the next phase of the cell cycle, thereby allowing time for DNA repair. *LINC01572* may simultaneously promote cell proliferation and DNA repair. Previous studies have reported that the G2M checkpoint and DNA repair signaling pathways play crucial roles in tumor promotion [36–39]. The present results suggest that the shorter OS and more advanced tumor stage observed in cases with high *LINC01572* expression could be caused by increased tumor cell proliferation and DNA damage repair.

The TME is considered an important index for the optimization of treatment strategies and for its influence on tumor initiation and elimination [40,41]. We therefore analyzed the correlation between *LINC01572* expression and immunity using the ESTIMATE and GSVA algorithms. *LINC01572* expression was clearly associated with an immune suppressive TME, as seen by its negative correlation with the infiltration of cytotoxic T cells, DC and macrophages, and thereby resulting in shorter OS.

Based on these interesting findings, we conducted further *in vitro* and *in vivo* experiments to confirm the re-

sults and to explore possible downstream mechanisms. The *in vitro* assay results showed that *LINC01572* expression was generally higher in tumor cell lines than in the 16HBE cell line. In addition, low cell activity after 96 h was detected with the CCK-8 assay in *shLINC01572* groups. The colony formation assay also showed that less colonies were observed in the *shLINC01572-1* and *shLINC01572-2* groups compared to control groups. Flow cytometry was performed to investigate whether low expression of *LINC01572* in tumor cells could affect the cell cycle. Compared to the shNC group, the proportion of cells in the G1 phase was significantly increased, and the proportion of cells in the G2 phase was significantly decreased in the *shLINC01572-1* groups of A549 and H1299 cells. Transwell assays were also performed to evaluate the migration ability of tumor cells. These showed that cell migration was significantly reduced in the *shLINC01572-1* groups of A549 and H1299 cells compared with the blank and shNC groups.

The *in vivo* growth rates in terms of tumor volume and weight were found to be lower in mice from the *shLINC01572-1* group compared to the shNC group. This result suggests that blocking *LINC01572* in tumor cells may reverse cancerous growth.

One of the limitations of this study was that the downstream mechanisms for *LINC01572* in tumor initiation and progression are still unclear, including for example its interactions with miRNA and mRNA. Further detailed investigations are needed to clarify this issue.

The results of multi-omics analysis and other experiments showed that *LINC01572* is overexpressed in tumor samples and may promote tumor initiation and progression by influencing the cell cycle and DNA repair. Together with the suppression of immunity in the TME, these factors could all contribute to the worse outcome observed for LUAD patients with high *LINC01572* expression. In conclusion, *LINC01572* may be an effective diagnostic biomarker, as well as a promising therapeutic biomarker for LUAD.

5. Conclusions

LINC01572 is overexpressed in tumor tissue relative to adjacent normal tissue. *LINC01572* is associated with tumor initiation, progression and immune dysregulation. It therefore has potential value as a novel biomarker and therapeutic target in LUAD.

Availability of Data and Materials

Bioinformatics datasets presented in this study can be found in online repositories. And the datasets used and/or analyzed during experiments are available from the corresponding author on reasonable request.

Author Contributions

DW, JC, LL and YZ conceived the study. YZ, ZZ took part in Writing original draft. YZ, ZZ, LW and XYL designed and carried out experiments. YZ, XYL, LW, XLL and LL collected and provided formal data analysis. DW, JC, LL and XYL took part in reviewing & editing the article. DW, LL, and XLL performed validation of the data and provided funding acquisition. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Experimental Animal Ethics Committee, Nanjing Hospital Affiliated to Nanjing Medical University has approved our related experiments. The approval number(s)/ID(s) is DWSY-23047355. The use of human tissues conformed to the guidelines of the Declaration of Helsinki, and informed consent was obtained from all subjects involved in the study.

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Conflict of Interest

The authors declare no conflict of interest.

References

- [1] Mao Y, Yang D, He J, Krasna MJ. Epidemiology of Lung Cancer. *Surgical Oncology Clinics of North America*. 2016; 25: 439–445.
- [2] Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, *et al.* Cancer statistics in China, 2015. *A Cancer Journal for Clinicians*. 2016; 66: 115–132.
- [3] Takamochi K, Ohmiya H, Itoh M, Mogushi K, Saito T, Hara K, *et al.* Novel biomarkers that assist in accurate discrimination of squamous cell carcinoma from adenocarcinoma of the lung. *BMC Cancer*. 2016; 16: 760.
- [4] Huang T, Li J, Zhang C, Hong Q, Jiang D, Ye M, *et al.* Distinguishing Lung Adenocarcinoma from Lung Squamous Cell Carcinoma by Two Hypomethylated and Three Hypermethylated Genes: A Meta-Analysis. *PLoS ONE*. 2016; 11: e0149088.
- [5] Pao W, Girard N. New driver mutations in non-small-cell lung cancer. *The Lancet Oncology*. 2011; 12: 175–180.
- [6] Hildebrandt MAT, Gu J, Wu X. Pharmacogenomics of platinum-based chemotherapy in NSCLC. *Expert Opinion on Drug Metabolism & Toxicology*. 2009; 5: 745–755.
- [7] Sacco JJ, Al-Akhrass H, Wilson CM. Challenges and Strategies in Precision Medicine for Non-Small-Cell Lung Cancer. *Current Pharmaceutical Design*. 2016; 22: 4374–4385.
- [8] Sattler M, Mambetsariev I, Fricke J, Tan T, Liu S, Vaidehi N, *et al.* A Closer Look at *EGFR* Inhibitor Resistance in Non-Small

Cell Lung Cancer through the Lens of Precision Medicine. *Journal of Clinical Medicine*. 2023; 12: 1936.

- [9] Chen MF, Chaffa JE. Early-stage anaplastic lymphoma kinase (ALK)-positive lung cancer: a narrative review. *Translational Lung Cancer Research*. 2023; 12: 337–345.
- [10] Król K, Mazur A, Stachyra-Strawa P, Grzybowska-Szatowska L. Non-Small Cell Lung Cancer Treatment with Molecularly Targeted Therapy and Concurrent Radiotherapy-A Review. *International Journal of Molecular Sciences*. 2023; 24: 5858.
- [11] Vaseq R, Sharma A, Li Y, Schmidt-Wolf IGH. Revising the Landscape of Cytokine-Induced Killer Cell Therapy in Lung Cancer: Focus on Immune Checkpoint Inhibitors. *International Journal of Molecular Sciences*. 2023; 24: 5626.
- [12] Kemper M, Krekeler C, Menck K, Lenz G, Evers G, Schulze AB, *et al.* Liquid Biopsies in Lung Cancer. *Cancers*. 2023; 15: 1430.
- [13] Zhang N, Lei T, Xu T, Zou X, Wang Z. Long noncoding RNA *SNHG15*: A promising target in human cancers. *Frontiers in Oncology*. 2023; 13: 1108564.
- [14] Zhong C, Xie Z, Zeng LH, Yuan C, Duan S. *MIR4435-2HG* Is a Potential Pan-Cancer Biomarker for Diagnosis and Prognosis. *Frontiers in Immunology*. 2022; 13: 855078.
- [15] Li H, Gao J, Liu L, Zhang S. *LINC00958*: A promising long non-coding RNA related to cancer. *Biomedicine & Pharmacotherapy*. 2022; 151: 113087.
- [16] Chaturvedi A, Som A. The LCNetWork: An electronic representation of the mRNA-lncRNA-miRNA regulatory network underlying mechanisms of non-small cell lung cancer in humans, and its explorative analysis. *Computational Biology and Chemistry*. 2022; 101: 107781.
- [17] Djebali S, Davis CA, Merkel A, Dobin A, Lassmann T, Mortazavi A, *et al.* Landscape of transcription in human cells. *Nature*. 2012; 489: 101–108.
- [18] Lu T, Wang Y, Chen D, Liu J, Jiao W. Potential clinical application of lncRNAs in non-small cell lung cancer. *OncoTargets and Therapy*. 2018; 11: 8045–8052.
- [19] Li G, Zhang H, Wan X, Yang X, Zhu C, Wang A, *et al.* Long noncoding RNA plays a key role in metastasis and prognosis of hepatocellular carcinoma. *BioMed Research International*. 2014; 2014: 780521.
- [20] Wilusz JE, Sunwoo H, Spector DL. Long noncoding RNAs: functional surprises from the RNA world. *Genes & Development*. 2009; 23: 1494–1504.
- [21] Chen WJ, Tang RX, He RQ, Li DY, Liang L, Zeng JH, *et al.* Clinical roles of the aberrantly expressed lncRNAs in lung squamous cell carcinoma: a study based on RNA-sequencing and microarray data mining. *Oncotarget*. 2017; 8: 61282–61304.
- [22] Song Z, Jia N, Li W, Zhang XY. *LINC01572* Regulates Cisplatin Resistance in Gastric Cancer Cells by Mediating miR-497-5p. *OncoTargets and Therapy*. 2020; 13: 10877–10887.
- [23] Volders PJ, Anckaert J, Verheggen K, Nuytens J, Martens L, Mestdagh P, *et al.* LNCipedia 5: towards a reference set of human long non-coding RNAs. *Nucleic Acids Research*. 2019; 47: D135–D139.
- [24] Cao Z, Pan X, Yang Y, Huang Y, Shen HB. The lncLocator: a subcellular localization predictor for long non-coding RNAs based on a stacked ensemble classifier. *Bioinformatics*. 2018; 34: 2185–2194.
- [25] Liu J, Lichtenberg T, Hoadley KA, Poisson LM, Lazar AJ, Cherniack AD, *et al.* An Integrated TCGA Pan-Cancer Clinical Data Resource to Drive High-Quality Survival Outcome Analytics. *Cell*. 2018; 173: 400–416.e11.
- [26] Becht E, Giraldo NA, Lacroix L, Buttard B, Elarouci N, Petitprez F, *et al.* Estimating the population abundance of tissue-infiltrating immune and stromal cell populations using gene expression. *Genome Biology*. 2016; 17: 218.
- [27] Yoshihara K, Shahmoradgoli M, Martínez E, Vegesna R, Kim H, Torres-García W, *et al.* Inferring tumour purity and stromal and immune cell admixture from expression data. *Nature Communications*. 2013; 4: 2612.
- [28] Hänzelmann S, Castelo R, Guinney J. GSEA: gene set variation analysis for microarray and RNA-seq data. *BMC Bioinformatics*. 2013; 14: 7.
- [29] Bindea G, Mlecnik B, Tosolini M, Kirilovsky A, Waldner M, Obenauf AC, *et al.* Spatiotemporal dynamics of intratumoral immune cells reveal the immune landscape in human cancer. *Immunity*. 2013; 39: 782–795.
- [30] Thorsson V, Gibbs DL, Brown SD, Wolf D, Bortone DS, Ou Yang TH, *et al.* The Immune Landscape of Cancer. *Immunity*. 2018; 48: 812–830.e14.
- [31] Herbst RS, Morgensztern D, Boshoff C. The biology and management of non-small cell lung cancer. *Nature*. 2018; 553: 446–454.
- [32] Spizzo R, Almeida MI, Colombatti A, Calin GA. Long non-coding RNAs and cancer: a new frontier of translational research? *Oncogene*. 2012; 31: 4577–4587.
- [33] Ti W, Wang J, Cheng Y. The Interaction Between Long Non-Coding RNAs and Cancer-Associated Fibroblasts in Lung Cancer. *Frontiers in Cell and Developmental Biology*. 2022; 9: 714125.
- [34] Lai S, Quan Z, Hao Y, Liu J, Wang Z, Dai L, *et al.* Long Non-Coding RNA *LINC01572* Promotes Hepatocellular Carcinoma Progression via Sponging *miR-195-5p* to Enhance PFKFB4-Mediated Glycolysis and PI3K/AKT Activation. *Frontiers in Cell and Developmental Biology*. 2021; 9: 783088.
- [35] Hong L, Wang X, Cui W, Wang F, Shi W, Yu S, *et al.* Construction of a ferroptosis scoring system and identification of *LINC01572* as a novel ferroptosis suppressor in lung adenocarcinoma. *Frontiers in Pharmacology*. 2023; 13: 1098136.
- [36] Bradbury A, Hall S, Curtin N, Drew Y. Targeting ATR as Cancer Therapy: A new era for synthetic lethality and synergistic combinations? *Pharmacology & Therapeutics*. 2020; 207: 107450.
- [37] Engeland K. Cell cycle arrest through indirect transcriptional repression by p53: I have a DREAM. *Cell Death and Differentiation*. 2018; 25: 114–132.
- [38] Abuetab H, Wu HH, Chai C, Al Yousef H, Persad S, Sergi CM, *et al.* DNA damage response revisited: the p53 family and its regulators provide endless cancer therapy opportunities. *Experimental & Molecular Medicine*. 2022; 54: 1658–1669.
- [39] Mandal J, Mandal P, Wang TL, Shih IM. Treating ARID1A mutated cancers by harnessing synthetic lethality and DNA damage response. *Journal of Biomedical Science*. 2022; 29: 71.
- [40] Wu Y, Yi M, Niu M, Mei Q, Wu K. Myeloid-derived suppressor cells: an emerging target for anticancer immunotherapy. *Molecular Cancer*. 2022; 21: 184.
- [41] Bai R, Li Y, Jian L, Yang Y, Zhao L, Wei M. The hypoxia-driven crosstalk between tumor and tumor-associated macrophages: mechanisms and clinical treatment strategies. *Molecular Cancer*. 2022; 21: 177.