OncoTargets and Therapy

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ORIGINAL RESEARCH

Long Noncoding RNA *LINC00551* Suppresses Glycolysis and Tumor Progression by Regulating *c-Myc*-Mediated *PKM2* Expression in Lung Adenocarcinoma

This article was published in the following Dove Press journal:
OncoTargets and Therapy

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Background: Lung adenocarcinoma (LUAD) is a leading cause of mortality associated with cancer globally. Thus, it is essential to elucidate its tumorigenesis and prognosis. Accumulating evidence shows that long noncoding RNAs (lncRNAs) play important roles in the occurrence and progression of tumors by regulating their glucose metabolism.

Methods: Bioinformatics analysis was performed to explore the expression of *LINC00551* in LUAD. The level of *LINC00551* in LUAD cells and tissues was detected by RT-qPCR. CCK-8, colony formation, EDU and transwell assays were conducted to evaluate the cell growth and migration of LUAD cells (A549 and PC9). High throughput sequencing was used to discover the downstream genes of *LINC00551*. The metabolic function of LUAD cells was identified by glucose uptake and lactate production assays. Furthermore, tumor xenografts were established to investigate the effects of *LINC00551* on tumor growth in vivo.

Results: Herein, we found that *LINC00551* was low-expressed in LUAD, and its level correlated with clinical prognosis. Ectopic expression of *LINC00551* inhibited the proliferation and migration of LUAD cells (A549 and PC9). High throughput sequencing and gene enrichment analysis revealed that LINC0551 may be involved in metabolic pathway. Glucose uptake and lactate production assays suggested that *LINC00551* suppressed glycolysis of LUAD cells. Mechanistically, our work revealed that *LINC00551* inhibited glycolysis in LUAD cells by impairing *c-Myc*-mediated transcription of an important glycolysis-related enzyme *PKM2*.

Conclusion: In summary, our study identifies *LINC00551* as a tumor suppressor in LUAD and implicates the *LINC00551*/*c-Myc*/*PKM2* axis in the glycolytic remodeling of LUAD.

Keywords: lung adenocarcinoma, *LINC00551*, *c-Myc*, *PKM2*, glycolysis

Introduction

Globally, lung cancer is the leading cause of cancer-associated mortality, with less than 20% survival rate in 5 years.[1] Lung adenocarcinoma (LUAD) accounts for 40% of all diagnosed lung cancers.[2] Although great advances have been made in the clinical treatment of LUAD,[3] overall survival time of patients with LUAD has not improved dramatically, principally due to a lack of reliable molecular biomarkers. Thus, a better understanding of the molecular mechanisms underlying LUAD initiation and progression may improve the diagnosis and therapy of this lethal disease.

Due to the advancement of sequencing technology, numerous long noncoding RNAs (lncRNAs) have been identified. LncRNAs are defined as transcripts with more than 200 nucleotides in length that usually lack protein-coding capacity.[4] A growing body of literature implicates lncRNAs in every step of physiological and pathological processes, at the genome level, transcriptional level, post-transcriptional level, translational level and posttranslational level.[5]

Metabolic remodeling is a defining hallmark of cancer and its common feature is the preference for the glycolytic pathway for ATP generation even in the presence of oxygen to meet the high energy demands for growth, invasion, and metastasis.[6] This phenomenon, also known as the Warburg effect or aerobic glycolysis,[7] results in increased glucose uptake, elevated lactate levels, decreased ATP production, and more readily available energy supply.[8] LncRNAs regulate glucose metabolism in tumor cells by modulating metabolic enzymes, regulatory molecules, signaling pathways, and related oncogenes.[9,10] A study by Song et al revealed that lncRNA *PVT1* is a molecular sponge that suppresses the *miR-497* expression, thus promoting glucose metabolism and cell growth in osteosarcoma via the *PVT1/miR-497/HK2*axis.[11] Zhao et al found that *LINC00092* is highly expressed in ovarian carcinoma where it binds glycolysis pathway enzyme *PKKFB2* (fructose-2,6-bisphosphatase) to facilitate glucose breakdown and thereby accelerating tumor metastasis and sustains localized cancer-related fibroblasts reinforcement.[12] Although the effects of lncRNAs on the glucose metabolism have been described, there is still a far way to clarify and discover the mechanisms underlying lncRNA regulation of glucose metabolism. Specifically, it is necessary to determine which other glycolytic enzymes and metabolic pathways lncRNAs are associated with. In the present research, we identified that *LINC00551* was downregulated in LUAD tissues and exerted suppressive roles in the progression and glycolysis of LUAD, partially by regulating the expression of *c-Myc*-mediated *PKM2*. Our study enhanced understanding on the complex networks of glucose metabolism in cancer and offered a possible molecular biomarker for clinical diagnosis and therapy of diseases.

Materials and Methods

Patient Tissue Samples

A total of 32 paired LUAD and adjacent healthy tissues were obtained from the Department of Cardiothoracic Surgery, Nanjing First Hospital, Nanjing Medical University between 2016 and 2019. After excision, the samples were rapidly frozen in liquid nitrogen. Comprehensive data concerning clinical features, tumor stage, and histopathological features were obtained under authorization by the Ethics Committee of Nanjing First Hospital. An informed consent for participation was obtained from patients prior to analysis. The clinical information of these 32 patients were shown in Table S1.

Cell Culture

Here, 4 human lung adenocarcinoma lines, A549, H1299, H1975, and PC9, and one normal bronchial epithelial cell, HBE were utilized. A549 and H1299 cell lines were obtained from Jiangsu Cancer Hospital, while PC9, H1975, and HBE cell lines were obtained from Jinling Hospital. All cell lines were approved by Nanjing Hospital affiliated to Nanjing Medical University. HBE, A549, and H1299 cells were cultivated in RPMI-1640 medium (Gibco, China) supplemented with 10% fetal bovine serum (FBS, Science Cell), 100 U/mL penicillin-streptomycin mixture, L-glutamine and, sodium pyruvate, in 5% CO_2 presence at 37 °C. H1975 and PC9 cells were maintained under the same conditions, in Dulbecco's Modified Eagle Medium (DMEM; Gibco, China).

Plasmid Transfection

The full-length complementary DNA (cDNA) of *LINC00551* and *c-Myc* were cloned into the pcDNA3.1+ expression vector. DNA Midiprep kits (OMEGA, Shanghai, China) was used to extract the plasmid vectors. Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA) and P3000 (Invitrogen) were utilized to transfect the plasmids into LUAD cell lines (A549 and PC9 cells) in a six-well plate according to the manufacture's protocols. Subsequent experiments were performed after 24 or 48 h transfection. The transfection efficacy was confirmed through quantitative PCR (RT-qPCR) prior to biological function experiments.

Total Extraction of RNA and RT-qPCR

Total RNA was extracted from LUAD tissue samples and cultured cells using Trizol reagent (Invitrogen, USA) according to the manufacture's protocol. The RNA was reverse transcribed into cDNA using PrimerScript Reverse Transcription Kit (Takara, RR047A) and amplified by qRT-PCR with TB Green™ Premix Ex Taq (Takara RR420A) in an ABI 7500 System (Applied Biosystems,

Foster City, CA, USA). Actin housekeeping gene acted as the internal control. The $2^{-\Delta\Delta Ct}$ method quantified the expression of the target gene. The primers used in this study were acquired from Invitrogen Shanghai Sangon Biotech (Invitrogen, China). The primers sequences were as follows: *LINC00551* Forward: 5′-TCAGCAGCCT TCAGTTGGAG-3′ Reverse: 5′-AGGCTCTGTTCAGCT GGTTC-3′; *c-Myc* Forward: 5′-GGCTCCTGGCAA AAGGTCA-3′ Reverse: 5′-CTGCGTAGTTGTGCTGA TGT-3′; *Actin* Forward: 5′-AGCGAGCATCCCCCAA AGTT-3′ Reverse: 5′- GGGCACGAAGGCTCATCATT-3′; *PKM2* Forward: 5′-ATGTCGAAGCCCCATAGTGAA -3′ Reverse: 5′-TGGGTGGTGAATCAATGTCC-3′.

The Cell Proliferation Assays

LUAD cells, A549 and PC9, were transfected with plasmids or small interfering RNAs (siRNAs) prior to cell proliferation assays. For the EDU (5-ethynyl-2′-deoxyuridine) assay (EdU Apollo®488 In Vitro Imaging Kit, RiboBio, Guangzhou, China), 15◊103 cells/100 μL/well were seeded into a 96-well plate. Once the cells adhered to the plate, 100 μL reagent A was added in the wells and allowed to incubate at 37 ºC for 2 h. Afterward, the cells were fixed with 4% paraformaldehyde (Biosharp), treated with 0.5% TritonX-100 (100 μL), then subjected to Apollo and Hoechst staining. After washing with phosphate-buffered saline (PBS) three times, the cells were photographed using a fluorescence microscope to calculate the proliferation rates. In CCK-8 assay, $2◊10^3$ cells/well were seeded into 96-well plates and 10 μL CCK-8 reagent was added. And then the plates were incubated for 2 h under 5% CO_2 environment. After 0-, 24-, 48-, 72-, and 96-h incubation, absorbance at 450 nm was recorded. To evaluate colony-formation, 400 cells/well were seeded into 6-well plate and cultivated for 10–14 days. Following incubation, the cells were fixed in 4% paraformaldehyde for 30 min, stained with 0.1% crystal violet solution, washed with PBS, and then photographed.

Transwell Migration Assay

The migrate potential of LUAD cells (A549 and PC9) was assessed using the Millicell Hanging Cell Culture Insert (Millicell MCEP24H48). According to c recommendations, $4 ◊ 10^4$ cells in 200 μL of serum-free medium were put in the upper chamber while lower chamber was filled with 800 μL 10% FBS supplemented medium. After incubating for 24-h at 37 °C, non-migrated cells were scraped out using cotton swabs. Subsequently, migrated cells were fixed, stained with 10% crystal violet solution, washed with PBS, and then photographed.

Analysis of Nuclear and Cytoplasmic RNA

Cytoplasmic and nuclear RNA was isolated using the PARIS Kit (Invitrogen), following the provided procedure. Briefly, LUAD cells (A549 and PC9) were trypsinized and washed twice with PBS. After cell pellets lysis using buffer I, and the cytoplasmic lysate was collected as supernatant. Nuclear pellets were further lysed in buffer II and the supernatants were collected as nuclear lysates following centrifugation. The cytoplasmic and nuclear portions were divided to extract RNA and for qRT-PCR. The GAPDH and U6 were applied as corresponding cytoplasmic and nuclear markers, respectively.

In vivo Tumorigenesis Assay

To assess *LINC00551* effect in vivo, male mice (age 6–8 weeks) were randomly classified into 2 groups of 5 mice. The mice were subcutaneously injected with $2 ◊ 10^6$ A549 cells/100 μL sterile PBS. The LUAD cells had been transfected with plasmids expressing *LINC00551* cDNA, or an empty pcDNA3.1+ vector (control). Starting one week after the injection, the mice were observed three times a week for six weeks, and tumor growth was evaluated by measuring harvested tumor nodules volume and mass. All animal studies were performed in accordance with NIH animal use guidelines. The study design was approved by Experimental Animal Ethics Committee of Nanjing Hospital affiliated to Nanjing Medical University.

Bioinformatic Study

Human exon arrays for LUAD and adjacent healthy tissues were downloaded from the NCBI's Gene Expression Omnibus (GEO, GSE19804 https://www.ncbi.nlm.nih.gov/gds/?term=GSE19804). Correlation analysis between expression of *c-Myc* and glycolysis enzymes was performed using GEPIA (http://gepia.cancer-pku.cn/index.html). The protein-coding potential of *LINC00551* was assessed using CPAT (http://lilab.research.bcm.edu/cpat/index.php) and LNCipedia (https://lncipedia.org/db). Survival analysis of *LINC00551* and *PKM2* was performed using GEPIA. Cancer Cell Line Encyclopedia (https://portals.broadinstitute.org/ccle/about) was queried for *LINC00551* expression in different cancer cell lines.

RNA Sequencing Assay

To further illustrate the molecular mechanism of *LINC00551* function in LUAD, we conducted high-throughput RNA sequencing in control and *LINC00551*-overexpressing A549 cells. The threshold set for upregulated and downregulated genes was a fold change >2.0 and a P value < 0.05. Differentially expressed genes were subjected to enrichment analysis to determine the biological processes that *LINC00551* may participate in.

Measurement of Glucose Uptake

Glucose levels were quantified with a Standard glucose assay kit (Thermo Fisher Scientific). In brief, the *LINC00551* overexpression plasmid and pCDNA3.1+ vector control plasmid was used to transfect A549 and PC9 cells for 24 h and the harvested cells were seeded into a 96-well plate (5 x 104 cells/well). Once the cells attached to the plate, the culture medium was removed and replaced by serum-free medium for 6 h to overnight. Subsequently, 50 μL of fluorescent 2-DG (Molecular Probes, Invitrogen) was added and the cells were incubated at 37 °C under 5% CO_2 environment for 30 min. The cells were washed twice with cold PBS and lysed before Assay Mixture (100 μL/well) was added, and kept at room temperature for 30–120 min. Cellular 2-DG uptake was measured by monitoring optical density ratio increase at 570/610 nm using a microplate reader.

Quantification of Lactate Levels

LUAD Cells (A549 and PC9) were seeded in a 6-well plate and transfected with a control plasmid or *LINC00551*-overexpressing plasmid. The lactate assay kit (Nanjing Jiancheng Bioengineering, China) was utilized to quantify the lactate levels in the culture medium based on the manufacturer's guidelines. Absorbance at 570 nm was recorded via a plate reader. The outcomes were standardized to the total protein concentration of each sample.

Statistical Analyses

All statistical studies were completed in GraphPad Prism (version 7.0, GraphPad, USA). Student's *t*-test was employed to compare group means while Log rank test and Kaplan-Meier plots were used to evaluate the survival differences. The correlation among the different genes' expression was assessed through Pearson correlation analysis. P values < 0.05 were considered statistically significant. All data are expressed as the mean ± standard deviation (SD).

Results

LINC00551 Was Downregulated in LUAD

Since the function of *LINC00551* has not been studied in LUAD, we sought to determine whether LINC00551 could play a role in LUAD tumorigenesis and development. *LINC00551* is located at 13q33.3, with 4 exons and 1967 bp in length (Figure S1). According to CPAT and LNCipedia, *LINC00551* has almost no protein-coding potential (coding probability: 0.11) (Figure 1A and B). Analysis of the GTEx dataset and Cancer Cell Line Encyclopedia revealed that *LINC00551* is highly expressed in normal lung tissue (Figure S2), but relatively low expressed in non-small-cell lung cancer cells (Figure S3). In GEPIA, *LINC00551* was downregulated in LUAD (Figure 1C), and correlated with tumor stage of LUAD patients. (Figure 1D). The overall survival analysis using GEPIA showed that low *LINC00551* expression predicted a poorer prognosis in LUAD patients (Figure 1E). In line with these results, *LINC00551* expression was markedly lower in LUAD cell lines (A549, H1299, H1975, and PC9) and tumor tissue than in normal lung epithelial cell line HBE (Figure 1F) and adjacent normal tissue, respectively (Figure 1G). Since the subcellular localization of lncRNAs largely influences their mechanism of action,[13] we analyzed the nuclear and cytoplasmic RNA fractions isolated from A549 and PC9 cells to estimate the localization of *LINC00551*. Approximately 75% of *LINC00551* located in the nucleus of LUAD cells (Figure 1H). These results implied that *LINC00551*was downregulated in LUAD cells and tissues and might exert its function in LUAD progression.

LINC00551 Overexpression Suppressed Proliferation, Colony Formation, and Migration of LUAD Cells in vitro

To further explore the biological effects of *LINC00551* on LUAD development, we overexpressed *LINC00551* in A549 and PC9 cells by plasmid transfection (Figure 2A). Overexpression of *LINC00551* inhibited cell proliferation (Figure 2B) and decreased colony formation (Figure 2C and B) and migration in both cell lines (Figure 2E–F). EDU staining confirmed that *LINC00551* impaired the proliferation of LUAD cells (Figure 2G–H).

A

Result for species name : hg19 with job ID :1581667855							
Data ID	Sequence Name	RNA Size	ORF Size	Ficket Score	Hexamer Score	Coding Probability	Coding Label
0	NC_000013.11:106617810-106631658	13849	420	0.8831	-0.0708280173695	0.1100864590506	no

B

PhyloCSF score	-101.0604
CPAT coding probability	4.94%
Bazzini small ORFs	0

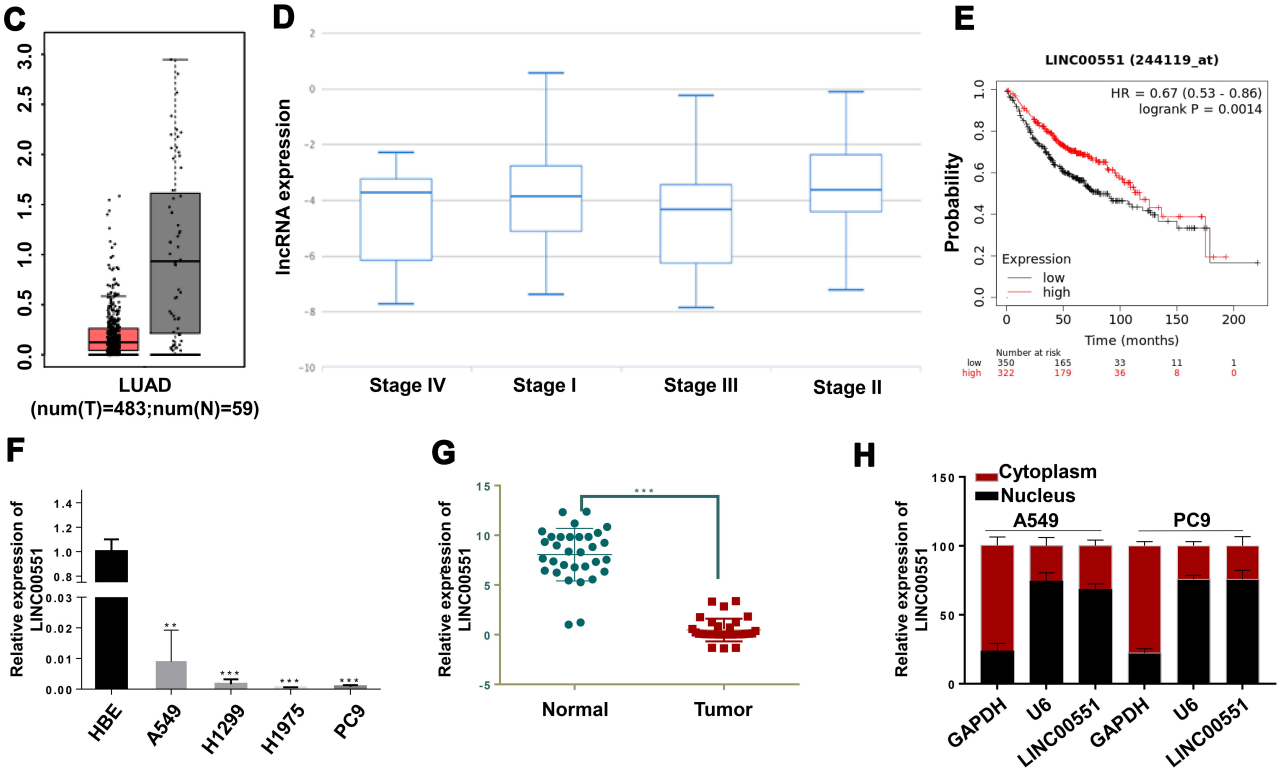

Figure 1 *LINC00551* was downregulated in lung adenocarcinoma and correlated with clinical prognosis. (**A and B**). Computational assessment of lncRNAs in CAPT and Incipedia database revealed that *LINC00551* was of limited protein-coding capacity (coding probability marked with red box). (**C**). *LINC00551* was downregulated in LUAD tissues compared with adjacent normal tissues in TCGA dataset. (**D**). *LINC00551* correlated with tumor stages of LUAD in TANRIC database. (**E**). Kaplan-Meier survival analysis showed that low expression of *LINC00551* was associated with a worse overall survival of lung cancer patients. (**F–G**). Expression profiles of *LINC00551* in human lung adenocarcinoma cell lines relative to one normal lung epithelial cell line (HBE) and 32 paired clinical samples of LUAD. 1h. Subcellular localization studies indicated that LINC00551 was mainly located in nucleus. (**$p < 0.01$; ***$p < 0.001$).

LINC00551 Overexpression Reduced *c-Myc* Levels and Affected Aerobic Glycolysis via *PKM2*

To clarify the underlying mechanisms of *LINC00551* in LUAD, we performed high-throughput RNA sequencing of *LINC00551*-overexpressing A549 cells (Figure 3A). Gene enrichment analysis of differentially expressed genes revealed that *LINC0551* may be involved in metabolic pathways (Figure 3B). Since altered glucose metabolism is a distinct tumor cell feature, we hypothesized that *LINC00551* participates in glucose metabolism. Indeed, overexpression of *LINC00551* decreased the levels of glycolytic enzymes, especially *PKM2* (Figure 3C), inhibited cellular uptake of glucose (Figure 3D), and reduced synthesis of lactate (Figure 3E) in the A549 and PC9 cells. To further elucidate the mechanisms of *LINC00551* in LUAD glycolysis, we found *MYC* mRNA level was significantly decreased among multiple differentially expressed genes as shown in Figure 3A consistent with the result of RT-qPCR (Figure 3F). *MYC* is a well-known oncogene encoding *c-Myc* transcription factor,[7]

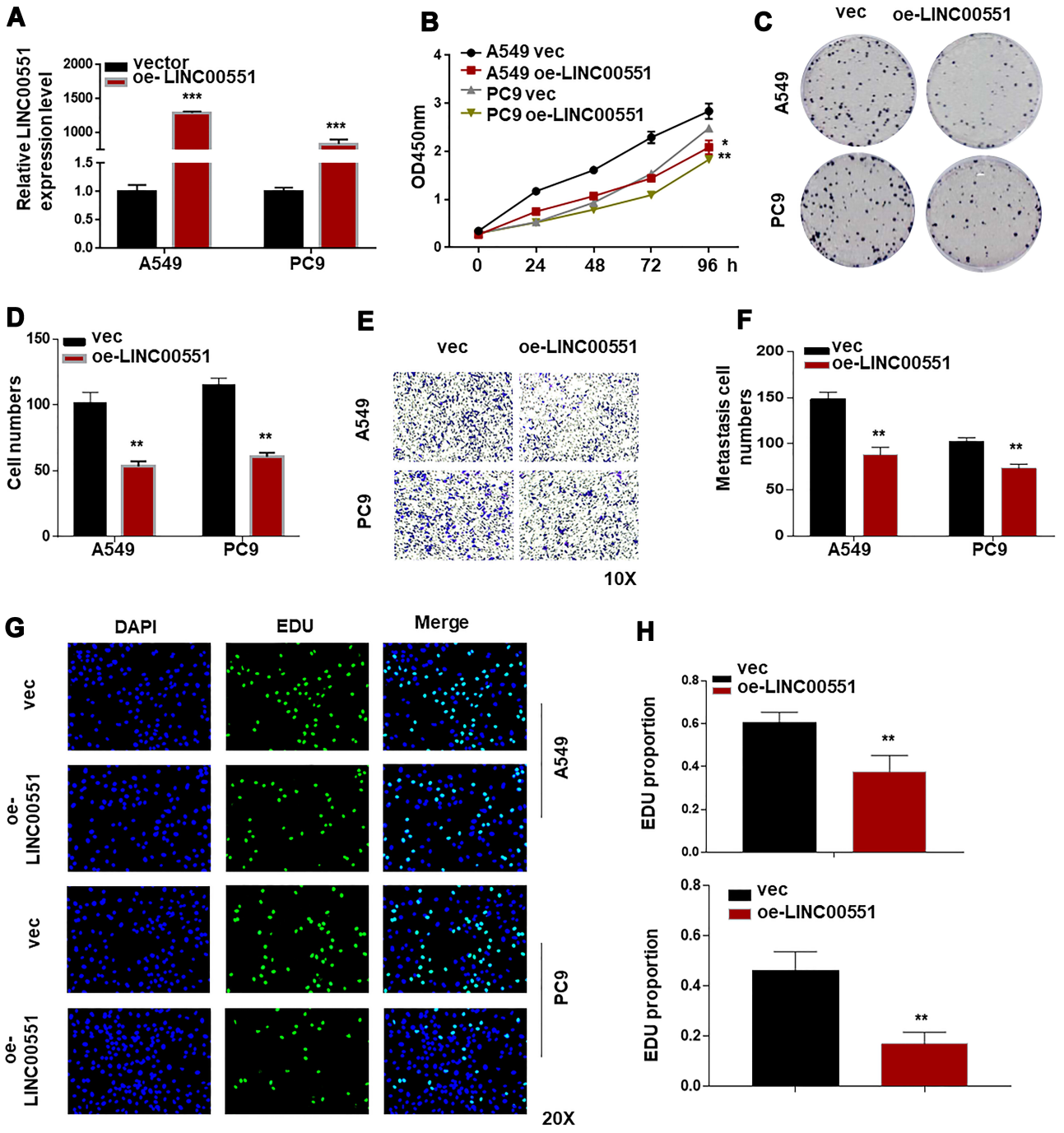

Figure 2 *LINC00551* suppressed the progression of LUAD cells. (**A**). Verification of *LINC00551* over-expression efficiency in A549 and PC9 cells by qRT-PCR. (**B**). CCK-8 assay demonstrated that *LINC00551* inhibited the proliferation of A549 and PC9 cells. (**C**–**D**). Overexpression of *LINC00551* inhibited the ability of colony formation of A549 and PC9 cells. (**E**–**F**). Transwell assay showed that *LINC00551* could inhibit the migration ability of A549 and PC9 cells. (**G**–**H**). Overexpression of *LINC00551* decreased cell proliferation of A549 and PC9 cells, as detected by EdU staining. The data were represented as the means ± SD of at least three independent experiments. (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

which enhances cell development and progression of[14] and controls the transcription of many of the genes involved in glucose metabolism, including *PKM2*.[15] Furthermore, the expression of *c-Myc* positively correlated with *PKM* in TCGA dataset (Figure 3G). Considering that *LINC00551* was mainly located in the nucleus (Figure 1H), we hypothesized that *LINC00551* regulates glucose metabolism through *c-Myc*-mediated transcription of *PKM2*.

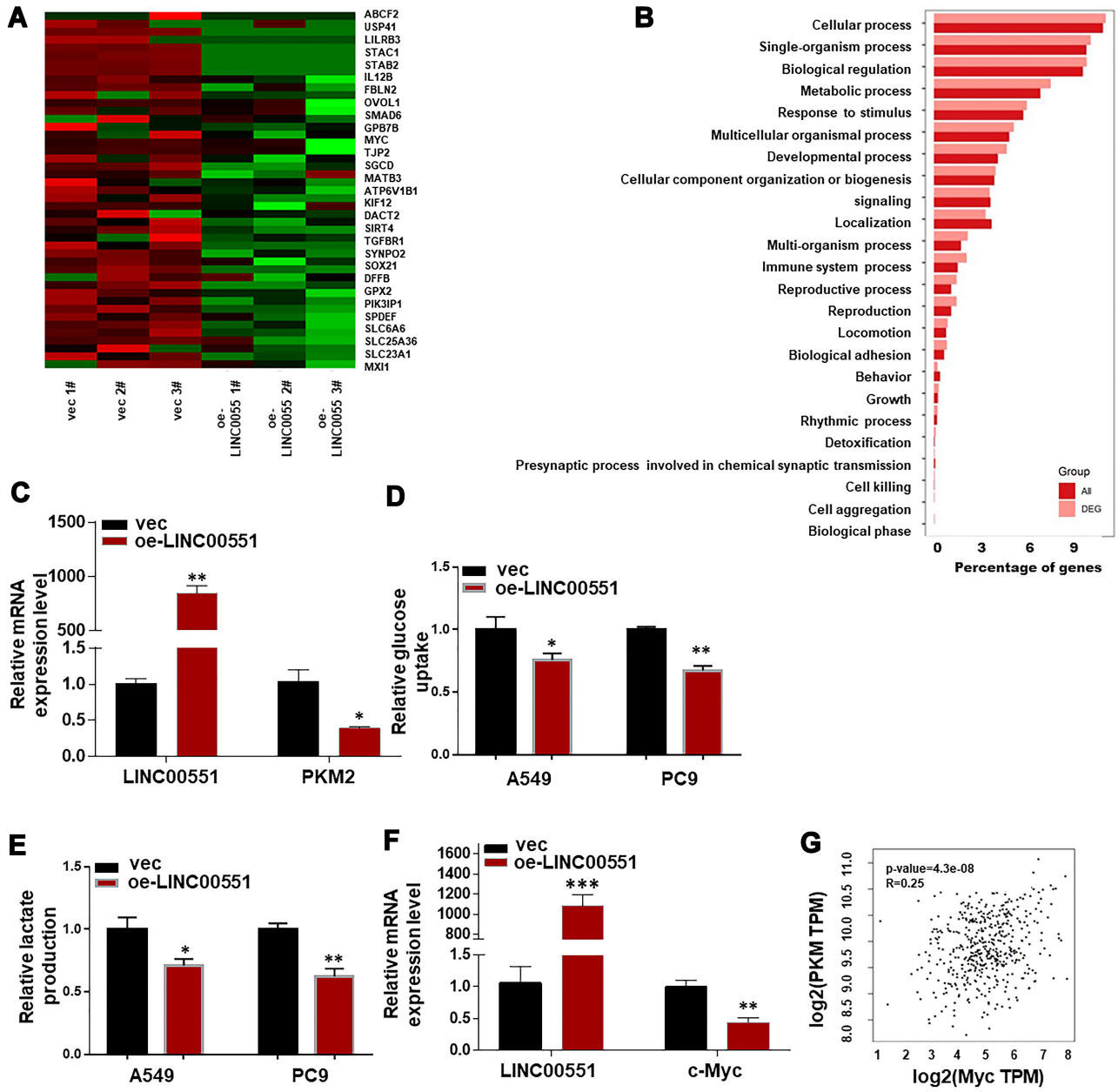

Figure 3 *LINC00551* overexpression reduced *c-Myc* expression and affected aerobic glycolysis via *PKM2*. (**A**). Differential expressed genes were compared between control and *LINC00551*-overexpression A549 cells. (**B**). Enrichment analysis of DEGs (Differential Expressed Genes) in biological process (BP) sets hinted that *LINC00551* might participate in metabolic process. (**C**). Overexpression of *LINC00551* decreased expression of *PKM2*. (**D**–**E**). *LINC00551* inhibited the aerobic glycolysis of LUAD cells (A549 and PC9), confirmed by glucose uptake and lactate production. (**F**). Overexpression of *LINC00551* down-regulated the expression of *c-Myc*. (**G**). The expression of *c-Myc*positively correlated with *PKM* in TCGA dataset. The data are represented as the means ± SD of at least three independent experiments. (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

c-Myc Overexpression in *LINC00551*-Overexpressing Cells Restored *PKM2* Expression and Warburg Effect, and Inhibited LUAD Progression

To test the hypothesis that the effect of *LINC00551* on glycolysis is mediated by *c-Myc*, we performed further experiments. The results showed that *PKM2* mRNA level could be restored by c-Myc in *LINC00551*-overexpressing A549 cells (Figure 4A). *c-Myc*could also rescue the inhibitory effect of *LINC00551* overexpression on glucose uptake, lactate production (Figure 4B and C), cell proliferation (Figure 4D-G), and metastasis in A549 and PC9 cells (Figure 4H-I). Overall, these results indicated that *LINC00551* inhibited development of LUAD cells by affecting tumor cell glycolysis via *c-Myc*-mediated transcription of *PKM2*.

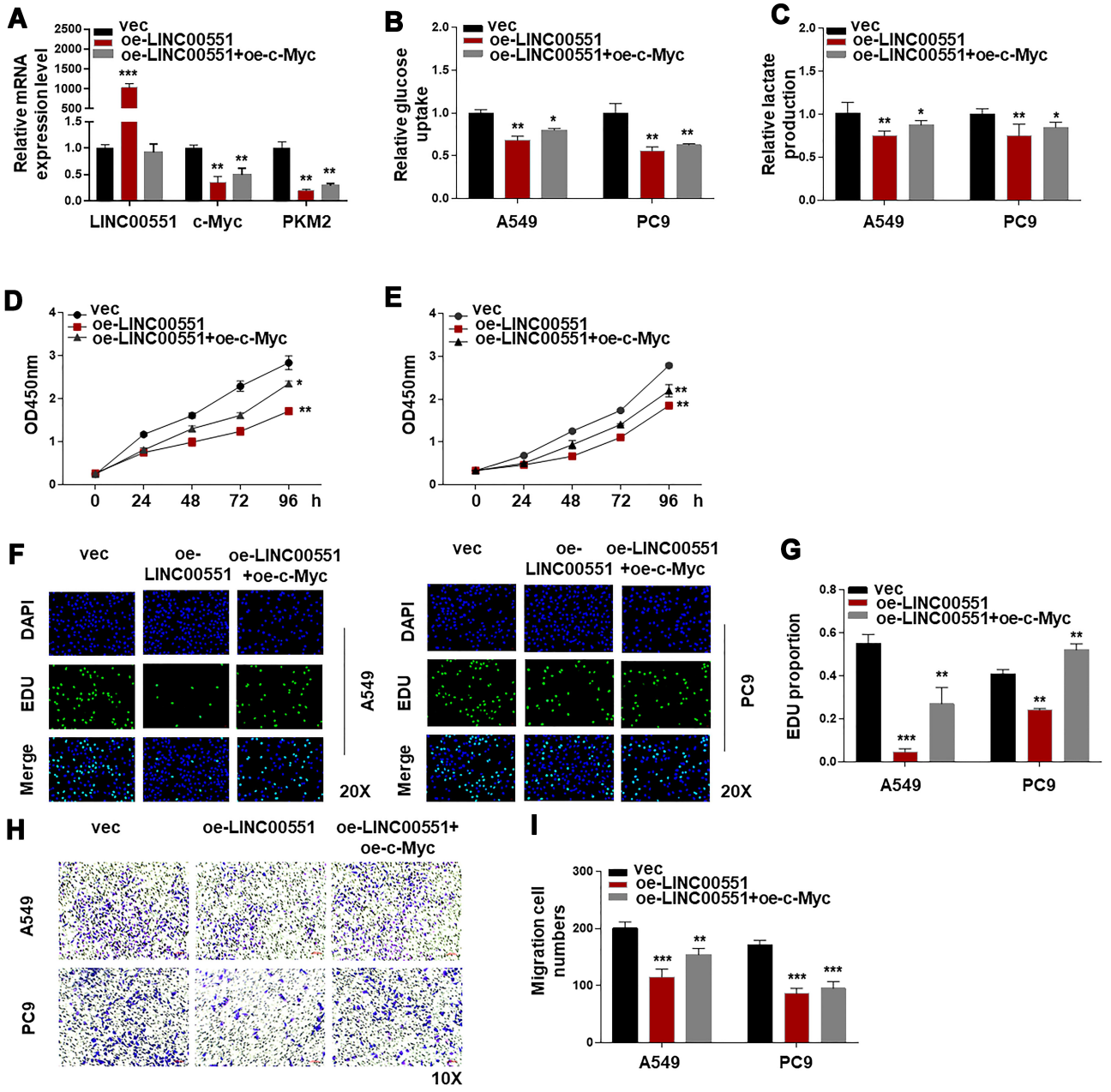

Figure 4 *LINC00551* regulated glucose metabolism of LUAD partly by controlling the expression of *c-Myc*. (**A**). c-Myc complementation in *LINC00551*-overexpressed cells (A549 and PC9) restored *PKM2* expression. (**B**–**C**). Ectopic overexpression of *c-Myc*rescued the influence of *LINC00551* on glucose metabolism of LUAD cells (A549 and PC9). (**D**–**I**). *c-Myc* partially reversed the effect of *LINC00551* on proliferation and migration of LUAD cells (A549 and PC9). (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

LINC00551 Overexpression Suppressed LUAD Development in vivo

To examine the function of *LINC00551* in vivo, xenograft tumors were utilized (Figure 5A). Overexpression of *LINC00551* markedly reduced tumor volume and weight (Figure 5B and C). We also testified that *LINC00551* was low expressed in harvested tumor nodules and plasma (Figure 5D-E). Additionally, high level of *PKM2* correlated with poor clinical prognosis in GEPIA dataset (Figure 5F). Altogether, these outcomes reveal that *LINC00551* suppresses the development of LUAD through *c-Myc* mediated glycolysis enzyme *PKM2* (Figure 5G).

Discussion

The late diagnosis and the complex processes involved in the development of cancer lead to poor LUAD prognosis.[16] Although the past decades saw significant progress in the treatment of the disease,[17] drug resistance

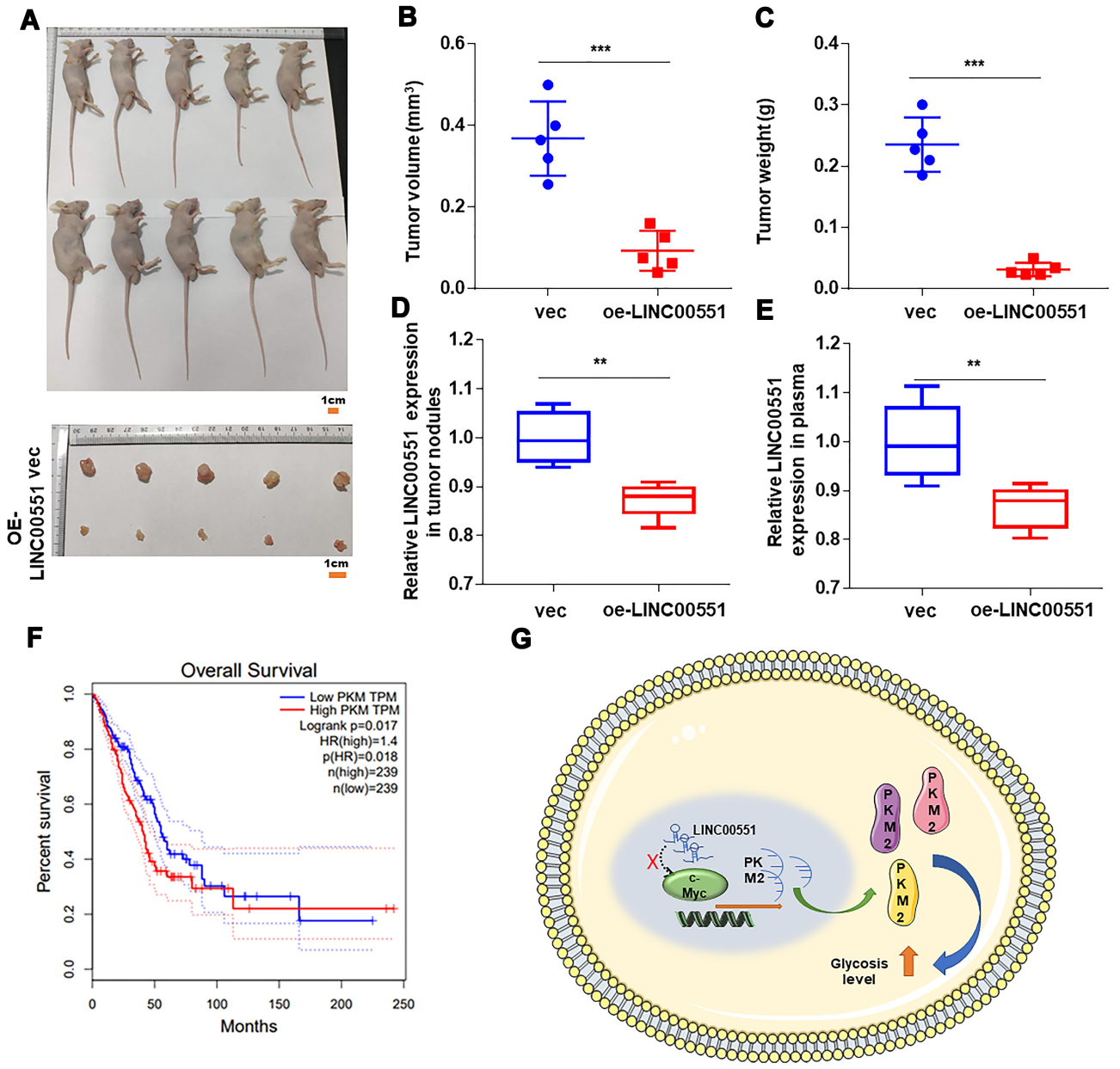

Figure 5 *LINC00551* repressed LUAD proliferation in vivo. (**A**). Harvested tumor nodules were shown. (**B**–**C**). The volume and weight of tumor nodules of each group are measured and the results revealed that *LINC00551* inhibited proliferation of LUAD. (**D**–**E**). *LINC00551* also low expressed in harvested tumor nodules and plasma. (**F**). Patients with high expression of *PKM* have a poor prognosis in GEPIA database. (**G**). Schematic diagram of *LINC00551* affects tumor progression through *c-Myc* regulation of *PKM*. The data are represented as the means ± SD (**$p < 0.01$ ***$p < 0.001$).

and side effects are significant obstacles to effective targeted therapy.[18,19] Thus, there is an urgent need to identify key molecular drivers of LUAD to yield new targets for its therapy. Recent studies revealed an aberrant expression of lncRNAs in many cancers which promotes tumor initiation and progression.[20]

Previously *LINC00551* has been reported to be downregulated in esophageal squamous cancer and inhibited cancer proliferation and invasion by increase in *HSP27*phosphorylation.[21] In the present study, we showed that *LINC00551* suppressed LUAD cell proliferation, and abnormally expressed *LINC00551* are related with clinical pathological characteristics and overall survival of LUAD patients. Moreover, we revealed that *LINC00551* participates in metabolic processes and thus affects tumor development. Altered glucose metabolism is a hallmark of cancer that is essential for the progression of many tumors.[22] Growing evidence suggests that lncRNAs regulate glucose metabolism in cancer cells[23–25] by modulating enzymes, regulatory molecules, metabolism-related signaling

pathways, and oncogenes.[22,26–30] In agreement with these findings, our results suggest that *LINC00551* exerts its anticancer effects by inhibiting glycolysis via repression of *c-Myc*-mediated transcription of *PKM2*. Most glucose metabolic pathway associated genes are transcriptional targets of *Myc*. For instance, *Myc* increases the expression of genes coding hexokinase and glucose transporters enhancing glucose uptake, and stimulates various glycolytic genes expression, such as that encoding *phosphoglucose isomerase, phosphofructokinase, glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase, enolase,* as well as *lactate dehydrogenase A*.[15,29,31,32] *Myc* also regulates the alternative pyruvate kinase (*PK*) splicing, a key determiner of the glycolytic pathway, by increasing the heterogeneous nuclear ribonucleoproteins (*hnRNP*) expression. *PK* is composed of two different isoforms: *PKM M1*, which is expressed in most adult tissues, and *PKM M2*, which is expressed mainly during embryonic development. While aerobic glycolysis activated by *PKM M2*, oxidative phosphorylation is endorsed by *PKM M1*. By upregulating the expression of *hnRNPA1* and *hnRNPA2*, *Myc* preserves an ultimate *PKM M2*/*PKM M1* ratio by increasing *hnRNPA1* and *hnRNPA2* expression, ensuring peak glycolysis when oxygen is available.[33]

LncRNAs, as regulators of metabolism, may constitute attractive targets for cancer therapy. Goldberg and Sharp found that siRNA-mediated knockdown of *PKM2* inhibited tumor development and increased cell apoptosis in vitro. Besides, this induced tumor regression in mouse xenograft model (in vivo).[34] Pusapati and colleagues showed that inhibition of glycolysis in combination with *mTOR* inhibitors prevented metabolic reprogramming and induced cancer cell apoptosis.[34] These antiglycolytic mediators might exhibit high efficacy when combined.

In conclusion, a better understanding of the mechanisms regulating aerobic glycolysis can help develop glycolytic inhibitors as anticancer agents. Our study showed that overexpression of *LINC00551* repressed *c-Myc*-mediated transcription of *PKM2*, thereby impairing the progression and glucose metabolism of LUAD cells. Thus, the novel lncRNA *LINC00551* is a possible biomarker as well as a prospective treatment target for LUAD.

Conclusions

In this study, we found lncRNA *LINC00551*, worked as a suppressor in regulating LUAD cells glucose metabolism through *c-Myc*-mediated transcription of *PKM2*, can be a possible treatment target for LUAD.

Abbreviations

LUAD, lung adenocarcinoma; lncRNA, long noncoding RNA; NSCLC, non-small-cell lung cancer.

Data Sharing Statement

All data involved in this study are contained in this published paper and its supplementary materials.

Ethics Statement

This study was complied with the Declaration of Helsinki. All human-based research in the study was approved by Nanjing Hospital affiliated to Nanjing Medical University. And All animal studies were conducted in accordance with NIH animal use guidelines and approved by Experimental Animal Ethics Committee of Nanjing Hospital affiliated to Nanjing Medical University.

Acknowledgment

The authors thank Nanjing Xinjia Medical Technology Co. Ltd for providing technical platform support.

Author Contributions

Jinsong Yang and Jing Luo designed and supervised the study. Li Wang, Huishan Wang and Bining Wu performed most of the experiments. Li Wang, Bining Wu, Hualin Yu and Xueyan Li participated in acquisition of data. Chengfeng Fan, Xiaoli Shi, and Chun Zhang denoted to analyze and interpret data. Jinsong Yang and Jing Luo agreed to submit to the current journal. All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. Consent for publication: All authors read and approved the final version of the manuscript. These authors contributed equally to the work and should be regarded as joint first authors: Li Wang, Huishan Wang, and Bining Wu.

Funding

This research was supported by the Medical Science Development Subject in Science and Technology Project of Nanjing (Grant No. ZKX13017) the Natural Science Foundation of Jiangsu province (No. BK20151086), the National Nature Scientific Foundation of China

(No.81673030). National Science Foundation for Natural Youth Grant (No. 81902354).

Disclosure

The authors report no conflicts of interest for this work.

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