Observational Study

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Long noncoding RNA *LINC00921* serves as a predictive biomarker for lung adenocarcinoma

An observational study

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Abstract

Lung adenocarcinoma (LUAD) is usually diagnosed at advanced stages. Hence, there is an urgent need to seek an effective biomarker to predict LUAD status. Long noncoding RNAs (lncRNAs) play key roles in the development of tumors. However, the relationship between *LINC00921* and LUAD remains unclear. The gene expression data of LUAD were downloaded from the Cancer Genome Atlas database to investigate the expression level of *LINC00921* in LUAD. Diagnostic ability analysis, survival analysis, tumor mutational burden analysis, and immune cell infiltration analysis of *LINC00921* in LUAD patients were performed simultaneously. According to the median expression value of *LINC00921*, patients were divided into *LINC00921* high- and low-expression groups. The function of *LINC00921* in LUAD was identified through difference analysis and enrichment analysis. Moreover, drugs that may be relevant to LUAD treatment were screened. Finally, blood samples were collected for real-time polymerase chain reaction. *LINC00921* was significantly lower in LUAD tumor tissues. Notably, patients with low expression of *LINC00921* had a shorter median survival time. Decreased immune cell infiltration in the tumor microenvironment in the low *LINC00921* expression group may contribute to poorer patient outcomes. Tumor mutational burden was significantly different in survival between the *LINC00921* high- and low-expression groups. In addition, *LINC00921* may exert an influence on cancer development through its regulation of target genes transcription. Glyceraldehyde-3-phosphate dehydrogenase-related drugs may be more likely to be therapeutically effective in LUAD. *LINC00921* was able to be used as the potential diagnostic indicator for LUAD.

Abbreviations: Cor = Pearson correlation coefficient, DEG = differentially expressed gene, FDR = false discovery rate, lncRNA = long noncoding RNA, LUAD = lung adenocarcinoma, NSCLC = nonsmall cell lung cancer, GO = gene ontology, PCG = protein-coding gene, ROC = receiver operating characteristic, SCLC = small cell lung cancer, ssGSEA = single-sample gene set enrichment analysis, TCGA = the Cancer Genome Atlas, TIME = tumor immune microenvironment, TMB = tumor mutational burden.

Keywords: biomarkers, *LINC00921*, lncRNAs, lung adenocarcinoma, prognostic diagnosis

1. Introduction

Lung cancer is widely recognized as the most prevalent form of cancer globally.[1] Nonsmall cell lung cancer (NSCLC) constitutes the majority, approximately 85%, of primary lung cancer cases.[2] Lung adenocarcinoma (LUAD), with characterization of high metastasis and mortality, is a dominating subtype of NSCLC, and its incidence has been steadily increasing over the years.[3] It is imperative to acknowledge that LUAD exhibits significant variations in pedigree. Recent studies have shown that individuals of East Asian ancestry with LUAD exhibit a comparatively more stable genome than individuals of European ancestry.[4] Moreover, smokers have a higher prevalence of LUAD.

An estimated 1.4 million lung cancer patients die from lung cancer each year, the highest mortality rate among all types of cancer.[2] The primary factor contributing to the elevated mortality rate is the advanced stage of lung cancer diagnosis in the majority of patients.[5,6] Some prognostic methods exhibit restricted efficacy, and achieving early detection continues to be a challenging objective. Recently, there has been significant progress in our understanding of tumor biology due to advancements in biomedical technology.[7]

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The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

All data are available in the article.

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Long noncoding RNAs (lncRNAs) play a crucial role in tumorigenesis, cancer progression, and prognosis.[8,9] Studies have shown that lncRNAs can participate in cancer progression by affecting cell proliferation, differentiation, and apoptosis.[10–13] A related study reported that lncRNA PTAR was significantly upregulated in mesenchymal subtype samples.[14] In epithelial ovarian cancer tissues, the expression of lncRNA ABHD11-AS1 was higher and exhibited a positive correlation with tumor stage.[15] At present, a variety of lncRNAs have been reported to be involved in the occurrence and progression of LUAD.[16,17] lncRNAs show great potential in the clinical application of LUAD diagnosis. For example, they can serve as tumorigenesis biomarkers to predict prognostic features.[18]

LINC00921 is a novel lncRNA, and studies have shown that *LINC00921* regulates tumorigenesis and epithelial–mesenchymal transition in triple-negative breast cancer.[19] In addition, *LINC00921* may be a potential marker for distinguishing malignant cancers from benign biliary diseases.[20] However, there are no studies reporting the potential of *LINC00921* in predicting LUAD. Here, we analyzed publicly sequencing data to investigate the expression level of *LINC00921* in LUAD. We used comparative analysis, differential analysis, survival analysis, and functional analysis to comprehensively and deeply analyze the relationship between *LINC00921* and LUAD and evaluated its potential as a prognostic marker for LUAD.

2. Materials and methods

2.1 Data collection and preprocessing

To understand the gene expression signature of LUAD, we downloaded gene expression profiles and clinical information from the Cancer Genome Atlas (TCGA) database. The TCGA-LUAD data were obtained from University of California, Santa Cruz Xena (https://gdc.xenahubs.net). The TCGA-LUAD data were processed according to the following process: the samples with no clinical follow-up information, unknown and less than 0 days survival time, and no survival status were removed; the probes were transferred to Gene Symbol, removing those that corresponded to more than one gene; the median of expression profiles with multiple gene symbols were taken. Finally, a total of 59 paracancerous and 513 cancer tissue samples were included in this study.

2.2 lncRNAs selection in LUAD

Our previous study suggested that *SATB1* was involved in the development of LUAD.[21] A total of 166 miRNAs interacted with *SATB1* were obtained with the encyclopedia of RNA interactomes (http://starbase.sysu.edu.cn/index.php).[22] The LUAD-related miRNA dataset was selected to obtain differentially expressed miRNAs with false discovery rate (FDR) < 0.05, and $|\log_2$ fold change (FC)| > 0.5. Then, 2 differentially expressed

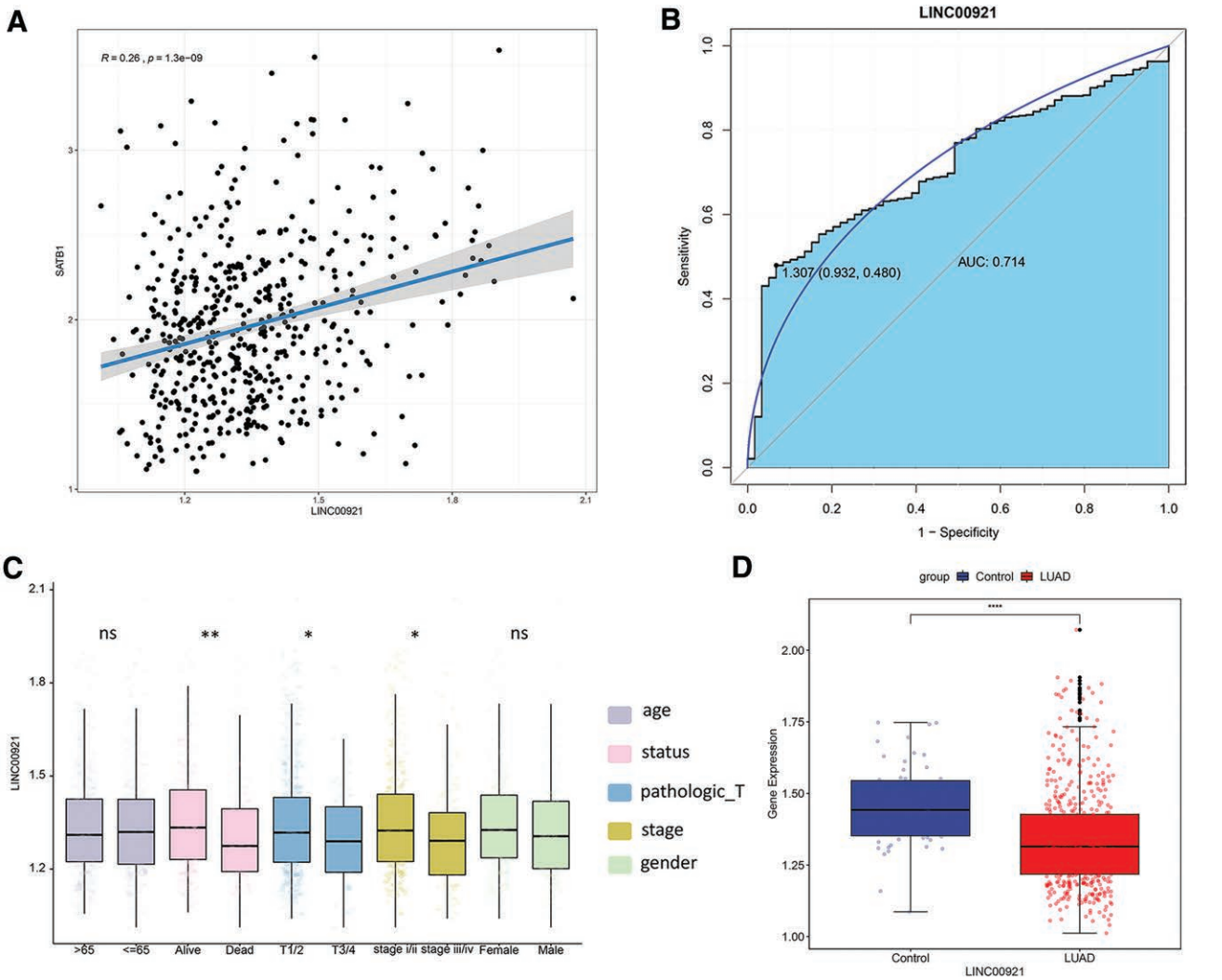

Figure 1. Characteristics of *LINC00921* in LUAD. (A) The dot plot showed the correlation between *LINC00921* and *SATB1*. (B) The expression level of *LINC00921* in LUAD patients. (C) The ROC curve for *LINC00921*. Ns, not significant; * and ** represent $P < .05$ and $P < .01$, respectively. (D) Expression level of *LINC00921* in different clinical variables. **** represents $P < .0001$. LUAD = lung adenocarcinoma, ROC = receiver operating characteristic.

miRNAs were found to reverse regulate the expression of *SATB1*, *hsa-miR-217*, and *hsa-miR-449a*. Based on the encyclopedia of RNA interactomes, 209 lncRNAs were found that may interact with these 2 miRNAs. Among them, 4 lncRNAs were found to be correlated with the overall survival of LUAD. Correlation analysis of these 4 lncRNAs with *SATB1* showed that *LINC00921* had the highest correlation with *SATB1*. Therefore, *LINC00921* was selected for further study.

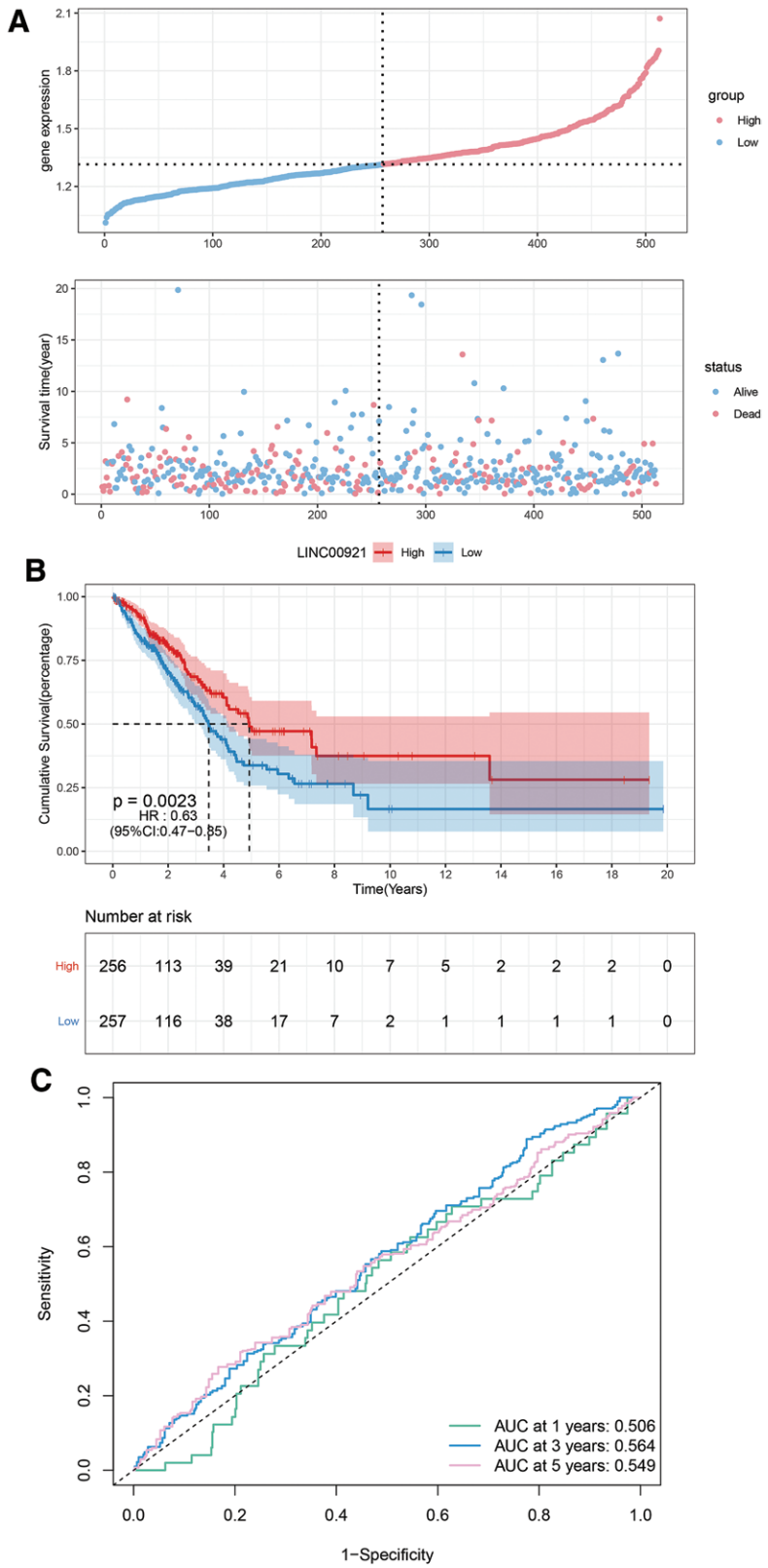

Figure 2. Survival analysis of *LINC00921* in LUAD. (A) Scatter plot showed the risk factor in different *LINC00921* expression group in LUAD patients. (B) The survival curve in different *LINC00921* expression group in LUAD patients. (C) Time-dependent ROC curve of *LINC00921* expression predicting LUAD overall survival. LUAD = lung adenocarcinoma, ROC = receiver operating characteristic.

2.3 Diagnostic efficacy analysis of LINC00921 in LUAD

The expression of *LINC00921* between LUAD tissues and paracancerous tissues was determined using the Wilcoxon test analysis. Then, the expression of *LINC00921* in different groups with various clinical indicators (such as age, gender, stage, T stage, and so on) was analyzed using T test. The pROC was

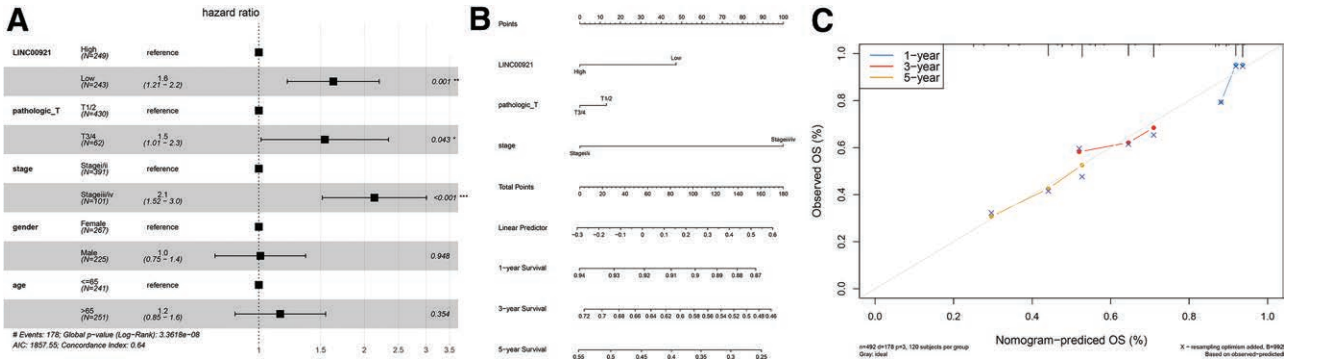

Figure 3. The relationship between *LINC00921* and clinical features in LUAD. (A) Multivariate Cox analysis of clinical characteristics and *LINC00921.* (B) The nomograph of clinical features and risk score. (C) The calibration chart of 1, 3, 5 years in nomogram.

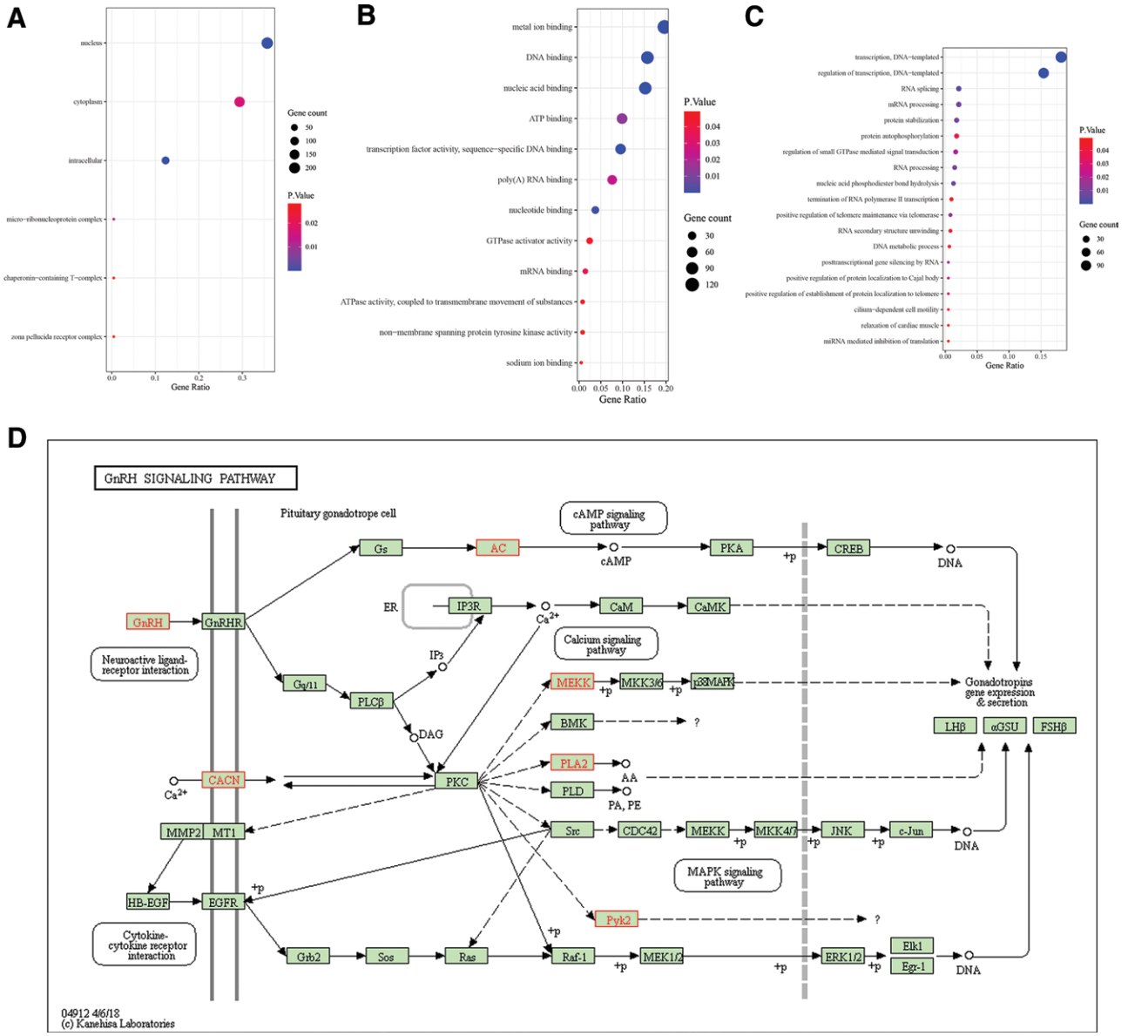

Figure 4. Function analysis of *LINC00921* in LUAD. (A–C) The GO analysis of *LINC00921,* respectively, represents 3 levels in turn, namely, cellular component, molecular function, and biological process. (D) KEGG analysis of *LINC00921.* GO = gene ontology, KEGG = Kyoto encyclopedia of genes and genomes.

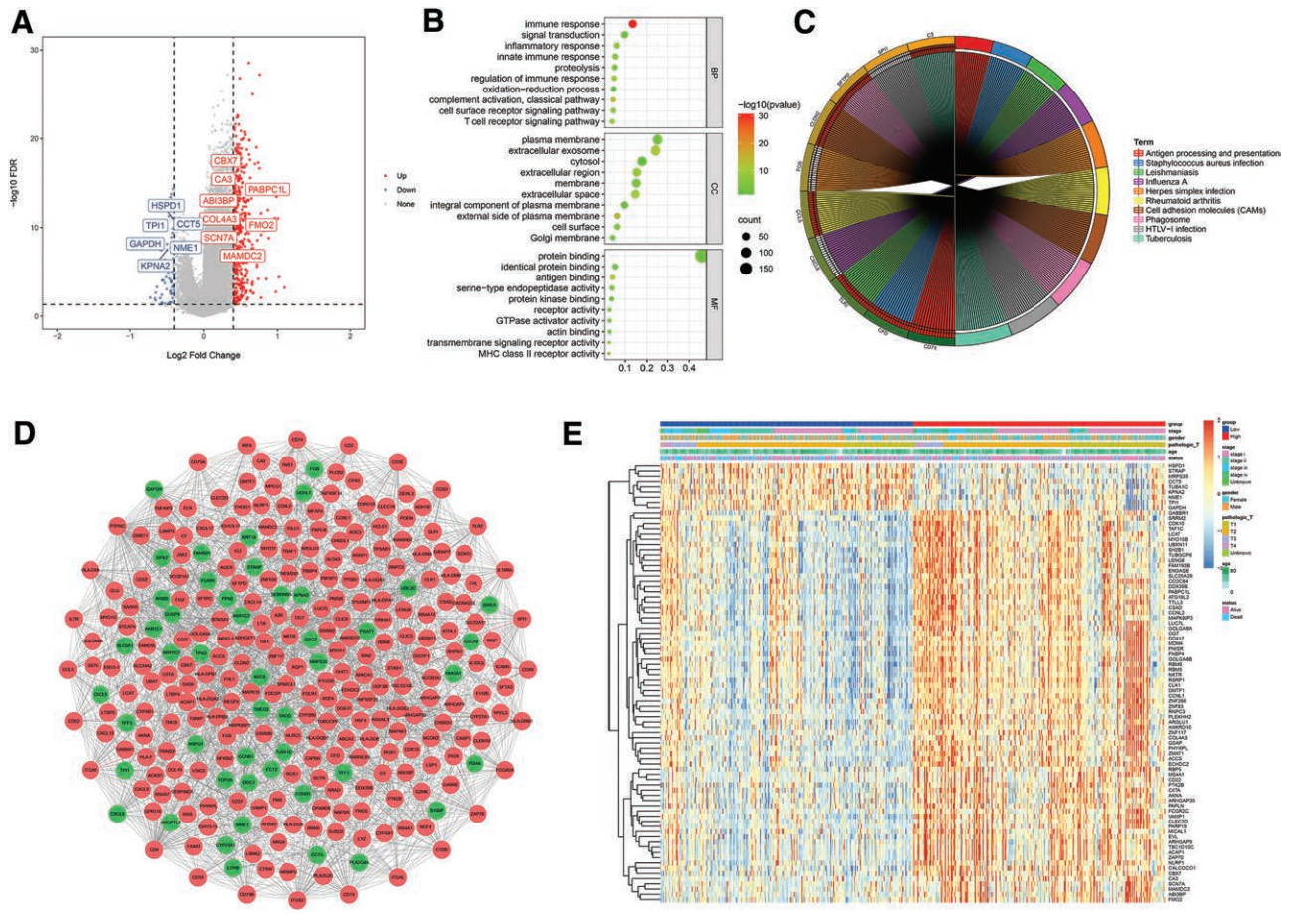

Figure 5. Differential expression analysis of different *LINC00921* expression level groups in LUAD. (A) The DEGs in different *LINC00921* expression level groups. (B) The GO analysis of DEGs in different *LINC00921* expression level groups. (C) KEGG analysis of DEGs in different *LINC00921* expression level groups. (D) The PPI network of DEGs in different *LINC00921* expression level groups. (E) The heatmap showed the expression trends of the intersection genes of DEGs and *LINC00921* coexpressed genes. DEGs = differentially expressed genes, GO = gene ontology, KEGG = Kyoto encyclopedia of genes and genomes, LUAD = lung adenocarcinoma, PPI = protein–protein interaction.

selected to perform the receiver operating characteristic (ROC) curve of *LINC00921*.[23]

2.4 Survival analysis of LINC00921 in LUAD

According to the median expression value of *LINC00921*, patients were divided into *LINC00921* high- and low-expression groups. Survival analysis was performed using Kaplan–Meier and log-rank tests,[24] the univariate and multivariate Cox proportional hazards regression models. Time-dependent ROC curves were performed by the survival package in R to assess the accuracy of LINC00921 in predicting LUAD prognosis. The rms package in R was utilized to evaluate the contribution of *LINC00921* expression to LUAD prognosis.[25] Further, the predictive value of the combination of *LINC00921* expression and clinical parameters for the prognosis of LUAD was assessed by combined effect survival analysis.

2.5 Enrichment analysis of coexpressed genes with LINC00921 in LUAD

It is well known that lncRNAs are noncoding RNAs that participate in regulating protein-coding genes (PCGs) in the posttranscriptional level. We used Pearson correlation coefficient (Cor) to identify *LINC00921*-related PCGs with $P < .05$ and $|Cor| > 0.4$. Functional enrichment analysis of these *LINC00921*-related PCGs was performed with the Database of Annotation, Visualization, and Integrated Discovery (v6.8) with $P < .05$.[26]

2.6 Identification of differentially expressed genes and enrichment analysis

To reveal the prognostic mechanism of *LINC00921* in LUAD, we used the limma (R package) to identify the differentially expressed genes (DEGs) between the *LINC00921* high- and low-expression groups with $|\log_2 FC| \geq 0.4$ and FDR < 0.05.[27] Database of Annotation, Visualization, and Integrated Discovery (v6.8) was utilized to identify biological functions and pathways between *LINC00921* high- and low-expression groups. Moreover, the search tool for recurring instances of neighbouring genes was used to reveal the protein–protein interaction (PPI) of these DEGs.

2.7 Drug selection of DEGs in LUAD

To explore drugs that may be related to LUAD treatment, we used the limma (R package) to identify DEGs between tumor tissues of LUAD patients and controls.[27] $|\log_2 FC| \geq 1$ and FDR < 0.05 was determined as drug-related DEGs. By intersecting these DEGs with the DEGs in the *LINC00921* high- and low-expression group and the PCGs related to *LINC00921*, the potential drugs that may target these intersecting genes were acquired by searching in the Drug-Gene Interaction Database (https://dgidb.org/).[28]

2.8 Tumor immune microenvironment infiltration analysis in LUAD

The single-sample gene set enrichment analysis (ssGSEA) was used to quantify the relative abundance of each cell infiltrate

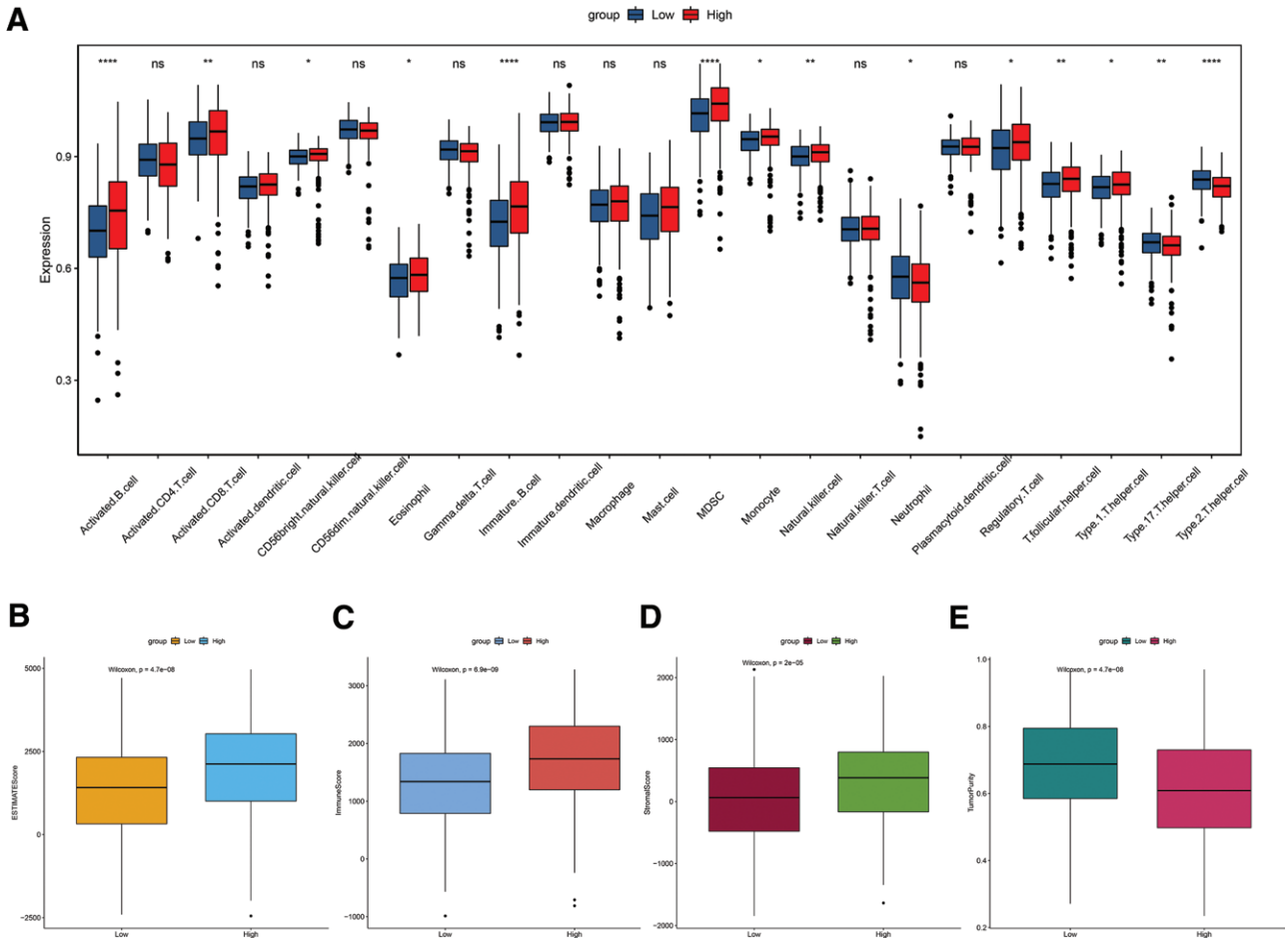

Figure 6. The relationship between the *LINC00921* expression and immune cell infiltration in LUAD. (A) The boxplots showing differences in immune cell infiltration between high and low *LINC00921* expression groups. *, **, ***, and **** represent $P < .05$, $P < .01$, $P < .001$, and $P < .00001$, respectively. Ns, not significant. (B) Differences in ESTIMATE scores between high and low *LINC00921* expression groups. (C) Differences in immune scores between high and low *LINC00921* expression groups. (D) Differences in stromal scores between high and low *LINC00921* expression groups. (E) Differences in tumor purity between high and low *LINC00921* expression groups. LUAD = lung adenocarcinoma.

in the tumor immune microenvironment (TIME) in LUAD patients.[29] A gene set marking each TIME infiltrating immune cell type was obtained from Charoentong's research.[30] Enrichment score calculated by ssGSEA was used to represent the relative abundance of each TIME infiltrating cell in each sample. The immune score, stromal score, tumor purity, and Estimation of STromal and Immune cells in MAlignant Tumour tissues using Expression data (ESTIMATE) score was calculated for each LUAD patient using "ESTIMATE" (R package). Differences in immune cell infiltration, immune score, stroma score, tumor purity, and ESTIMATE score were evaluated by using the Wilcoxon test analysis.

2.9 Correlation analysis between LINC00921 and tumor mutational burden in LUAD

Tumor mutational burden (TMB) is defined as the total number of nonsynonymous mutations in each coding region of the tumor genome.[31] Recently, high TMB has been identified as a genetic trait related to favorable outcomes of immune checkpoint inhibitor therapy. Mutation data of LUAD were derived from TCGA database. TMB score was calculated using the "maftools" (R package). The association of *LINC00921* with TMB was assessed using Spearman correlation coefficient. And a survival analysis of TMB in the *LINC00921* high- and low-expression groups was performed.

2.10 Expression verification of LINC00921, SATB1, and hsa-miR-449a in LUAD

To further understand the potential value of *LINC00921*, *SATB1*, and *hsa-miR-449a* in LUAD, electronic expression analysis was performed based on GSE174302 (including 35 LUAD plasma samples and 46 healthy control plasma samples), GSE20189 (including 73 LUAD plasma samples and 80 healthy control plasma samples), and GSE152702 (including 38 LUAD plasma samples and 7 healthy control plasma samples) datasets, respectively. Wilcoxon test was used to statistically analyze the expressions of *LINC00921*, *SATB1*, and *hsa-miR-449a* in the control and LUAD groups. In addition, 6 LUAD blood samples and 9 healthy control blood samples were collected for real-time polymerase chain reaction (PCR) to verify the expression of *LINC00921*, *SATB1*, and *hsa-miR-449a*. Total RNA in blood samples was extracted by RNAliquid overspeed whole blood (liquid sample) total RNA extraction kit. Reverse transcription and real-time PCR were performed using FastQuant cDNA first strand Synthesis Kit and SuperReal PreMix Plus (SYBR Green), respectively (mRNA and lncRNA). The miRNA reverse transcription and real-time PCR were performed using miRNA First Strand cDNA Synthesis kit (Tailing Reaction) and miRNA fluorescence quantitative PCR kit, respectively. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), ACTB, and hsa-U6 were used as internal control for gene detection. hsa-U6 is the internal reference of miRNA. The hsa-U6 primers and reverse primer

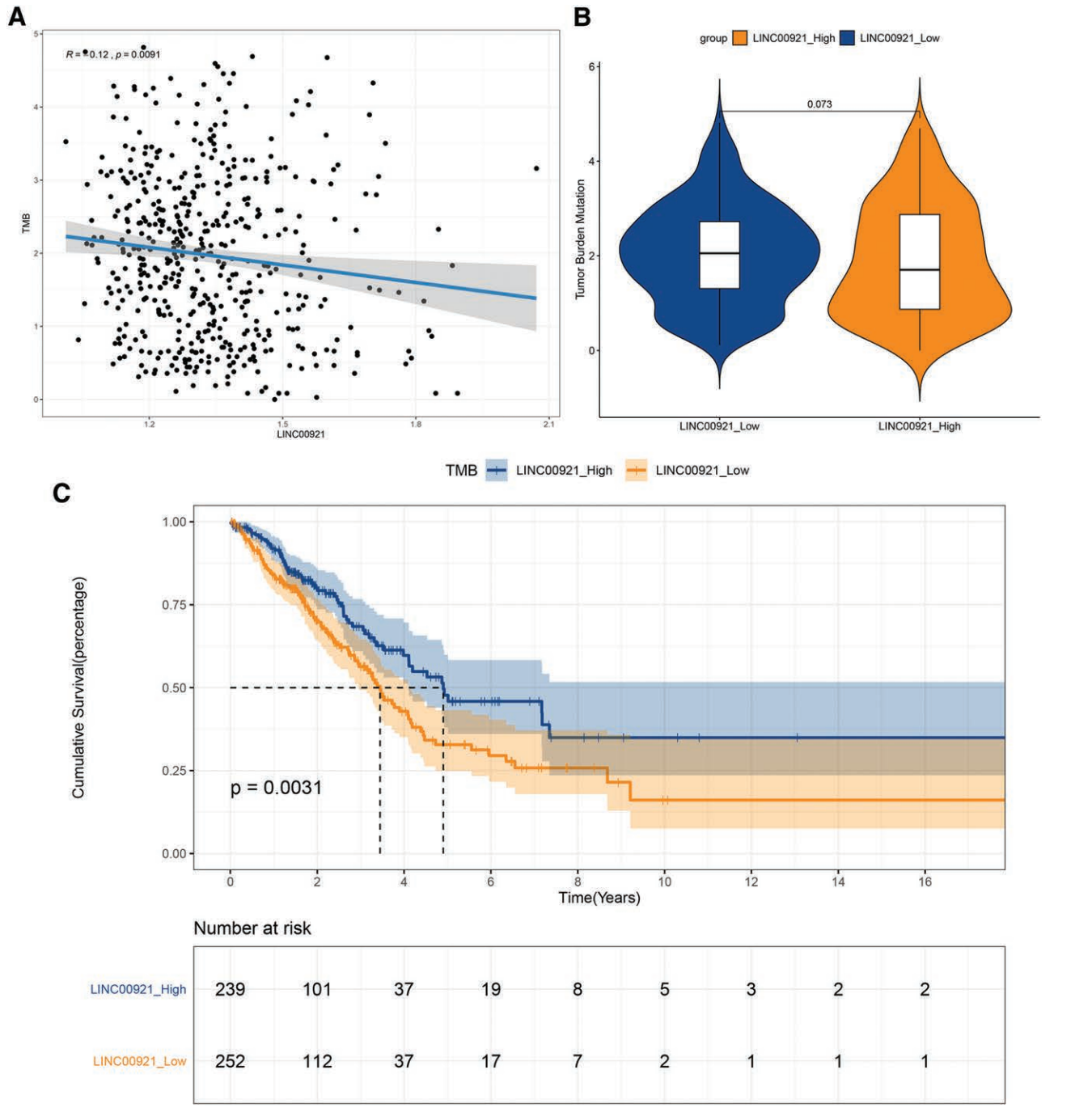

Figure 7. Relationship between *LINC00921* and TMB in LUAD. (A) Correlation linear regression analysis between *LINC00921* and TMB. (B) Differences in TMB between high and low *LINC00921* expression groups. (C) Survival analysis of TMB in high and low *LINC00921* expression groups. LUAD = lung adenocarcinoma, TMB = tumor mutational burden.

of *hsa-miR-449a* were obtained from the miRNA fluorescence quantitative PCR kit. The $2^{-\Delta\Delta Ct}$ method was used for relative quantitative analysis of data. The study was conducted in accordance with the Declaration of Helsinki and approved by the ethics committee of the 363 Hospital. Written informed consent was obtained from every participant.

2.11 Statistical analysis

Statistical analysis was performed using R version 3.5.3. Differences in *LINC00921* gene expression levels were compared between LUAD tumor samples and cancer samples using the Wilcoxon test analysis. Survival curves were generated using the Kaplan–Meier method. Differences between groups were compared using the log rank test. Univariate and multivariate analyses were performed through Cox regression models, combined with other clinical features to determine the independent prognostic value of *LINC00921*. The predictive efficiency of risk models for 1-, 3-, and 5-year overall survival was estimated using ROC curves. $P < .05$ was considered statistically significant.

3. Results

3.1 Characteristics of LINC00921 in LUAD

Role of *SATB1* in LUAD is a long-term research direction of our group, and certain results have been achieved.[21]

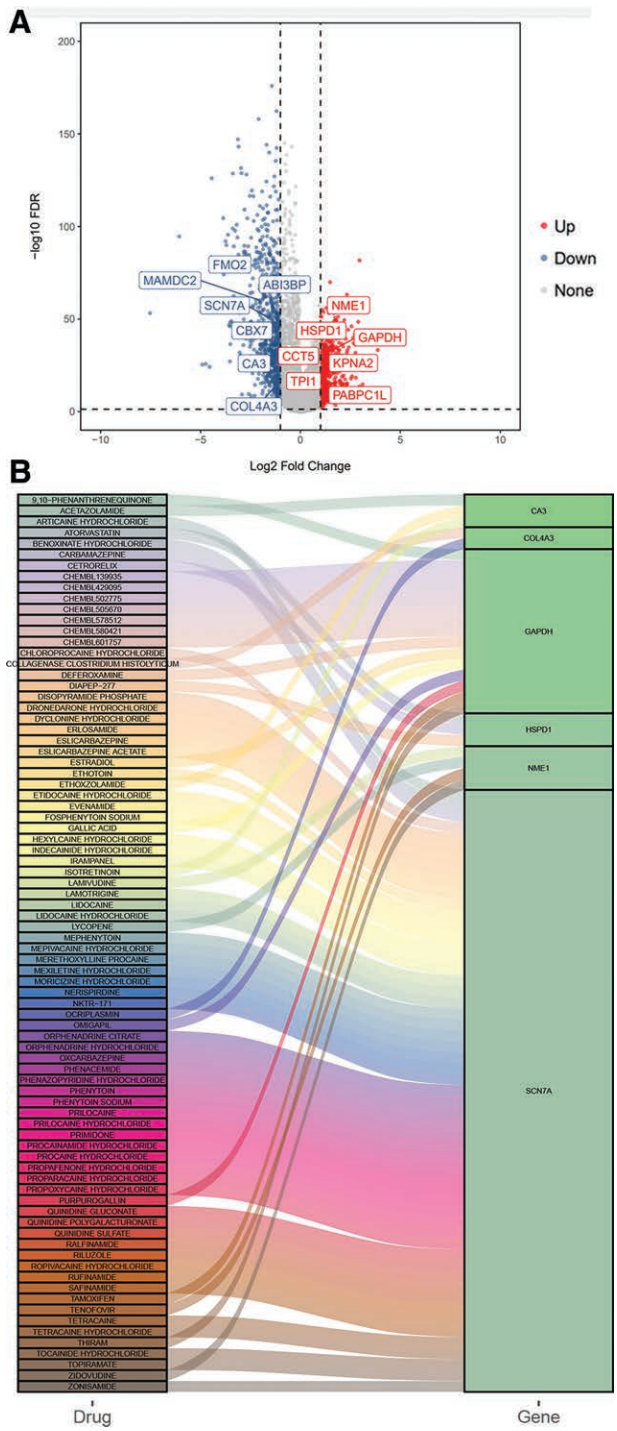

Figure 8. Drug prediction of LUAD treatment. (A) The DEGs in LUAD patients compared to normal controls. (B) Drug prediction of LUAD treatment. DEGs = differentially expressed genes, LUAD = lung adenocarcinoma.

Bioinformatics analysis found that *LINC00921* was significantly positively correlated with *SATB1*, suggesting that *LINC00921* may affect LUAD process by regulating *SATB1* (Fig. 1A). To investigate the significance of *LINC00921* in LUAD patients, we first analyzed the differential expression characteristics of *LINC00921* in cancer tissues. The expression of *LINC00921* in cancer tissues was significantly lower (Fig. 1B). Next, the ROC curve of *LINC00921* was plotted using pROC. The results showed that the area under the curve value reached 0.714 (Fig. 1C). Then, the expression profile of the *LINC00921* in different clinical variables was also indicated. Significant differences were found in T stage, survival status, and stage (Fig. 1D).

3.2 Survival analysis of LINC00921 in LUAD

To determine the prognostic-indicative function of *LINC00921* in LUAD patients, we first divided LUAD patient samples into *LINC00921* high- and low-expression groups based on the median expression of *LINC00921*. Survival analysis showed that low expression of *LINC00921* was significantly related to poor prognosis in LUAD and increased the risk of cancer-related death in LUAD patients (log-rank $P = .0023$; adjusted hazard ratio = 0.63; 95% confidence interval = 0.47–0.85). Patients with low *LINC00921* expression had a shorter median survival time compared to patients with high *LINC00921* expression (Fig. 2A and B). The expression level of *LINC00921* had a certain prognostic value for long-term survival of LUAD patients. The areas under the ROC curve for 1-, 3- and 5-year survival were 0.506, 0.564, and 0.549, respectively (Fig. 2C).

3.3 Relationship between LINC00921 and clinical features in LUAD

Age and tumor grade are important clinical information for sample patients. Clarifying the relationship between tumor risk score and clinical characteristics is crucial for formulating reasonable treatment plans. First, multivariate Cox analysis indicated that *LINC00921* was an independent prognostic factor different from age, stage, grade, and T stage (Fig. 3A). We constructed nomograms to predict the probability of overall survival at 1, 3, and 5 years by combining independent prognostic indicators with significance (*LINC00921*, stage, and T stage). As shown in Figure 3B, each factor was assigned proportionally to its risk contribution to survival. Calibration curves indicated that the combined model (nomogram) showed high accuracy at 1- and 3-year overall survival (Fig. 3C).

3.4 Functional analysis of LINC00921-related PCGs in LUAD

Coexpression analysis of *LINC00921* was performed with threshold conditions of $P < .05$ and $|Cor| > 0.4$, and 616 PCGs associated with *LINC00921* were screened. Among these 616 PCGs, 30 PCGs were negatively associated with *LINC00921*, while 586 PCGs were positively correlated with *LINC00921*. Cellular component analysis of gene ontology (GO) showed that these PCGs were mainly distributed in the nucleus, cytoplasm, and intracellular regions (Fig. 4A). Molecular function analysis of GO suggested that these PCGs were involved in DNA binding, nucleic acid binding, etc (Fig. 4B). Biological process analysis of GO indicated that these PCGs participate in transcription, DNA-template, regulation of transcription, etc (Fig. 4C). These PCGs were closely related to gonadotropin-releasing hormone and ATP-binding cassette transporters signaling pathways (Fig. 4D).

3.5 Differential expression analysis in LUAD

DEGs between low and high *LINC00921* expression groups were identified under the criterion of $|\log_2 FC| \geq 0.4$ and FDR < 0.05. Totally, 510 (58 downregulated and 452 upregulated) DEGs were obtained (Fig. 5A). GO analysis showed that these DEGs may be distributed in the plasma membrane, extracellular exosome, and participated in immune response, inflammatory response, T cell receptor signaling pathway, and other immune-related biological processes through molecular functions such as protein binding (Fig. 5B). These DEGs were mainly enriched in antigen processing and presentation, cell adhesion molecules and other signaling pathways, etc (Fig. 5C).

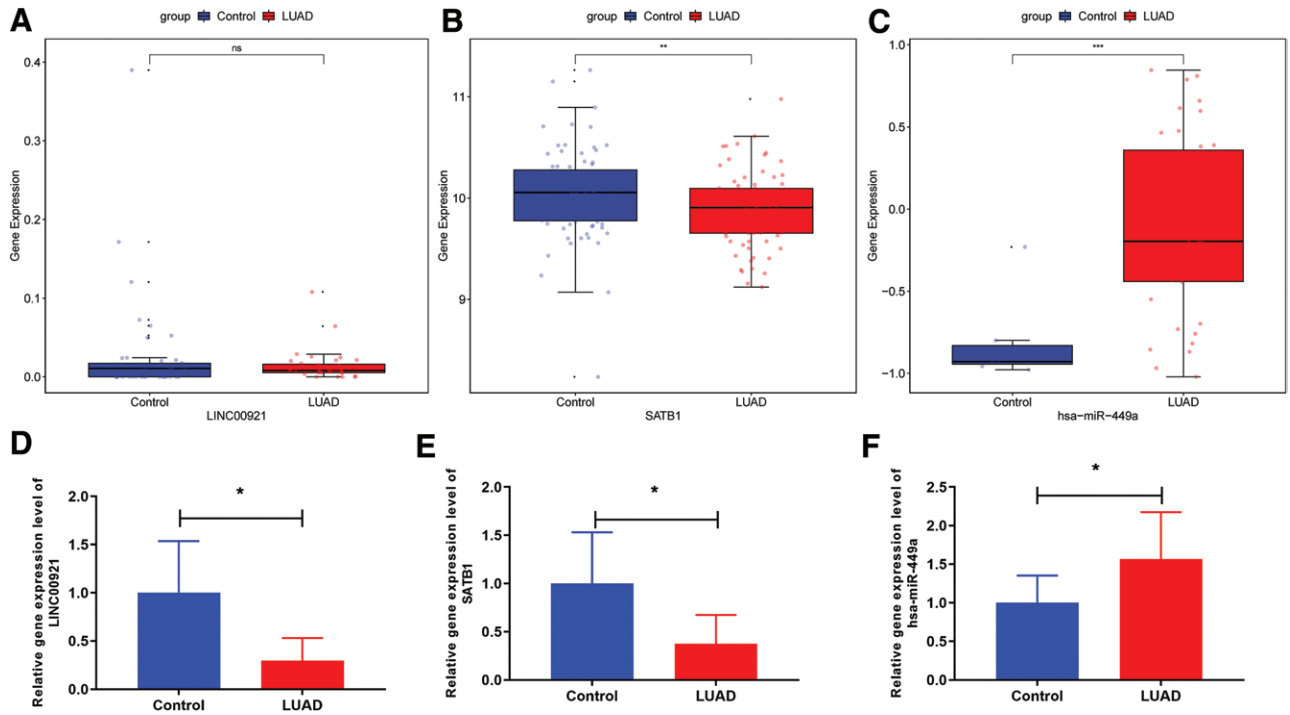

Figure 9. Electronic expression and real-time PCR verification of *LINC00921*, *SATB1*, and *hsa-miR-449a*. (A) Validation of *LINC00921* expression based on GSE174302 dataset. (B) Validation of *SATB1* expression based on GSE20189 dataset. (C) Validation of *hsa-miR-449a* expression based on GSE152702 dataset. (D–F) Expression validation of *LINC00921*, *SATB1*, and *hsa-miR-449a* in LUAD blood samples by real-time PCR. *, **, and *** represent $P < .05$, $P < .01$, and $P < .001$, respectively. Ns, not significant. PCR = polymerase chain reaction.

PPI analysis suggested that these DEGs had a complex network of regulatory interactions (Fig. 5D). There were 84 intersection genes of these DEGs and *LINC00921* coexpressed genes (Fig. 5E).

3.6 Relationship between LINC00921 and immune cell infiltration in LUAD

To explore the relationship between *LINC00921* and the TIME, the ssGSEA was used to evaluate the status of 23 immune cell infiltration in the TCGA_LUAD dataset. The infiltration degree of most immune cells in the high *LINC00921* expression group was significantly higher than that in the low expression group, such as activated B cells, activated $CD8^+$ T cells, myeloid suppressor cells, immature B cells, etc, while the infiltration of cells such as Type 17 T helper cells and Type 2 T helper cells was significantly lower than that in the low *LINC00921* expression group (Fig. 6A). Further, the immune score, stromal score, tumor purity, and ESTIMATE score of each LUAD patient was calculated. In the high *LINC00921* expression group, the immune score, stromal score, and ESTIMATE score were significantly higher than those in the low *LINC00921* expression group, while tumor purity was significantly lower than that in the low *LINC00921* expression group (Fig. 6B–E).

3.7 Relationship between LINC00921 and TMB in LUAD

TMB could determine the individual's response to cancer immunotherapy. Correlation analysis showed that *LINC00921* and TMB exhibited a negative correlation (Fig. 7A). There is no difference of TMB between the high and low *LINC00921* expression groups (Fig. 7B). However, survival analysis of TMB in different *LINC00921* expression groups found that TMB had significant survival differences in high *LINC00921* expression group compared to low *LINC00921* expression group (Fig. 7C).

3.8 Drug prediction associated with LUAD treatment

To explore drugs that may be related to LUAD treatment, we screened DEGs between the LUAD group and the control group with $|\log_2 FC| \geq 1$ and FDR < 0.05. Of a total of 1377 DEGs, 783 downregulated and 594 upregulated were identified (Fig. 8A). We intersected the DEGs in the high and low *LINC00921* expression groups and the PCGs related to *LINC00921* to obtain 84 genes and 1377 drug-related DEGs between the LUAD and the control groups, and then 14 genes were obtained. The potential drugs target these 14 genes that were screened based on the Drug-Gene Interaction Database, of which 8 genes had no corresponding drugs, namely, *TPI1*, *PABPC1L*, *MAMDC2*, *KPNA2*, *FMO2*, *CCT5*, *CBX7*, and *ABI3BP*. The remaining genes are *SCN7A*, *NME1*, *HSPD1*, *GAPDH*, *CA3*, and *COL4A3* (Fig. 8B). Interestingly, we found that *GAPDH* was also a hub gene in the PPI network constructed by DEGs in the high and low *LINC00921* expression group. So, we suggested that *GAPDH*-related drugs may have a higher possibility of therapeutic effects on LUAD.

3.9 Expression verification of LINC00921, SATB1, and hsa-miR-449a in LUAD by real-time PCR

Electronic expression verification of *LINC00921*, *SATB1*, and *hsa-miR-449a* was performed based on GSE174302, GSE20189, and GSE152702 datasets. Compared with the control group, the expression of *LINC00921* in LUAD plasma showed a downregulated trend (Fig. 9A). Compared with the control group, *SATB1* was significantly downregulated and *hsa-miR-449a* was significantly upregulated in LUAD plasma (Fig. 9B and C). The expression trend of *LINC00921* in LUAD plasma samples is consistent with that in LUAD tissue samples, but lacks significance, which may be related to the small amount of plasma samples. In addition, blood samples were collected for real-time PCR validation of *LINC00921*, *SATB1*,

and *hsa-miR-449a*. The results showed that *LINC00921* and SATB1 were significantly downregulated and hsa-miR-449a was significantly upregulated in LUAD group compared with control group (Fig. 9D–F), which was consistent with the results of public database analysis.

4. Discussion

LUAD is the predominant type of NSCLC and the diagnosis of LUAD usually occurs at an advanced stage due to the absence of clinical symptoms in the early stages of LUAD.[32,33] Therefore, the discovery of therapeutic and prognostic biomarkers for LUAD is a great boon to patients. Emerging evidence suggests that lncRNAs serve as key regulators of gene expression, the dysregulation of which has been implicated in carcinogenesis and has the potential to be prognostic biomarkers for tumor therapy.[34,35] In the present study, we investigated the relationship between *LINC00921* and LUAD for the first time through systematic bioinformatics analysis and found that *LINC00921* has the potential to serve as a prognostic marker for LUAD.

We used LUAD expression data downloaded from the TCGA database to investigate the expression signature of *LINC00921* in cancerous and paracancerous tissues. The expression of *LINC00921* in LUAD cancerous tissues was lower than that in paracancerous tissues, suggesting a potential association between the low expression of *LINC00921* and the occurrence of cancer. Moreover, survival analysis indicated that low expression of *LINC00921* was associated with poorer prognosis, which is in line with the study in breast cancer.[19] *LINC00921* can be used as a prognostic factor independent of other clinical features. lncRNAs regulate cancer development and progression through different pathways in vivo, one of the main ways is by regulating the expression of target genes.[36,37] Function analysis of *LINC00921*-related PCGs showed that *LINC00921* may exert an influence on cancer development through its regulation of target genes transcription. Taken together, these results suggested that *LINC00921* is associated with the development of LUAD and has the potential to predict the prognosis of LUAD.

Immunotherapy plays an important role in cancer treatment, increasing the likelihood that cancer can be cured.[38] Recently, the relationship between lncRNAs and immune-related functions has been explored in a variety of cancers, including LUAD.[39,40] The tumor microenvironment was found to be associated with cancer progression.[41] Analysis of immune cell infiltration in tumor microenvironment is very effective for revealing tumor treatment prognosis.[42,43] Therefore, we investigated the relationship between *LINC00921* and immune cell infiltration in TIME of LUAD. Interestingly, we found that the infiltration degree of most immune cells was reduced in the tumor microenvironment in the low *LINC00921* expression group, which could be responsible for poor patient outcomes. TMB may determine an individual's response to immunotherapy, and it is associated with improved survival in a variety of tumor types.[44] Survival analysis showed that TMB was significantly different in survival between the *LINC00921* high- and low-expression groups. Moreover, we screened *LINC00921*-related drugs that might treat LUAD and found that drugs related to *GAPDH* may be more likely to be therapeutically effective in LUAD.[28] Overall, these results provided new insights into the treatment of LUAD.

A study has demonstrated that *SATB1* deficiency is associated with poor prognosis in lung squamous cell carcinoma.[45] Moreover, our previous study suggested that *SATB1* was involved in regulating LUAD.[21] *hsa-miR-449a* plays an important role in regulating tumorigenesis and development.[46] *hsa-miR-449a* is involved in regulating the radiosensitivity of LUAD cells.[47] In addition, *hsa-miR-449a* is also involved in regulating the occurrence of lung cancer.[48] In this study, it was found that *LINC00921* was significantly correlated with *SATB1* and *hsa-miR-449a*. Therefore, it is speculated that the *SATB1-hsa-miR-449a-LINC00921* axis could potentially serve as a significant pathway in the regulation of the occurrence and development of LUAD. In addition, the expression of *SATB1*, *hsa-miR-449a*, and *LINC00921* showed differences between LUAD patients and normal subjects verified by real-time PCR and public database analysis, which further suggested the importance of *SATB1-hsa-miR-449a-LINC00921* axis in LUAD.

This study has some limitations. The results are mainly based on the analysis of data from public databases, and the sample size for verification is relatively small. The expression levels of *LINC00921*, *hsa-miR-449a*, and *SATB1* will be further validated in our following research with a larger sample size. Further in-depth functional studies will be continued, including at the cellular and animal levels, followed by further exploring the mechanisms and clinical applications of *LINC00921* in carcinogenesis.

After a series of bioinformatics analysis on *LINC00921*, the current data suggested that *LINC00921* was able to be used as a prognostic indicator for LUAD, and high expression of *LINC00921* was closely associated with good prognosis of LUAD.

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