Low expression of *LINC00982* and *PRDM16* is associated with altered gene expression, damaged pathways and poor survival in lung adenocarcinoma

WENWEN LV[1,2*], XIAO YU[1*], WENXING LI[3,4], NANNAN FENG[1], TIENAN FENG[1,2], YU WANG[1], HONGYAN LIN[1] and BIYUN QIAN[1,2]

[1]Hongqiao International Institute of Medicine, Shanghai Tongren Hospital/School of Public Health; [2]Clinical Research Center, Shanghai Jiao Tong University School of Medicine, Shanghai 200025; [3]State Key Laboratory of Genetic Resources and Evolution, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, Yunnan 650223; [4]Kunming College of Life Science, University of Chinese Academy of Sciences, Kunming, Yunnan 650204, P.R. China

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Abstract. Recently, long non-coding RNAs (lncRNAs) have been shown to play critical roles in lung adenocarcinoma (LUAD). The present study aimed to explore the effect of *LINC00982* and *PRDM16* on clinical features and survival in LUAD. We found that LUAD patients demonstrated lower expression and copy number variation but higher methylation of long intergenic non-protein coding RNA 982 (*LINC00982*) and PR domain containing 16 (*PRDM16*) compared with controls. Thus, we divided the LUAD patients into two groups according to the median expression of *LINC00982* and *PRDM16*. Through differential expression, KEGG pathway enrichment and Ingenuity® Pathway Analysis (IPA), we found that patients with low expression of both *LINC00982* and *PRDM16* presented with more deregulated genes, as well as more significant pathways, than patients with high expression of these two genes. In addition, Kaplan-Meier curves and Cox proportional hazards models revealed that patients with low expression of *LINC00982*, *PRDM16* or both, showed poorer survival than the groups with high expression of *LINC00982*, *PRDM16*. We further used multivariate survival models to verify these results. Furthermore, we confirmed that the expression of *LINC00982* and *PRDM16* was significantly decreased in LUAD cell lines compared to normal cell lines *in vitro*. In conclusion, the present study revealed that *LINC00982* and *PRDM16* may serve as biomarkers or potential drug targets for the diagnosis and therapy of LUAD.

Correspondence to: Professor Biyun Qian, Hongqiao International Institute of Medicine, Shanghai Tongren Hospital/School of Public Health, Shanghai Jiao Tong University School of Medicine, 227 South Chongqing Road, Shanghai 200025, P.R. China
E-mail: qianbiyun@stju.edu.cn

*Contributed equally

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Introduction

Lung cancer is one of the most common malignancies in humans and is the main cause of cancer-related deaths (1). Non-small cell lung cancer (NSCLC), accounts for ~80% of all lung cancer cases and can be divided into different histological types, including adenocarcinoma, squamous cell carcinoma and large cell carcinoma, of which lung adenocarcinoma (LUAD) is the most common subtype (2). Although significant progress has been made in regards to surgery, chemotherapy, radiation therapy and molecular-targeted therapy in the past few years, the overall 5-year survival rate of lung cancer patients is still only ~15% (3). This is due to the lack of effective early diagnostic methods and the limited efficacy of current therapies. Thus, the importance of discovering simple and effective biomarkers is not only reflected in early diagnosis, but also in improving the prognosis of lung cancer patients.

Compared with the study of biomarkers of protein-coding genes, human studies on non-coding RNA are relatively few. However, the human genome contains more than 98% non-protein coding sequences, with the vast majority transcribed into long non-coding RNAs (lncRNAs) that are >200 bases in length. In recent years, lncRNAs have been reported to serve as diagnostic and prognostic markers in cancer (4-8). In lung cancer, lncRNAs, such as *MALAT-1* (9) and *HOTAIR* (10), have been associated with cancer development. We also previously identified a series of differentially expressed lncRNAs in 12 pairs of NSCLC tumors and adjacent tumor tissues (8), and levels of long intergenic non-protein coding RNA 982 (*LINC00982*) and PR domain containing 16 (*PRDM16*) were lower in NSCLC tumors than levels in adjacent non-tumor tissues (Fig. 1A and B). In the present study, we explored the expression and prognostic value of *LINC00982* in lung cancer.

LINC00982, located on chromosome 1p36.32, has 2 transcripts and has been reported to be a tumor suppressor in gastric cancer (11,12). However, no studies are available regarding the biological function of *LINC00982* in lung cancer. In the present study, we observed that *LINC00982* and *PRDM16* share the same enhancer, ACTRT2 (enhancer ID: GH01F003274). Dysfunction of PRDM16 has been found in many diseases. In astrocytoma patients, poor prognosis can be predicted by the hypomethylation status of the *PRDM16* promoter (13). Some recent studies have reported that PRDM16 plays a significant role in the development of cancer such as prostate (14), colorectal (15,16) and myeloid cancers (17,18). As in lung cancer, the *PRDM16* promoter has been reported to be methylated and upregulated *PRDM16* suppressed lung cancer cell growth (19), but the value of this gene for diagnosis and prognosis has not been fully explored.

Herein, we first analyzed the independent effect of *LINC00982* and *PRDM16* expression on the clinical features and survival status of LUAD patients and further explored the combined effect of *LINC00982* and *PRDM16* expression on global gene expression, potentially affected pathways and biological functions and the prognosis of LUAD patients.

Materials and methods

Data sources. LUAD transcriptome and clinical data were downloaded from The Cancer Genome Atlas (TCGA, https://tcga-data.nci.nih.gov/) and the cBioPortal (http://www.cbioportal.org/) database in May 2016 (20,21). LUAD DNA promoter methylation data were collected from MethHC (http://methhc.mbc.nctu.edu.tw/php/search.php?opt=gene). In total, we downloaded TCGA level 3 data from 515 LUAD patients and 59 controls. All samples had RNA sequencing on the Illumina HiSeq 2000 version 2 platform and were normalized by the 'RNA-Seq by Expectation-Maximization' (RSEM) method. Copy number data on *LINC00982* (also called FLJ42875), *PRDM16* and epidermal growth factor receptor (*EGFR*) were also downloaded from cBioPortal database. We divided the LUAD patients into groups according to the median expression of *LINC00982* (3.89) and *PRDM16* (7.42). Patients in the high-PRDM16 group (≥7.42) and high-LINC00982 group (≥3.89) were designated as the 'both-high' group, and those with a low expression of *PRDM16* (<7.42) and *LINC00982* (<3.89) were considered as the 'both-low' group. In addition, we analyzed the expression profiles of *PRDM16* and *LINC00982* in lung squamous cell carcinoma (LUSC). Gene expression and clinical data on LUSC (including 501 patients and 51 controls) were also downloaded from TCGA and analyzed in the same way as the LUAD data.

Differential expression analysis. A paired sample t-test was used to analyze the differential gene expression and DNA methylation of *LINC00982* and *PRDM16* between the 59 paired tumor tissue and adjacent normal tissues. In order to illustrate the association between copy number variation and gene expression of *LINC00982* and *PRDM16*, we matched LUAD patient IDs and then divided these patients into high- and low-expression groups according to the median expression of *LINC00982* and *PRDM16*. The Mann-Whitney U statistic was used to calculate the differences between the two groups. We extracted expression information on *EGFR* and used it as a reference. Spearman's correlation analysis was used to explore the association of *LINC00982* and *PRDM16* expression. A P-value <0.05 was considered to indicate a statistically significant difference.

Differentially expressed genes in the 'both-high' and 'both-low' groups were analyzed using R v 3.3.3 (https://www.r-project.org/) and the bioconductor ibrary (https://bioconductor.org/packages). The empirical Bayes algorithm (function 'eBayes') in the limma package (22) was used to detect differentially expressed genes between the 'both-high' and 'both-low' groups and controls. We converted all gene expression values to z-scores and used heatmaps in the 'pheatmap' package(https://CRAN.R-project.org/package=pheatmap) to show the results. Significantly differentially expressed genes (upregulated or downregulated) were considered as an absolute value of the logarithmic transformed fold-change (log2 (FC)) ≥1 and a false discovery rate (FDR)-adjusted P-value ≤0.05. A Venn diagram was used to compare the upregulated and downregulated genes and affected pathways between the 'both-high' and 'both-low' groups, respectively.

Pathway enrichment analysis. We performed KEGG pathway enrichment analysis using differentially expressed genes in the 'both-high' and 'both-low' groups. The following formula was used to conduct the enrichment analysis:

$$P(X=k)=1-\frac{C_m^k \cdot C_{N-m}^{n-k}}{C_N^n}$$

Where N is the number of all genes in the dataset, m represents the number of differentially expressed genes in the dataset, n is the number of all genes in the enriched KEGG pathway and k is the number of differentially expressed genes in the KEGG pathway. An FDR P-value ≤0.05 was considered significantly enriched. The enrichment percentage in each subsystem was calculated as the number of differentially expressed genes divided by the number of all genes.

Gene co-expression with *PRDM16* and *LINC00982* was defined by the Spearman's correlation coefficient between each gene and *PRDM16* and *LINC00982* expression. Genes with an absolute Spearman's correlation coefficient >0.3 were considered to be co-expressed with *PRDM16* and *LINC00982*. In LUAD, the Spearman's correlation coefficient information was downloaded from cBioPortal (http://www.cbioportal.org/index.do). Co-expressed genes were uploaded into the Ingenuity Pathway Analysis software (Qiagen Redwood City Inc., Redwood City, CA, USA) to compare enriched pathways.

Clinicopathological and survival analysis. For clinical data analysis, categorical variables (i.e., sex, race, residual tumors, primary site, stage and smoking history) were given as numbers and percentages. Continuous variables (e.g., age) are presented as the mean ± standard deviation (SD). Student's t-tests were used to compare the means for continuous variables in two groups, and χ^2 tests were used to compare the prevalence of categorical variables. Kaplan-Meier survival curves were constructed to compare differences in overall survival

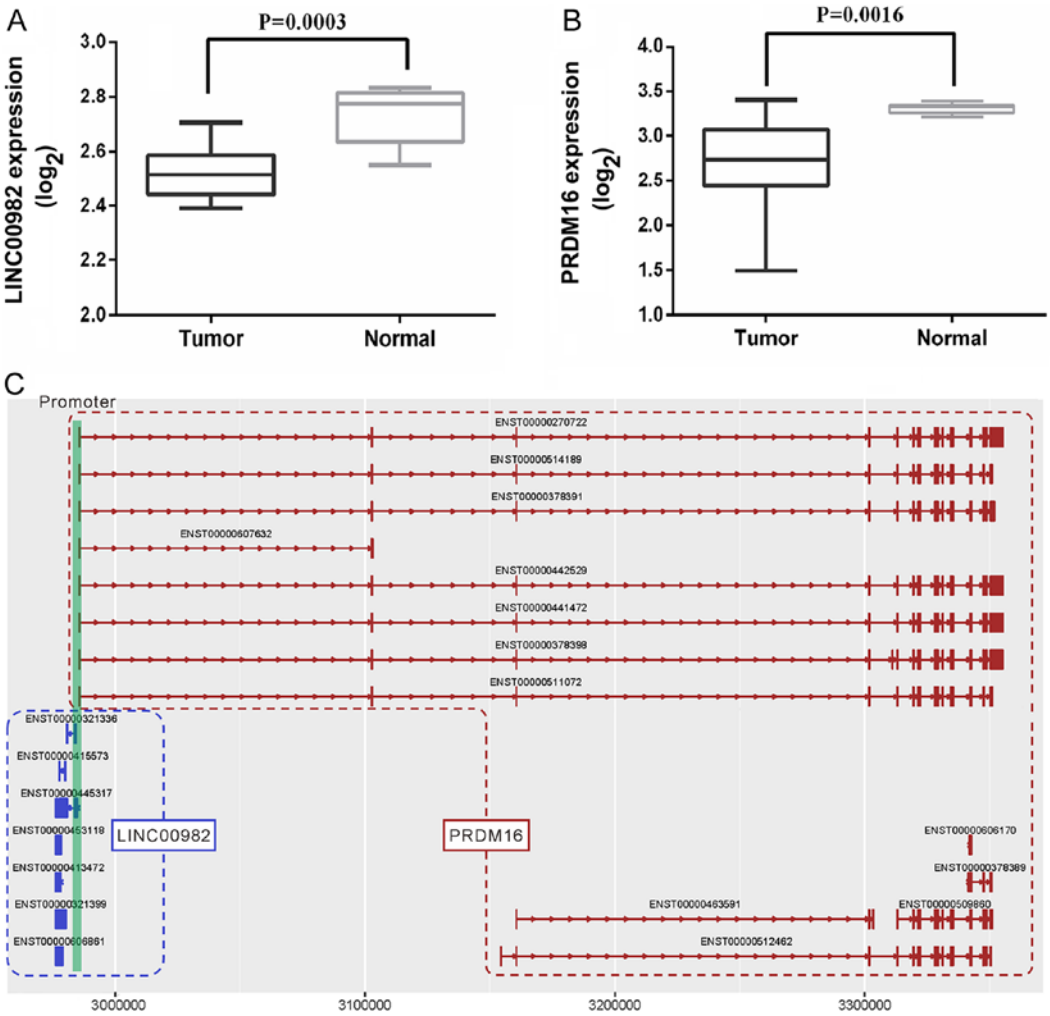

Figure 1. Expression profiles of (A) *LINC00982* and (B) *PRDM16* in NSCLC and (C) their gene structures on chromosome 1. *LINC00982*, long intergenic non-protein coding RNA 982; *PRDM16*, PR domain containing 16; NSCLC, non-small cell lung cancer.

and disease-free survival between the high-LINC00982 and low-LINC00982 groups and the high-PRDM16 and low-PRDM16 groups, as well as the 'both-low' and 'both-high' groups. The log-rank test was used to assess differences in survival between groups using the 'survival' package in R. Furthermore, we analyzed the association of *LINC00982* and *PRDM16* expression on overall survival and disease-free survival stratified by tumor stage. The effect of *LINC00982* and *PRDM16* expression and other clinicopathological factors (sex, age, residual tumors, primary site, stage and smoking status) on overall survival and disease-free survival was analyzed by using univariate Cox regression models. A multivariate Cox regression model was used to compare the independent effect of *LINC00982* and *PRDM16* expression on overall survival and disease-free survival and adjusted for corresponding covariates (smoking history, primary site, residual tumors and stage).

Cell lines. Human LUAD cell lines A549, H1299 and H1975 and a normal lung epithelium cell line (BEAS-2B) were obtained from the Chinese Academy of Sciences Committee on Type Culture Collection Cell Bank (Shanghai, China). All cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM; Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS; Sigma-Aldrich; Merck KGaA, Darmstadt, Germany), 25 U/ml penicillin and 25 μg/ml streptomycin at 37°C in 5% CO_2.

Quantitative real-time PCR (RT-qPCR) analysis. Total RNA was extracted from cell lines samples with Invitrogen™ TRIzol reagent (Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. Reverse-transcription PCR was performed with the Prime-Script RT Reagent kit (Tiangen Biotech, Beijing, China). Gene expression levels were determined by RT-qPCR and normalized against an endogenous control (*β-actin*) using SYBR Premix Ex Taq (ABI; Thermo Fisher Scientific, Inc.). Data were analyzed using the ΔΔCt approach and expressed as the target gene/β-actin ratio [$2^{-\Delta Ct\ (target\ gene\ -\ \beta\text{-}actin)}$]. The primers of the longer transcription of *LINC00982* (NR_015440.1, termed LINC00982-1) were as follows: Forward: 5'-CCGGCCCTCTTAGCTTCAAA-3' and reverse, 5'-GTGGAAAAGAAACCCACCGC-3'. The primers of the shorter transcription of LINC00982 (NR_024371.1, termed LINC00982-2) were as follows: Forward: 5'-GCT TCCCTTCCGTTCACTCA-3' and reverse: 5'-GGCTGAGTC TTTCTGGACCC-3'. Primers for *PRDM16* were as follows:

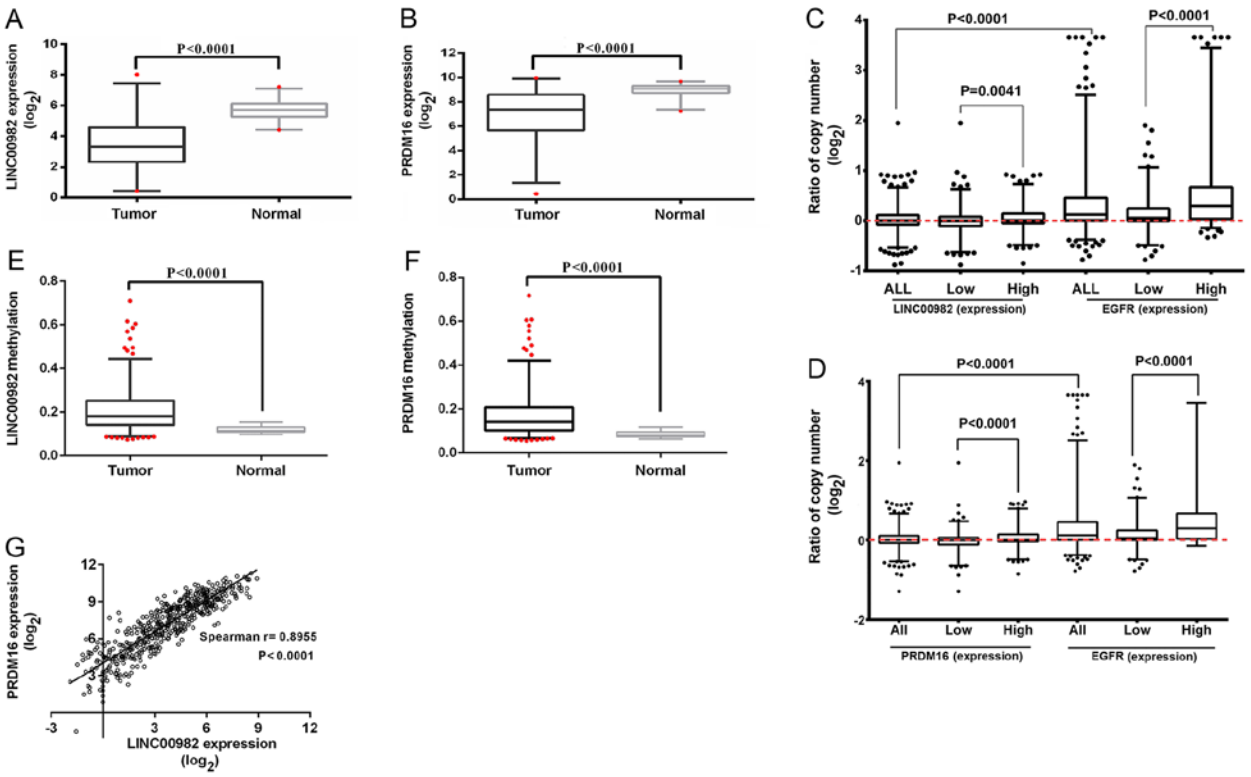

Figure 2. Effect of the expression of *LINC00982* and *PRDM16* on lung adenocarcinoma. (A and B) Gene expression of *LINC00982* and *PRDM16* in tumor samples and adjacent normal tissues. (C and D) Copy number variations of LINC00982 and PRDM16 in tumor samples and adjacent normal tissues; the EGFR was used as a reference. (E and F) Methylation status of *LINC00982* and *PRDM16* in tumor samples and adjacent normal tissues. (G) Spearman's correlation between *LINC00982* and *PRDM16* expression. *LINC00982*, long intergenic non-protein coding RNA 982; *PRDM16*, PR domain containing 16; EGFR, epidermal growth factor receptor.

forward: 5'-GTTCTGCGTGGATGCAAATCA-3' and reverse: 5'-GGTGAGGTTCTGGTCATCGC-3'. Data analysis was conducted using GraphPad Prism 6 (GraphPad Software, Inc., La Jolla, CA, USA). Data were analyzed using one-way analysis of variance (ANOVA) followed by the Least Significant Difference (LSD) method.

Results

LINC00982 and PRDM16 expression profiles in NSCLC. The expression of *LINC00982* and *PRDM16* in 59 human LUAD tissues was significantly decreased compared to the paired adjacent normal lung tissues (Fig. 2A and B). In addition, the same trend was observed in the LUSC tissues (data not shown). Furthermore, we stratified LUAD patients by the median expression of *LINC00982* and *PRDM16* and found that the low-*LINC00982* (<3.89) and low-*PRDM16* (<7.42) groups were decreased in tumors compared to adjacent normal lung tissues, whereas the high-*LINC00982* (≥3.89) and high-*PRDM16* (≥7.42) groups had no significant changes (data not shown). Analysis of copy number variations revealed that the copy number of the two genes was also lower in patients with low gene expression (Fig. 2C and D). *EGFR* expression was positively associated with gene copy number (Fig. 2C and D) as had been previously observed (23). In addition, the low level of expression was consistent with the hypermethylation of the promoter region of these two genes in tumor samples compared with adjacent tissues (Fig. 2E and F). We analyzed the Spearman's correlation between *LINC00982* and *PRDM16* expression and found that they were positively correlated (Fig. 2G).

We used Kaplan-Meier curves to explore the effect of *LINC00982* and *PRDM16* expression on LUAD patient survival status. The results indicated that patients with low expression of *LINC00982* or *PRDM16* showed poor overall survival and disease-free survival than the high-expression corresponding groups, although the effect of *LINC00982* on disease-free survival did not reach the significance threshold (Fig. 3). We also analyzed the effect of these two genes on survival in LUSC patients; however, neither affected survival status (data not shown). Therefore, we focused on the influence of *LINC00982* and *PRDM16* on LUAD in the subsequent analyses. In order to study the combined effect of *LINC00982* and *PRDM16* on patient survival, we combined the low-LINC00982 group and low-PRDM16 group into the 'both-low' group, as well as combining the high-LINC00982 group and high-PRDM16 group into the 'both-high' group. The Kaplan-Meier curves revealed that compared with the 'both-low' group, patients with high expression of these two genes presented with significantly prolonged overall survival (HR=0.55, P<0.001) and disease-free survival (HR=0.72, P=0.042) (Fig. 4). We also observed a consistent trend in patients with early-stage disease (I/II) (data not shown).

Gene expression and pathway analysis of LINC00982 and PRDM16 in LUAD. In Fig. 5 the gene expression profiles and KEGG pathway enrichment results in LUAD patients are displayed. Genes with an expression value of zero were removed.

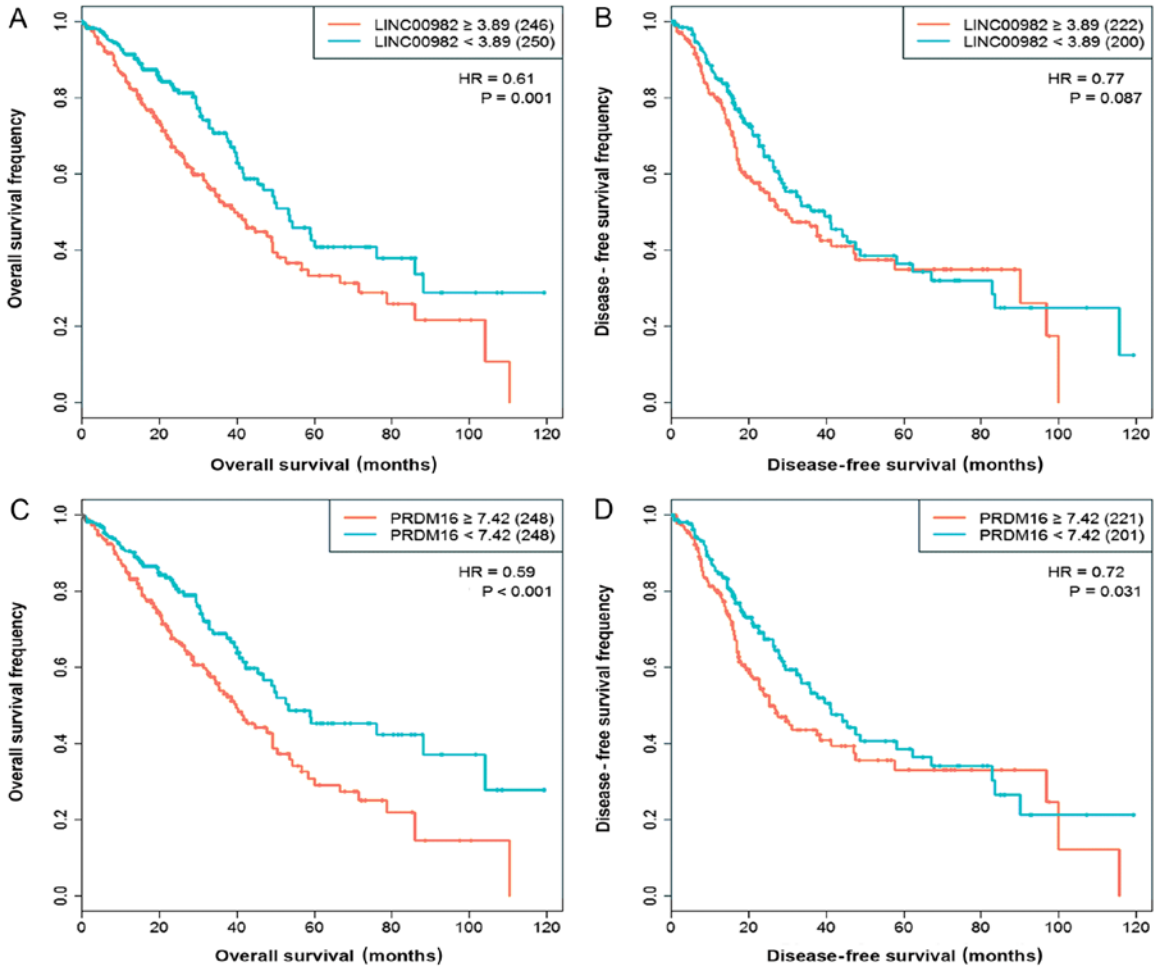

Figure 3. Effect of *LINC00982* and *PRDM16* expression on LUAD patient survival. (A) Effect of *LINC00982* expression on overall survival. (B) Effect of *LINC00982* expression on disease-free survival. (C) Effect of *PRDM16* expression on overall survival. (D) Effect of *PRDM16* expression on disease-free survival. LUAD, lung adenocarcinoma; HR, hazard ratio; *LINC00982*, long intergenic non-protein coding RNA 982; *PRDM16*, PR domain containing 16.

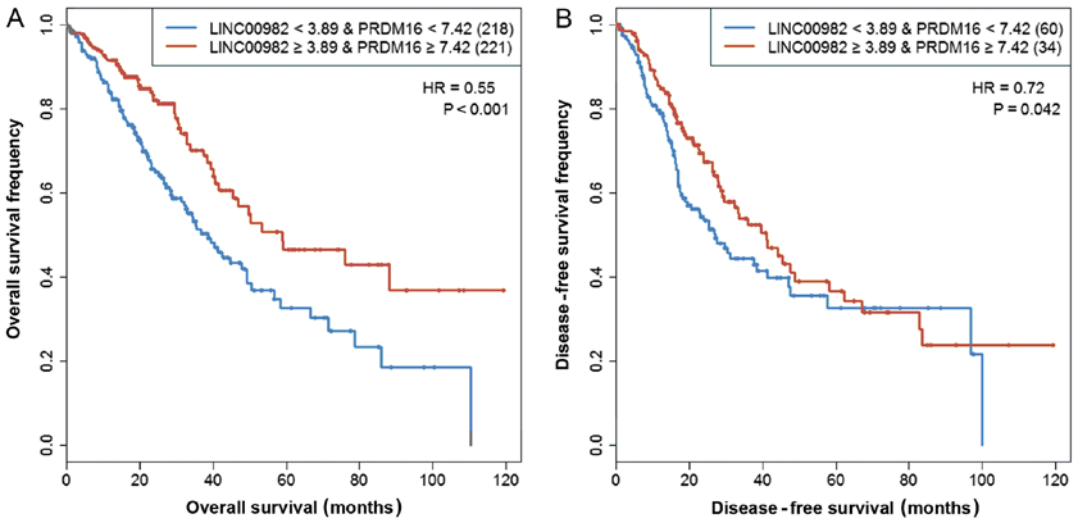

Figure 4. Interaction effect of LINC00982 and PRDM16 expression on (A) overall survival and (B) disease-free survival in LUAD patients. HR, hazard ratio; *LINC00982*, long intergenic non-protein coding RNA 982; *PRDM16*, PR domain containing 16; LUAD, lung adenocarcinoma.

In total, we assessed the gene expression of 19,606 genes in 515 LUAD patients and 59 controls (data not shown). The global gene expression in the high-LINC00982 group and low-LINC00982 group revealed a relatively large difference, as well as the high-PRDM16 and low-PRDM16 patients. Other characteristics (sex, age, race, smoking status and stage) were

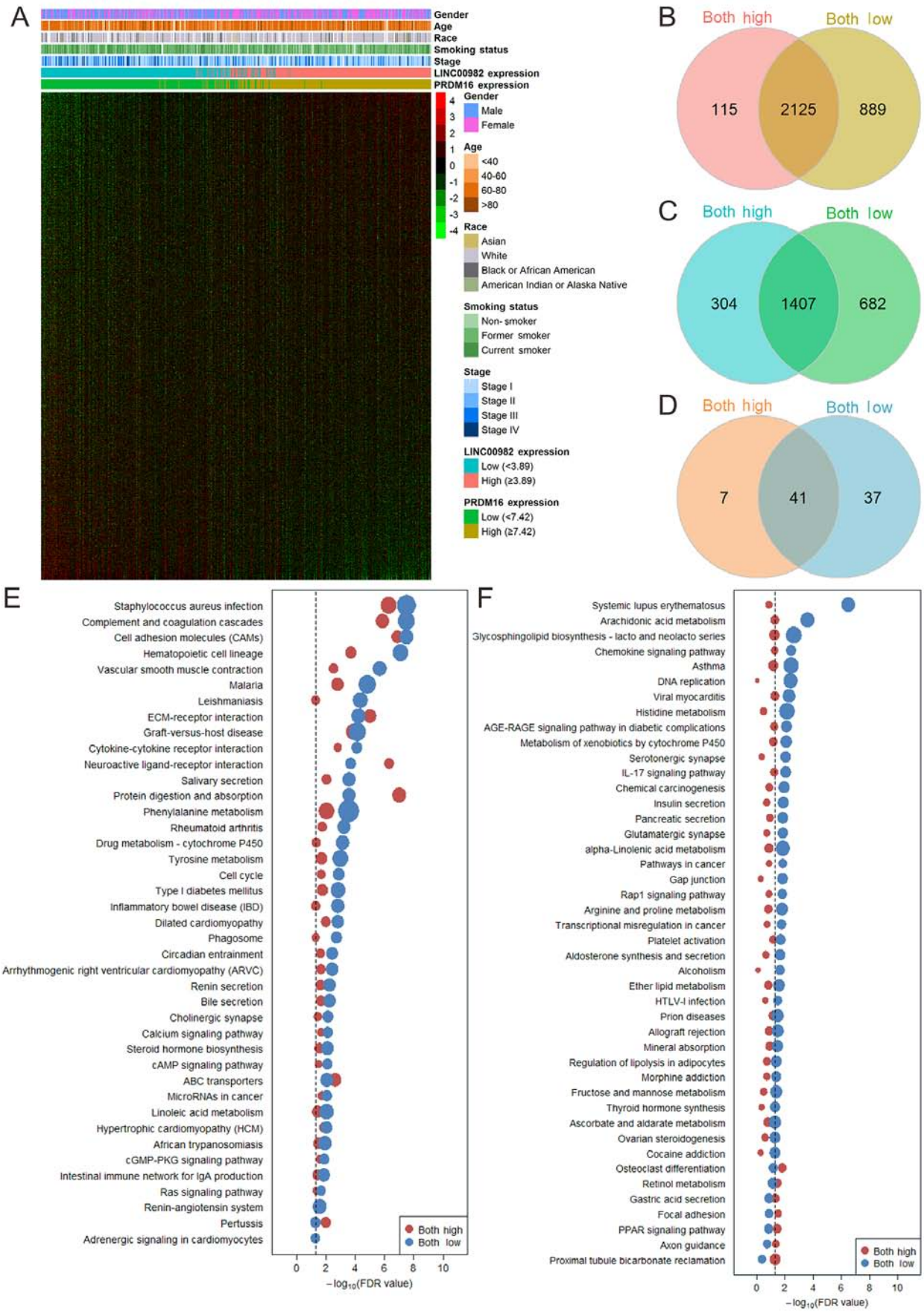

Figure 5. *LINC00982* and *PRDM16* genes expression profiles and pathway enrichment results. (A) Global gene expression of LUAD patients. All expression values were converted to z-score. (B and C) Venn diagram of upregulated and downregulated genes in both-high and both-low group. (D) Enriched KEGG pathways in 'both-high' and 'both-low' group. (E and F) Commonly and differentially enriched pathways in 'both-high' and 'both-low' group. The circle size indicates the enrichment percentages of the KEGG pathway. The dashed line indicates the significance. *LINC00982*, long intergenic non-protein coding RNA 982; *PRDM16*, PR domain containing 16; LUAD, lung adenocarcinoma.

Table I. Lung adenocarcinoma patient characteristics stratified by *LINC00982* and *PRDM16* expression.

Patient characteristics	*LINC00982* expression			*PRDM16* expression		
	High (≥3.89)	Low (<3.89)	P-value	High (≥7.42)	Low (<7.42)	P-value
Sex, n (%)						
Male	107 (41.5)	130 (50.8)	0.043	101 (39.1)	136 (53.1)	0.002
Female	151 (58.5)	126 (49.2)		157 (60.9)	120 (46.9)	
Mean age, years	65.8±9.4	64.9±10.1	0.297	65.9±9.1	64.8±10.3	0.221
Race, n (%)						
Asian	5 (2.2)	3 (1.4)	0.768	4 (1.7)	4 (1.8)	0.541
White	199 (87.3)	189 (85.5)		204 (87.9)	184 (84.8)	
Black/African American	23 (10.1)	28 (12.7)		22 (9.5)	29 (13.4)	
American Indian/Alaska Native	1 (0.4)	1 (0.5)		2 (0.9)	0	
Residual tumor, n (%)						
R0	163 (94.7)	181 (95.8)	0.896	159 (94.1)	185 (96.4)	0.549
R1	7 (4.1)	6 (3.2)		8 (4.7)	5 (2.5)	
R2	2 (1.2)	2 (1.0)		2 (1.2)	2 (1.0)	
Primary site, n (%)						
L-lower	46 (18.3)	32 (12.9)	0.245	40 (16)	38 (15.3)	0.626
L-upper	53 (21.1)	70 (28.2)		56 (22.4)	67 (26.9)	
R-lower	50 (19.9)	46 (18.5)		51 (20.4)	45 (18.1)	
R-middle	12 (4.8)	9 (3.6)		13 (5.2)	8 (3.2)	
R-upper	90 (35.9)	91 (36.7)		90 (36)	91 (36.5)	
Smoking, n (%)						
Never smoked	49 (19.7)	26 (10.5)	0.002	49 (19.6)	26 (10.6)	<0.001
Current smoker	47 (19)	72 (29)		46 (18.4)	73 (29.7)	
Former smoker	152 (61.3)	150 (60.5)		155 (62)	147 (59.8)	
Stage, n (%)						
I	152 (60.6)	123 (48.2)	0.009	149 (59.4)	126 (49.4)	0.076
II	59 (23.5)	63 (24.7)		59 (23.5)	63 (24.7)	
III	29 (11.6)	55 (21.6)		33 (13.1)	51 (20.0)	
IV	11 (4.4)	14 (5.5)		10 (4.0)	15 (5.9)	

LINC00982, long intergenic non-protein coding RNA 982; *PRDM16*, PR domain containing 16.

approximately randomly distributed, indicating that these variables contributed less to gene expression changes. A Venn diagram of differentially expressed genes in the 'both-high' and 'both-low' groups compared with adjacent normal tissues is shown in Fig. 5B and C. In total, 3,951 and 5,103 differentially expressed genes were observed in the 'both-high' group (data not shown) and the 'both-low' group (data not shown), respectively. Furthermore, there were 2,125 common downregulated and 1,407 common upregulated genes between the 'both-high' and 'both-low' groups (Fig. 5B and C). From the KEGG enrichment results, there were 48 significantly enriched pathways in the 'both-high' group (data not shown) and 78 significantly enriched pathways in the 'both-low' group (data not shown). There were 41 commonly and 44 differentially enriched KEGG pathways between the two groups (Fig. 5D). The enrichment profiles indicated that most of the pathways resulted in serious damage in the 'both-low' group as compared to the 'both high'-group (Fig. 5E and F). We also analyzed the biological pathway enrichment of the genes co-expressed with *LINC00982* and *PRDM16* using Ingenuity Pathway Analysis (Fig. 6). There were several common pathways associated with co-expression of *LINC00982* and *PRDM16*, such as cyclins and cell cycle regulation, NSCLC signaling and ERK/MAPK signaling.

LINC00982 and PRDM16 expression, clinicopathological variables and patient survival. Table I depicts the 515 LUAD patient characteristics in the high- and low-expressed *LINC00982* and *PRDM16* groups. We observed that females exhibited higher expression of *LINC00982* (P=0.043) and *PRDM16* (P=0.002) compared with males. There were no differences in age, race, residual tumor or primary site between the high-LINC00982 and low-LINC00982 groups, or between the high-PRDM16 and low-PRDM16 groups. Furthermore, patients in the low-LINC00982 group showed higher smoking frequency (P=0.002) and more serious disease stage (P=0.009)

Table II. Association of *LINC00982* and *PRDM16* expression, clinicopathological characteristics and survival status.

		Overall survival		Disease-free survival	
Variable	Total N	Hazard ratio (95% CI)	P-value	Hazard ratio (95% CI)	P-value
LINC00982 expression (continuous)	515	0.87 (0.81-0.94)	<0.001	0.93 (0.86-1.00)	0.038
PRDM16 expression (continuous)	515	0.89 (0.83-0.95)	<0.001	0.92 (0.86-0.98)	0.011
LINC00982 expression (categorical, above or below 3.88)	515	0.57 (0.42-0.76)	<0.001	0.74 (0.55-0.99)	0.040
PRDM16 expression (categorical, above or below 7.41)	515	0.58 (0.43-0.79)	<0.001	0.71 (0.53-0.95)	0.022
Sex (male vs. female)	514	0.94 (0.70-1.26)	0.672	0.97 (0.73-1.30)	0.846
Age (continuous)	495	1.01 (0.99-1.02)	0.333	1.00 (0.99-1.02)	0.354
Residual tumor (R0 vs. R1 or R2)	361	2.22 (1.37-3.60)	0.001	3.64 (1.83-7.23)	<0.001
Primary site (L-site vs R-site)	499	1.04 (0.77-1.40)	0.814	1.11 (0.82-1.51)	0.493
Smoking (never smoker vs. current smoker or former smoker)	496	0.92 (0.61-1.38)	0.672	1.04 (0.68-1.58)	0.873
Stage (stage I or II vs. stage III or IV)	506	2.65 (1.95-3.62)	<0.001	1.73 (1.21-2.47)	0.003

LINC00982, long intergenic non-protein coding RNA 982; *PRDM16*, PR domain containing 16; CI, confidence interval.

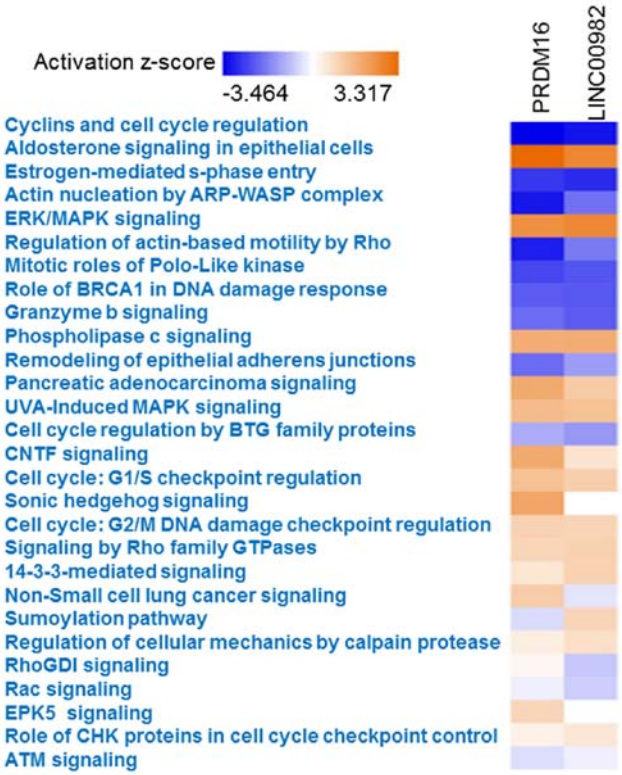

Figure 6. IPA analysis pathway enrichment in LUAD tissues of *LINC00982* and *PRDM16* co-expression genes in the TCGA cohort. All expression values were converted to z-score. All pathway P<0.05. LUAD, lung adenocarcinoma; *LINC00982*, long intergenic non-protein coding RNA 982; *PRDM16*, PR domain containing 16.

compared with the high-LINC00982 group. We also observed more patients who currently smoke in the low-PRDM16 group compared with the high-PRDM16 group (P<0.001). The results for smoking status, stage and gene expression of *LINC00982* and *PRDM16* are not shown.

We used univariate Cox proportional hazards models to analyze the effect of *LINC00982* and *PRDM16* expression, as well as other clinicopathological variables, on patient survival status (Table II). We found that high expression of *LINC00982* in the continuous and categorical models all showed prolonged overall survival and disease-free survival (all P<0.05). Furthermore, the expression of *PRDM16* in the continuous and categorical models also associated with survival (all P<0.05). Other clinicopathological variables such as residual tumors and stage also showed significant association with survival status. Therefore, we used multivariate Cox proportional hazards models adjusting for covariates including residual tumor and stage to verify the effect of *LINC00982* and *PRDM16* expression on patient survival status (Table III). The results indicated that the *LINC00982* and *PRDM16* low-expression groups were both associated with decreased overall survival (all P<0.05). However, the expression of these two genes did not affect disease-free survival. The above analysis showed that *LINC00982* and *PRDM16* independently affected overall survival.

RT-qPCR validation. We explored *LINC00982-1*, *LINC00982-2* and *PRDM16* expression in LUAD cell lines (A549, H1299 and H1975) and a normal lung epithelium cell line (BEAS-2B) by RT-qPCR (Fig. 7). We found that *LINC00982-1*, *LINC00982-2*, the two transcripts of *LINC00982* and *PRDM16* expression were significantly decreased in LUAD cell lines (A549, H1299 and H1975) compared to normal cell lines (BEAS-2B).

Discussion

Our results revealed that the combined effect of *LINC00982* and *PRDM16* expression was a risk factor that affected global gene expression, altered cancer-related pathways and biological functions, and decreased patient survival in LUAD. Additionally, our experimental results revealed that *LINC00982* and *PRDM16* transcripts were down-regulated in LUAD cell lines compared with the normal BEAS-2B cell line.

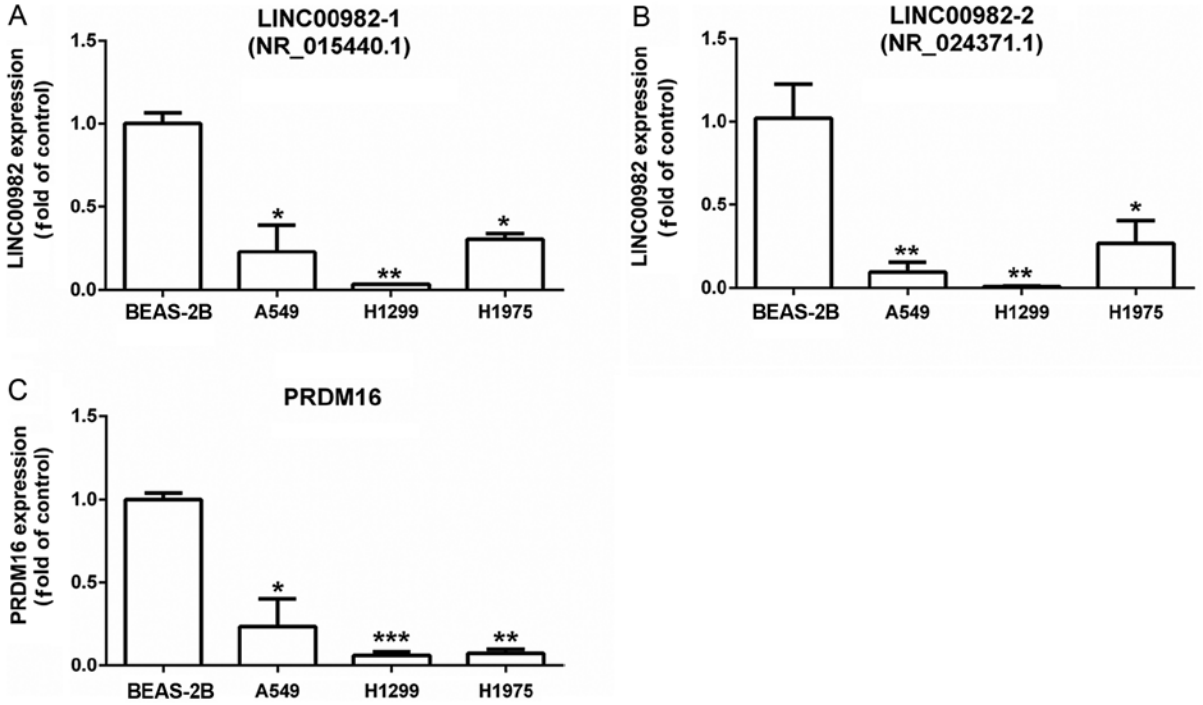

Figure 7. *LINC00982-1*, *LINC00982-2* and *PRDM16* expression in LUAD cell lines *in vitro*. BEAS-2B was used as the control, Bars, SD; *P<0.01; **P<0.001; ***P<0.0001. LUAD, lung adenocarcinoma; *LINC00982*, long intergenic non-protein coding RNA 982; *PRDM16*, PR domain containing 16.

Table III. Multivariate survival model of *LINC00982* and *PRDM16* expression on survival status.

Variable	Hazard ratio (95% CI)	P-value[a]
Overall survival		
LINC00982 expression (continuous)	0.89 (0.82-0.98)	0.015
LINC00982 expression (categorical, above or below 3.88)	0.66 (0.46-0.95)	0.023
PRDM16 expression (continuous)	0.91 (0.84-0.98)	0.019
PRDM16 expression (categorical, above or below 7.41)	0.62 (0.44-0.89)	0.008
Disease-free survival		
LINC00982 expression (continuous)	0.95 (0.87-1.04)	0.288
LINC00982 expression (categorical, above or below 3.88)	0.75 (0.53-1.08)	0.123
PRDM16 expression (continuous)	0.94 (0.87-1.03)	0.177
PRDM16 expression (categorical, above or below 7.41)	0.76 (0.53-1.08)	0.124

[a]Adjusted for residual tumor and stage. *LINC00982*, long intergenic non-protein coding RNA 982; *PRDM16*, PR domain containing 16; CI, confidence interval.

LUAD is a complex disease that is associated with altered gene expression, DNA methylation, protein modification and non-coding RNA dysfunction (24). In particular, lncRNAs play a significant role in cellular homeostasis and tumorigenesis and often serve as markers of prognosis and diagnostic targets for therapy (25). A recent study suggested that *CCAT2*, a LUAD-specific lncRNA, promoted invasion and metastasis of LUAD (26). In a previous study, Fei *et al* reported that *LINC00982* is dysregulated in gastric cancer patients, and the high expression of *LINC00982* was related to better overall survival (11). *LINC00982* is located about 0.5 kb telomeric at the 5' untranslated region of *PRDM16*, which suggests that these transcripts have the same enhancer, ACTRT2 (27). The *PRDM16* gene is not only associated with myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) (28), but also solid tumors such as lung cancer. Some studies have indicated that *PRDM16* expression is downregulated in lung cancer cells due to the methylation of its promoter (19), which was consistent with our results.

In the present study, compared with adjacent normal tissues, *LINC00982* and *PRDM16* expression were significantly decreased in tumor samples. Considering the increased methylation level of the promoter region and the decreased copy number in LUAD patients, we speculated that the dysregulation of *LINC00982* and *PRDM16* may be caused by DNA methylation and gene copy number disorders. Furthermore,

we used three survival analysis models to show that high expression of *LINC00982* and *PRDM16* was associated with higher patient survival, especially overall survival. In addition, patients with high expression of *LINC00982* and *PRDM16* demonstrated better overall survival and disease-free survival than patients with low expression of these two genes. Notably, these associations were consistent in patients with early tumor stages (stage I and II), combined with the evidence that high expression of *LINC00982* and *PRDM16* were related to low TNM stage, which may aid the early diagnosis of LUAD and improve the prognosis of affected patients, especially with a combination of changes in their expression.

Through pathway enrichment analysis, we found that PRDM16-associated genes were enriched in many canonical pathways, which are consistent with LINC00982-associated gene enriched pathways. However, the extents of the impact are differential, such as cyclins and cell cycle regulation, aldosterone signaling in epithelial cells, and estrogen-mediated S-phase entry. We observed that *LINC00982* and *PRDM16* were negatively associated with cyclins and cell cycle regulation in LUAD. Tumors are characterized by malignant cell growth and proliferation. Abnormalities in cell proliferation, differentiation and apoptosis are involved in the development and progression of tumors, and cell cycle disorder is the most important mechanism of tumor growth (29). The cell cycle is a highly orderly process. As a regulatory factor, cyclin overexpression is associated with carcinogenesis (30-32). In many tumor cells and proliferating cells, cyclin is overexpressed, and many tumor-suppressor genes such as *p53* (29), *BRCA1* (33) and *Rb* (34) play crucial roles in blocking the cell cycle. Consistent with our findings, a recent study reported that *LINC00982* inhibited cell proliferation and rendered cell cycle arrest in gastric cancer cells (11) and *PRDM16* was also reported to alter cell cycle distribution in stem (35), indicating that *LINC00982* and *PRDM16* may impede the occurrence and development of LUAD by mediating cell cycle arrest.

In recent years, precision medicine has increasingly been used in the treatment of cancer, especially in exploring and identifying biomarkers (36). The treatment of LUAD is typically carried out with multiple targeted therapies. Therefore, a better understanding of both coding genes and non-coding RNAs will help to improve the diagnosis and prognosis of human LUAD (37,38). In the present study, we identified *LINC00982* and *PRDM16* gene markers for predicting overall and disease-free survival based on RNA-Seq data that was obtained from TCGA. Additionally, after correcting for covariates, low expression of both *LINC00982* and *PRDM16* remained associated with reduced overall survival by Cox analysis models. Furthermore, by stratified analysis, low expression of both *LINC00982* and *PRDM16* was associated with poor overall survival and disease-free survival in stage I and II patients. In addition, we found that the risk ratio of *LINC00982* or *PRDM16* expression was lower than both *LINC00982* and *PRDM16* expression based on survival analysis. We therefore concluded that the interaction of *LINC00982* and *PRDM16* may play a significant role in the prognosis of LUAD patients than single *LINC00982* or *PRDM16* expression, and it was better to use these two genes as prognostic markers than using only one gene. However, we observed no association between the expression of *LINC00982* and *PRDM16* with patient survival in LUSC. This difference may be due to tumor heterogeneity if the genes that drive LUAD and LUSC are different (39). Finally, we observed that *LINC00982* and *PRDM16* were substantially decreased in human LUAD cell lines compared with a normal cell line. Therefore, we hypothesized that a combination of *LINC00982* and *PRDM16* expression may help to facilitate the prognosis of LUAD.

In conclusion, in the present study we found that *LINC00982* and *PRDM16* had low expression in tumor samples compared with adjacent normal tissues, and their expression levels were associated with their methylation status and copy number variations. Furthermore, patients with low expression of *LINC00982* and *PRDM16* were associated with more altered gene expression and influenced pathways compared with high-expression groups. In addition, independently and jointly, low expression of *LINC00982* and *PRDM16* was associated with poor patient survival, revealing that this combination had prognostic and diagnostic value. Our findings may also provide useful information to obtain a better understanding of LUAD. However, there are also several limitations in this study. Firstly, the biological functions of *LINC00982* and *PRDM16* need to be validated in cell and animal experiments. Secondly, it was a retrospective study, and as these findings are based on the reanalysis of TCGA data, prospective random population studies are needed to confirm these promising results. Lastly, data concerning drug therapy and prognosis of LUAD patients are not available and limit the analysis of outcomes in our study. Given the limitations of this study, further large-sample and in-depth studies are required to confirm these results.

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Availability of data and materials

LUSC and LUAD transcriptome and clinical data were downloaded from The Cancer Genome Atlas (TCGA, https://tcga-data.nci.nih.gov/) and the cBioPortal (http://www.cbioportal.org/) database. LUAD DNA promoter methylation data were collected from MethHC (http://methhc.mbc.nctu.edu.tw/php/search.php?opt=gene). The other datasets used during the present study are available from the corresponding author upon reasonable request.

Authors' contributions

BQ, XY and WLv designed this study. XY and WLv performed data collection. WLv, WLi, TF and XY conducted data

analysis. XY, NF, YW and HL performed the experiment. All authors wrote the manuscript. WLv, WLi, XY and BQ revised the manuscript. The final version of the manuscript has been read and approved by all authors, and each author believes that the manuscript represents honest work.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J and Jemal A: Global cancer statistics, 2012. CA Cancer J Clin 65: 87-108, 2015.
2. Gazdar AF: Should we continue to use the term non-small-cell lung cancer? Ann Oncol 21 (Suppl 7): vii225-vii229, 2010.
3. Zonderman AB, Ejiogu N, Norbeck J and Evans MK: The influence of health disparities on targeting cancer prevention efforts. Am J Prev Med 46 (3 Suppl 1): S87-S97, 2014.
4. Kang YH, Kim D and Jin EJ: Down-regulation of phospholipase D stimulates death of lung cancer cells involving up-regulation of the long ncRNA ANRIL. Anticancer Res 35: 2795-2803, 2015.
5. Liu X, Xiao ZD, Han L, Zhang J, Lee SW, Wang W, Lee H, Zhuang L, Chen J, Lin HK, *et al*: LncRNA *NBR2* engages a metabolic checkpoint by regulating AMPK under energy stress. Nat Cell Biol 18: 431-442, 2016.
6. Wang Y, Guo Q, Zhao Y, Chen J, Wang S, Hu J and Sun Y: BRAF-activated long non-coding RNA contributes to cell proliferation and activates autophagy in papillary thyroid carcinoma. Oncol Lett 8: 1947-1952, 2014.
7. Zhao Y, Guo Q, Chen J, Hu J, Wang S and Sun Y: Role of long non-coding RNA HULC in cell proliferation, apoptosis and tumor metastasis of gastric cancer: A clinical and in vitro investigation. Oncol Rep 31: 358-364, 2014.
8. Feng N, Ching T, Wang Y, Liu B, Lin H, Shi O, Zhang X, Zheng M, Zheng X, Gao M, *et al*: Analysis of microarray data on gene expression and methylation to identify long non-coding RNAs in non-small cell lung cancer. Sci Rep 6: 37233, 2016.
9. Schmidt LH, Spieker T, Koschmieder S, Schäffers S, Humberg J, Jungen D, Bulk E, Hascher A, Wittmer D, Marra A, *et al*: The long noncoding MALAT-1 RNA indicates a poor prognosis in non-small cell lung cancer and induces migration and tumor growth. J Thorac Oncol 6: 1984-1992, 2011.
10. Loewen G, Jayawickramarajah J, Zhuo Y and Shan B: Functions of lncRNA HOTAIR in lung cancer. J Hematol Oncol 7: 90, 2014.
11. Fei ZH, Yu XJ, Zhou M, Su HF, Zheng Z and Xie CY: Upregulated expression of long non-coding RNA LINC00982 regulates cell proliferation and its clinical relevance in patients with gastric cancer. Tumour Biol 37: 1983-1993, 2016.
12. Cao WJ, Wu HL, He BS, Zhang YS and Zhang ZY: Analysis of long non-coding RNA expression profiles in gastric cancer. World J Gastroenterol 19: 3658-3664, 2013.
13. Lei Q, Liu X, Fu H, Sun Y, Wang L, Xu G, Wang W, Yu Z, Liu C, Li P, *et al*: miR-101 reverses hypomethylation of the PRDM16 promoter to disrupt mitochondrial function in astrocytoma cells. Oncotarget 7: 5007-5022, 2016.
14. Zhu S, Xu Y, Song M, Chen G, Wang H, Zhao Y, Wang Z and Li F: PRDM16 is associated with evasion of apoptosis by prostatic cancer cells according to RNA interference screening. Mol Med Rep 14: 3357-3361, 2016.
15. Burghel GJ, Lin WY, Whitehouse H, Brock I, Hammond D, Bury J, Stephenson Y, George R and Cox A: Identification of candidate driver genes in common focal chromosomal aberrations of microsatellite stable colorectal cancer. PLoS One 8: e83859, 2013.
16. Momi N, Wali RK, Chhaparia A, Calderwood AH, Tiwari AK, Ledbetter SE, DeLaCruz M and Roy HK: Su2000 PRDM16 is a novel proto-oncogene in colorectal cancer (CRC): Modulation of the early warburg effect in field carcinogenesis. Gastroenterology 150: S606, 2016.
17. Matsuo H, Goyama S, Kamikubo Y and Adachi S: The subtype-specific features of EVI1 and PRDM16 in acute myeloid leukemia. Haematologica 100: e116-e117, 2015.
18. Li L, Zhao CT, Cui BL, Wu SL, Liu XD, Su Z, Yang J, Wang W, Cui ZG and Zhao HG: Expression of HOXB4, PRDM16 and HOXA9 in patients with acute myeloid leukemia and its clinical significance. Zhongguo Shi Yan Xue Ye Xue Za Zhi 24: 326-331, 2016 (In Chinese).
19. Tan SX, Hu RC, Liu JJ, Tan YL and Liu WE: Methylation of PRDM2, PRDM5 and PRDM16 genes in lung cancer cells. Int J Clin Exp Pathol 7: 2305-2311, 2014.
20. Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, Jacobsen A, Byrne CJ, Heuer ML, Larsson E, *et al*: The cBio cancer genomics portal: An open platform for exploring multidimensional cancer genomics data. Cancer Discov 2: 401-404, 2012.
21. Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, Sun Y, Jacobsen A, Sinha R, Larsson E, *et al*: Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci Signal 6: pl1, 2013.
22. Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W and Smyth GK: limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res 43: e47, 2015.
23. Hirsch FR, Varella-Garcia M, Bunn PA Jr, Di Maria MV, Veve R, Bremmes RM, Barón AE, Zeng C and Franklin WA: Epidermal growth factor receptor in non-small-cell lung carcinomas: Correlation between gene copy number and protein expression and impact on prognosis. J Clin Oncol 21: 3798-3807, 2003.
24. Cancer Genome Atlas Research Network: Comprehensive molecular profiling of lung adenocarcinoma. Nature 511: 543-550, 2014.
25. Castillo J, Stueve TR and Marconett CN: Intersecting transcriptomic profiling technologies and long non-coding RNA function in lung adenocarcinoma: Discovery, mechanisms, and therapeutic applications. Oncotarget 8: 81538-81557, 2017.
26. Qiu M, Xu Y, Yang X, Wang J, Hu J, Xu L and Yin R: CCAT2 is a lung adenocarcinoma-specific long non-coding RNA and promotes invasion of non-small cell lung cancer. Tumour Biol 35: 5375-5380, 2014.
27. Lavallée VP, Lemieux S, Boucher G, Gendron P, Boivin I, Girard S, Hébert J and Sauvageau G: Identification of MYC mutations in acute myeloid leukemias with NUP98-NSD1 translocations. Leukemia 30: 1621-1624, 2016.
28. Duhoux FP, Ameye G, Montano-Almendras CP, Bahloula K, Mozziconacci MJ, Laibe S, Wlodarska I, Michaux L, Talmant P, Richebourg S, *et al*: PRDM16 (1p36) translocations define a distinct entity of myeloid malignancies with poor prognosis but may also occur in lymphoid malignancies. Br J Haematol 156: 76-88, 2012.
29. Vermeulen K, Van Bockstaele DR and Berneman ZN: The cell cycle: A review of regulation, deregulation and therapeutic targets in cancer. Cell Prolif 36: 131-149, 2003.
30. Pagano M and Draetta G: Cyclin A, cell cycle control and oncogenesis. Prog Growth Factor Res 3: 267-277, 1991.
31. Yue W, Zhao X, Zhang L, Xu S, Liu Z, Ma L, Jia W, Qian Z, Zhang C, Wang Y and Yang X: Cell cycle protein cyclin Y is associated with human non-small-cell lung cancer proliferation and tumorigenesis. Clin Lung Cancer 12: 43-50, 2011.
32. Lamb R, Lehn S, Rogerson L, Clarke RB and Landberg G: Cell cycle regulators cyclin D1 and CDK4/6 have estrogen receptor-dependent divergent functions in breast cancer migration and stem cell-like activity. Cell Cycle 12: 2384-2394, 2013.
33. Jhanwar-Uniyal M: BRCA1 in cancer, cell cycle and genomic stability. Front Biosci 8: s1107-s1117, 2003.
34. Paternot S, Bockstaele L, Bisteau X, Kooken H, Coulonval K and Roger PP: Rb inactivation in cell cycle and cancer: The puzzle of highly regulated activating phosphorylation of CDK4 versus constitutively active CDK-activating kinase. Cell Cycle 9: 689-699, 2010.

35. Chuikov S, Levi BP, Smith ML and Morrison SJ: Prdm16 promotes stem cell maintenance in multiple tissues, partly by regulating oxidative stress. Nat Cell Biol 12: 999-1006, 2010.
36. Wishart DS: Emerging applications of metabolomics in drug discovery and precision medicine. Nat Rev Drug Discov 15: 473-484, 2016.
37. Zhang EB, Yin DD, Sun M, Kong R, Liu XH, You LH, Han L, Xia R, Wang KM, Yang JS, *et al*: P53-regulated long non-coding RNA TUG1 affects cell proliferation in human non-small cell lung cancer, partly through epigenetically regulating HOXB7 expression. Cell Death Dis 5: e1243, 2014.
38. Yang X, Song JH, Cheng Y, Wu W, Bhagat T, Yu Y, Abraham JM, Ibrahim S, Ravich W, Roland BC, *et al*: Long non-coding RNA HNF1A-AS1 regulates proliferation and migration in oesophageal adenocarcinoma cells. Gut 63: 881-890, 2014.
39. Jamal-Hanjani M, Wilson GA, McGranahan N, Birkbak NJ, Watkins TB, Veeriah S, Shafi S, Johnson DH, Mitter R, Rosenthal R, *et al*: Tracking the evolution of non-small-cell lung cancer. N Engl J Med 376: 2109-2121, 2017.