Jiao *et al. Respiratory Research* (2025) 26:64
https://doi.org/10.1186/s12931-025-03122-y

Respiratory Research

RESEARCH Open Access

Long non-coding RNA PRKG1-AS1 promotes cell proliferation and migration in lung adenocarcinoma

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Abstract
In previous studies, we have discovered a significant correlation between cGMP-dependent protein kinase I antisense RNA 1 (PRKG1-AS1) and the prognosis of lung adenocarcinoma (LUAD). Through analysis of The Cancer Genome Atlas (TCGA) database and expression data from non-small cell lung cancer tissues and cells, we found that PRKG1-AS1 is overexpressed in LUAD tissues. High expression of PRKG1-AS1 is associated with poor prognosis in LUAD patients. Cox regression analysis revealed that PRKG1-AS1 is an independent factor affecting the prognosis of LUAD. Gene Set Enrichment Analysis (GSEA) results indicated that PRKG1-AS1 might participate in various cancer-related biological processes and signaling pathways, including immune response. Furthermore, our study demonstrated that knockdown of PRKG1-AS1 expression inhibited proliferation and metastasis in LUAD. Correlation analysis between PRKG1-AS1 and protein-coding genes (PCGs) revealed a positive correlation between PRKG1-AS1 and dickkopf-1 (DKK1). Downregulation of PRKG1-AS1 led to decreased expression of DKK1, which is highly expressed in LUAD and is associated with poor prognosis. In summary, our findings suggest that PRKG1-AS1 may function as an oncogene contributing to the development of LUAD and holds significant prognostic value.

Keywords PRKG1-AS1, DKK1, LUAD, Proliferation, Metastasis

Introduction

Lung cancer caused deaths account for 23 and 24% of tumor-related deaths in females and males, respectively [1]. Most lung cancer are diagnosed at an advanced stage, which leading to the 5-year survival rate is only 18% [2]. Lung adenocarcinoma (LUAD) is the most common subtype of non-small cell lung cancer (NSCLC), which caused more than 500,000 deaths per year worldwide [1]. Thus, identifying some novel and effectively diagnostic or prognostic biomarkers is essential to improve the survival of LUAD.

With the progression of sequencing and gene chip technology, increasing number of long non-coding RNA (lncRNA) were identified [3]. LncRNA has been found to play crucial role in the occurrence and development of various cancers, although it has no ability to encode proteins [4–6]. Evidences suggested that lncRNA involved in many biological processes, such as metastasis [7], drug resistance [8], epigenetic [9], and so on. Recent years, the potential of lncRNA acted as diagnostic or prognostic biomarkers has been continuously concerned. For instance, LncRNA PANDAR could serve as a prognostic biomarker in Chinese cancer patients, and its high expression appears predictive of poor overall survival [10]. LncRNA-D16366 has great value in the diagnosis

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and prognosis of hepatocellular carcinoma [11].Our research team conducted a differential expression analysis on LUAD and non-tumor tissue RNA sequencing data downloaded from The Cancer Genome Atlas (TCGA) database. Through this analysis, we identified a LUAD prognostic prediction model consisting of 5 lncRNAs [12]. Notably, this model includes cGMP-dependent protein kinase type I antisense RNA 1 (PRKG1-AS1), located at 10q21.1. Previous studies have indicated that high expression of PRKG1-AS1 is associated with poor prognosis in oral squamous cell carcinoma [13], head and neck squamous cell carcinoma [14], gastric cancer [15], promoting cell proliferation, migration, and invasion. However, the specific function and mechanism of PRKG1-AS1 in lung cancer remain unclear.

In this study, we analyzed the expression of PRKG1-AS1 in TCGA database, as well as in 21 pairs of LUAD tissues and corresponding adjacent non-tumor tissues, and non-small cell lung cancer cell lines. We evaluated the prognostic value of PRKG1-AS1 in the TCGA database and examined its relationships with other clinical indicators. Additionally, we conducted Gene Set Enrichment Analysis (GSEA) to analyze the tumor-related biological processes and signaling pathways associated with PRKG1-AS1. Furthermore, we investigated the subcellular localization of PRKG1-AS1 and designed antisense oligonucleotides (ASO) targeting PRKG1-AS1 to knock down its expression. We validated the changes in proliferation and migration abilities of LUAD cell lines after PRKG1-AS1 knockdown. Using bioinformatics analysis, we predicted the target genes of PRKG1-AS1 and studied their expression patterns and functions in LUAD.

Table 1 Chi-square analysis between PRKG1-AS1 and clinicopathological indicators

Clinical factors	PRKG1-AS1 expression		*P* value
	Low	High	
Vital status			0.004**
Alive	164(73.2)	135(60.3)	
Dead	60 (26.8)	89(39.7)	
Gender			0.570
Female	123(54.9)	117(52.2)	
Male	101(45.1)	107(47.8)	
Age (years)			0.006**
≤ 65	121(54.0)	92(41.1)	
> 65	103(46.0)	132 (58.9)	
Stage			0.272
I	125(55.8)	116 (51.8)	
II	58(25.9)	53(23.7)	
III/IV	41(18.3)	55(24.6)	
Tumor stage			0.366
T1	73(32.6)	78(34.8)	
T2	127(56.7)	114(50.9)	
T3/4	34(10.7)	32(14.3)	
Node metastasis			0.835
N0	150(67.0)	144(64.3)	
N1	42(18.8)	45(20.1)	
N2	32(14.3)	35(15.6)	

** $P < 0.01$

Materials and methods

Data acquired from TCGA database

We obtained RNA sequencing data and clinical information from lung cancer patients via the TCGA website. The dataset comprised RNA expression profiles of 535 LUAD tissues and 59 non-tumor tissues. Differential expression analysis utilized the R package "edgeR" [16]. Subsequently, 448 LUAD patients were selected for further study based on a filter criterion of an overall survival period exceeding 60 days and having complete clinical data. Clinical factors considered included survival status, age, gender, T stage, N stage, and TNM stage. Detailed information regarding these 448 LUAD patients is provided in Table 1.

Tissues collected

From January 2016 to December 2019, we collected 21 paired samples of LUAD and corresponding para-carcinoma tissues at the Second Affiliated Hospital of Xi'an Jiaotong University. Ethical approval was obtained from the hospital's ethics committee. The ethics number is 2,023,179.

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

Lung tissue RNA was extracted using the FAST1000 system (Pioneer, China), followed by reverse transcription into cDNA using PrimerScript™ RT (takara, Japan). Gene amplification was detected using TB Green® Premix Ex Taq™ II (taraka, Japan), with GAPDH serving as the internal reference. The sequence of primer is on supplementary material Table 1.

Gene set enrichment analysis (GSEA)

Gene set enrichment analysis employed GSEA software V4.0.3, utilizing expression profiles of PCGs obtained from the TCGA database. Gene Ontology (GO) enrichment analysis focused on biological processes (BP), and BP results were visualized using the Cytoscape plug-in Enrichment Map [17]. Gene sets with a P value < 0.05, determined through 1000 random sample permutations, were labeled as "enriched."

Antisense oligonucleotide (ASO) transfection

Cells were cultured in 6-well plates until reaching the logarithmic growth phase and adherent. A mixture of ASOs, RPMI-1640 medium, and Lipofectamine 3000 was prepared and added to the culture wells. Following 24 h

of incubation, the medium was replaced, and subsequent cellular experiments were conducted. The sequence of ASO is on supplementary material Table 2.

CCK8 assay

Cells that underwent transfection were seeded into a 96-well plate. Following incubation for 0, 24, 48, 72, and 96 h, the cells were exposed to 100 μL of CCK-8 working solution (prepared by mixing complete culture medium with CCK-8 at a ratio of 9:1) for 1 h. Absorbance readings were taken at 450 nm using a spectrophotometer.

EDU assay

After transfection, culture the cells in a 96-well plate for 24 h, followed incubation for 2 h with 100 μL EdU working solution. Then cells were fixed with 4% paraformaldehyde, stained with Apollo staining solution to detect EdU-positive cells, and counterstained with Hoechst for DNA visualization. Subsequently, fluorescence images were captured using a fluorescence microscope.

Colony formation assay

Transfected cells were cultured and replicated in a 6-well culture plate for two weeks. Following this, the cells were fixed with formaldehyde for 30 min and stained with crystal violet for an additional 45 min. Subsequently, images were captured, and the number of cell colonies was quantified.

Wound healing assay

Transfected cells were cultured in a 6-well plate for 24–48 h until reaching full confluence. Draw a straight line perpendicular to the bottom of the plate. The scratches were observed at 0, 12, 24, 36, and 48 h. Images were captured, and the scratch closure rate was calculated.

Transwell cell migration assay

After transfection, cells were resuspended in RPMI-1640 medium. The serum-free cell suspension was added into the upper chamber, while medium containing 10% serum was added to the lower chamber. After 24 h, remove the medium in the upper chamber. Fix the cells with 4% paraformaldehyde for 30 min. Subsequently, crystal violet staining solution was applied for an additional 30 min. Then recorded for each well.

Screening of co-expressed genes and analysis of key genes

We performed co-expression analysis using the Pearson's correlation coefficient method to calculate the protein-coding genes (PCGs) associated with PRKG1-AS1 expression. The criteria for selecting co-expressed genes of PRKG1-AS1 were set as |Pearson's correlation coefficient| > 0.40 and $p < 0.01$.

Statistical analysis

Data analysis was conducted using R software and Prism 7.0 Software (GraphPad, USA). Statistical differences were assessed using student's t-test, one-way ANOVA, and chi-square analysis, with significance defined as $p < 0.05$.

Results

PRKG1-AS1 is overexpressed in LUAD tissues

Differential expression analysis revealed significant upregulation of PRKG1-AS1 in LUAD tumor tissues (LogFC = 1.21, $p < 0.001$, Fig. 1A) compared to non-tumor tissues. Then we assessed PRKG1-AS1 expression in 21 paired lung tumor and corresponding para-carcinoma tissues. The results indicated elevated expression of PRKG1-AS1 in LUAD tumor tissues ($p = 0.008$, Fig. 1B) compared to the corresponding non-tumor tissues. We subsequently validated the expression of PRKG1-AS1 in five non-small cell lung cancer cell lines, SPC-A-1, H1975, A549, PC-9, and H1299, along with the normal bronchial epithelial cell line BEAS-2B. The results revealed elevated expression of PRKG1-AS1 in the SPC-A-1 ($p = 0.0004$), H1975 ($p = 0.0023$), A549 ($p = 0.0133$), PC-9 ($p < 0.0001$), and H1299 ($p < 0.0001$) cell lines compared to the BEAS-2B (Fig. 1C).

PRKG1-AS1 has great prognostic value in LUAD patients

Kaplan-Meier survival analysis indicated that elevated PRKG1-AS1 expression was associated with poor outcomes ($p < 0.001$, Fig. 2A) in LUAD patients. Among 448 LUAD patients, the median survival of the high-expression group (3.11 years) was lower than that of the low-expression group (4.38 years) using the median value of PRKG1-AS1 as a cut-off. The 1-, 3-, and 5-year survival rates were 81.7%, 49.8%, and 27.6% in the high-expression group, and 95.6%, 67.8%, and 41.2% in the low-expression group, respectively. Univariate Cox regression analysis results indicated that PRKG1-AS1 expression was a prognostic risk factor significantly associated with overall survival ($p < 0.001$, HR = 1.788, Fig. 2B). To assess PRKG1-AS1's prognostic significance in conjunction with other clinical parameters, multivariate Cox regression analysis was performed. The results demonstrated that PRKG1-AS1 remained significantly associated with overall survival after adjusting for other clinical factors ($p < 0.001$, HR = 2.023, Fig. 2C). These findings suggest that PRKG1-AS1 has potential as an independent prognostic biomarker for LUAD patients.

Relationship between PRKG1-AS1 and other clinical factors

To explore the function of PRKG1-AS1 in LUAD, we analyzed the relationship between PRKG1-AS1 and other clinical factors. Analysis of the TCGA dataset revealed a significant correlation between PRKG1-AS1

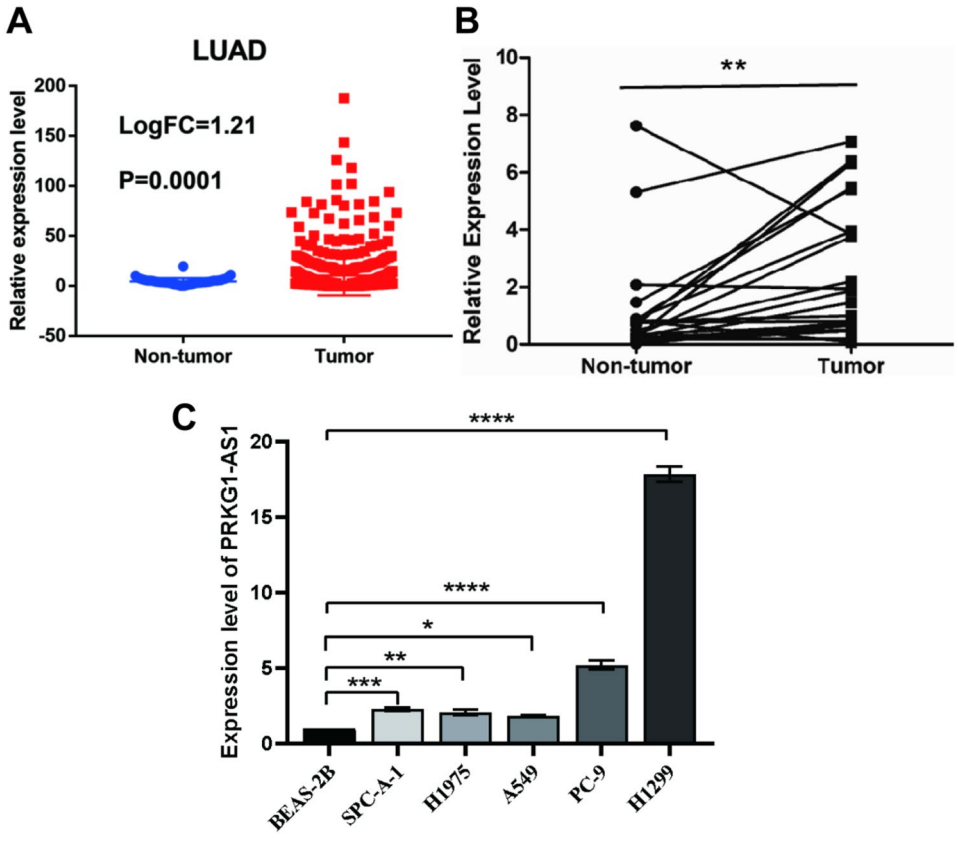

Fig. 1 PRKG1-AS1 is overexpressed in LUAD tumor tissues. **(A)** The expression level of PRKG1-AS1 in 535 LUAD tumor and 59 non-tumor tissues. **(B)** The expression of PRKG1-AS1 was detected in 21 paired LUAD tumor and corresponding non-tumor tissues. **(C)** The expression level of PRKG1-AS1 in non-small cell lung cancer cell lines. PRKG1-AS1, Protein kinase cGMP-dependent type I-Antisense RNA 1, LUAD, lung adenocarcinoma. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$

expression and TNM stage ($p = 0.021$, Fig. 3A), as well as N stage ($p = 0.041$, Fig. 3B). Patients in advanced TNM or N stages tended to exhibit higher levels of PRKG1-AS1 expression. However, there were no statistical difference between PRKG1-AS1 and other clinical factors, including T stage ($p = 0.188$, Fig. 3C), age ($p = 0.540$, Fig. 3D) and gender ($p = 0.088$ Fig. 3E). Although there were no significantly statistical difference, the patients in advanced T stage have a higher expression level of PRKG1-AS1 compared with those in early stage.

GO terms and KEGG pathways

GSEA results indicated an association between PRKG1-AS1 and numerous tumor-related biological processes and Kyoto Encyclopedia of Genes and Genome (KEGG) pathways. The significantly enriched biological process terms were visualized in Fig. 4A using an enrichment map, and those BP terms mainly involved in "immune response" "NF-kB signal pathway" "Receptor-related signaling pathways" "Cell adhesion" "Notch signal pathway" "Histone acetylation", and so on. The significantly enriched KEGG pathway mainly included "small lung cancer" and "P53 signal pathway" (Fig. 4B). The results of enrichment analysis indicated that PRKG1-AS1 was closely related to tumor development.

PRKG1-AS1 predominantly localizes to the cell nucleus

Subcellular localization analysis of PRKG1-AS1 was conducted using the LncATLAS online database. We observed negative RCI values greater than 1 in multiple cell lines, indicating that PRKG1-AS1 primarily functions within the cell nucleus, as illustrated in Fig. 5A. Subsequently, we utilized PRKG1-AS1 as the target gene, with U6 and GAPDH serving as references, to assess the separation levels of nuclear and cytoplasmic RNA. Cell nucleus-cytoplasm RNA separation and RT-qPCR experiments were performed in the LUAD cell line H1299, as depicted in Fig. 5B. The results demonstrated that approximately 98% of GAPDH was localized in the cytoplasm and 2% in the nucleus in H1299 cells. U6 was distributed with approximately 36% in the cytoplasm and 64% in the nucleus. PRKG1-AS1 exhibited an approximate distribution of 13% in the cytoplasm and 87% in the nucleus. By comparing the expression ratios of PRKG1-AS1 in the nucleus and cytoplasm, it was evident that PRKG1-AS1 primarily localizes to the cell nucleus. These

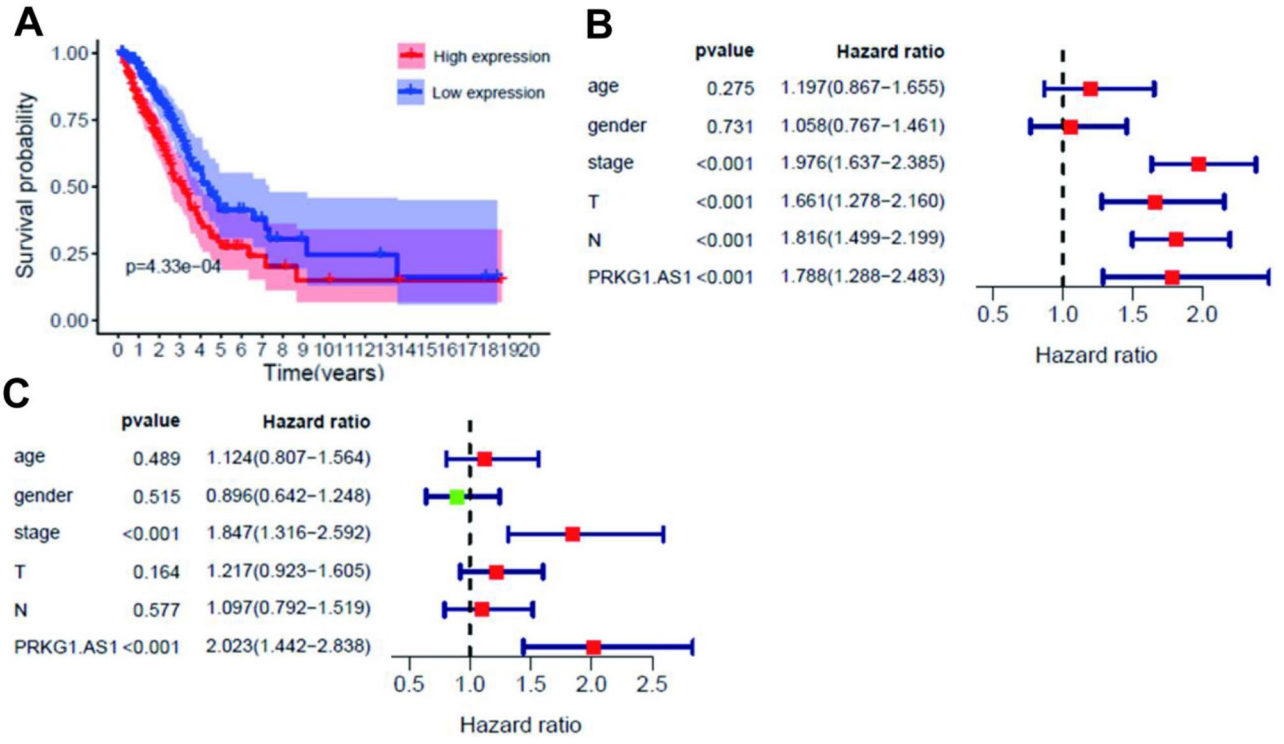

Fig. 2 The prognostic value of PRKG1-AS1 in LUAD patients in TCGA dataset. **(A)** The Kaplan-Meier survival analysis curve of PRKG1-AS1 in 448 LUAD patients. **(B)** Univariate cox regression analysis of clinical factors in 448 LUAD patients. **(C)** Multivariate cox regression analysis of clinical factors in 448 LUAD patients. TCGA, The cancer genome atlas

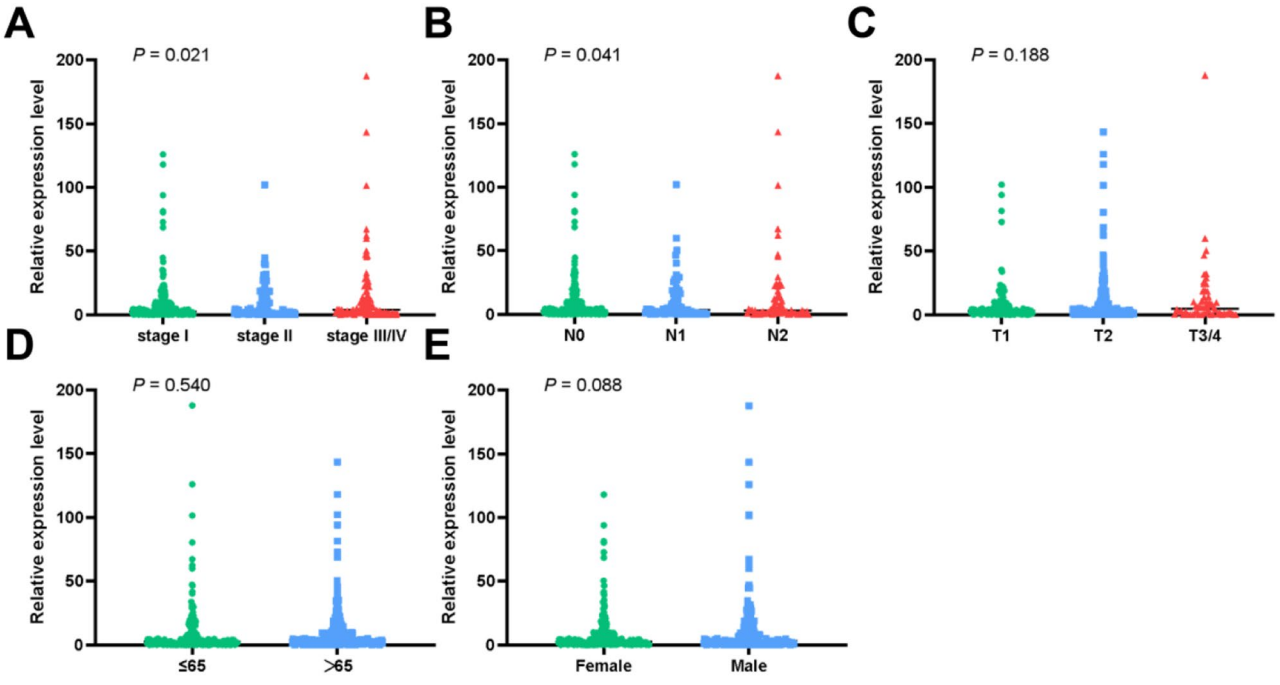

Fig. 3 The correlation between PRKG1-AS1 and clinical factors. The relationship between PRKG1-AS1 and **(A)** TNM stage, **(B)** N stage, **(C)** T stage, **(D)** age and **(E)** gender in TCGA dataset

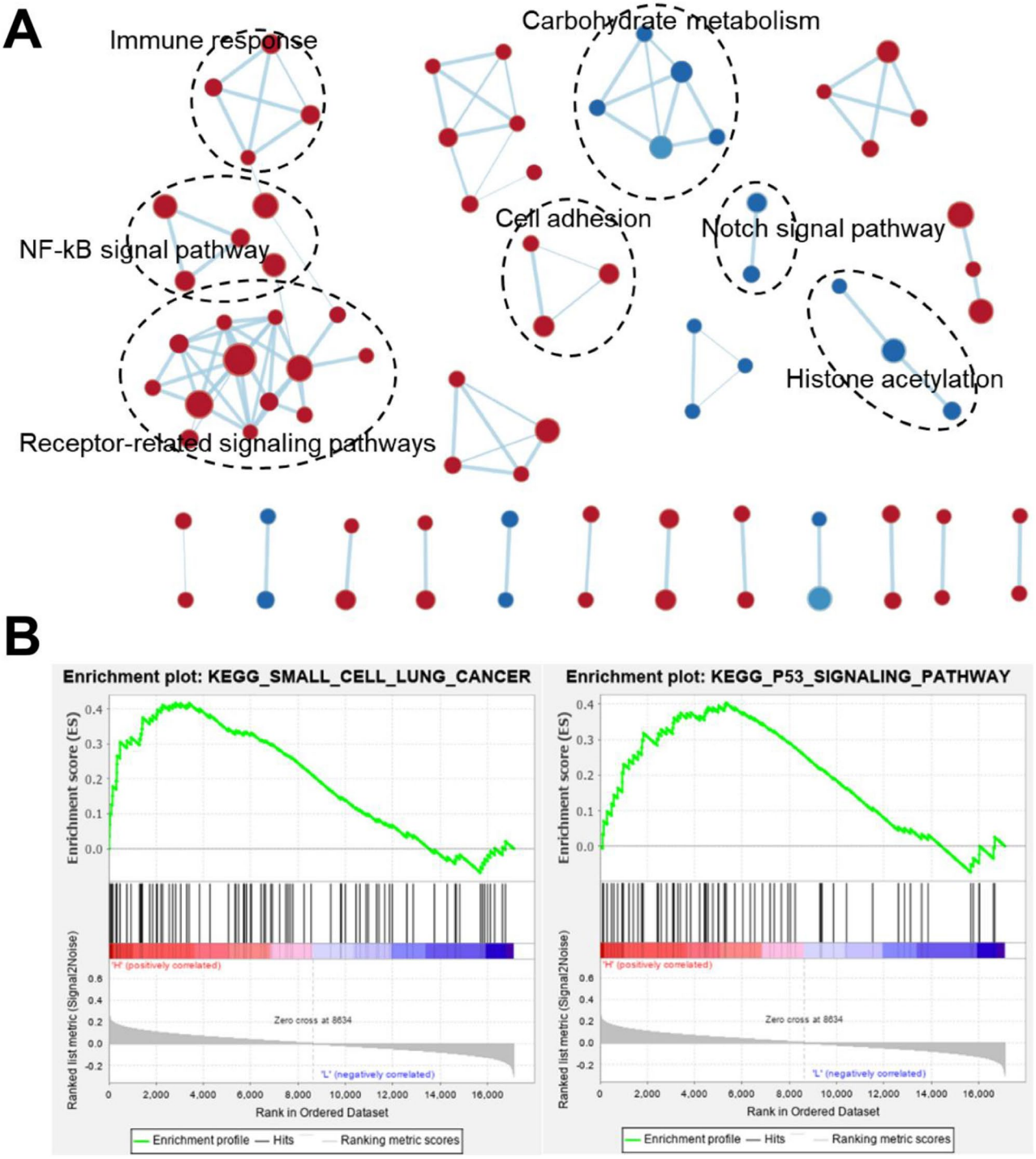

Fig. 4 The results of GSEA which were based on the data from TCGA database. **(A)** The significantly enriched GO terms which were visualized using cytoscape. **(B)** The significantly enriched pathways. GO, Gene Ontology, KEGG, Kyoto Encyclopedia of Genes and Genomes

findings confirm the predominant nuclear localization of PRKG1-AS1.

Knocking down PRKG1-AS1 inhibits LUAD Proliferation

We transfected PC9 and H1299 cells with ASOs and evaluated their knockdown efficiency using qRT-PCR. Significant knockdown efficiency was observed in H1299 cells with ASO-80 (86.36%), ASO-340 (79.85%), and ASO-503 (72.49%). In PC-9 cells, ASO-80 achieved a knockdown efficiency of 39.35%, ASO-340 40.61%, and ASO-503 22.62% (Fig. 6A-B). Based on knockdown efficiency, ASO-80 and ASO-340 were selected

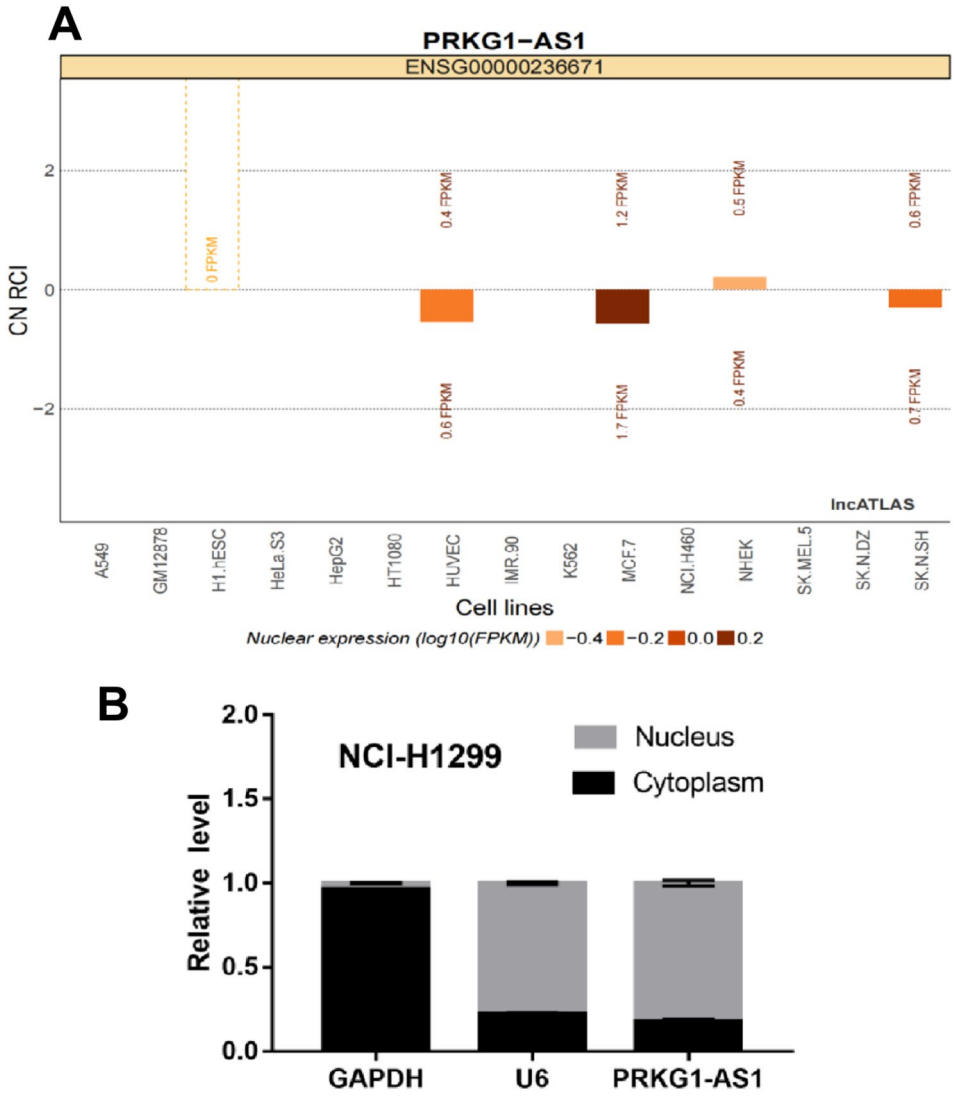

Fig. 5 PRKG1-AS1 predominantly localizes to the cell nucleus. **(A)** Subcellular localization analysis of PRKG1-AS1 in the LncATLAS online database. **(B)** Validation of subcellular localization of PRKG1-AS1 in H1299 cell line

for subsequent experiments and denoted as ASO1 and ASO2, respectively. CCK-8 assays indicated a significant decrease in cell viability over time in both H1299 ($p < 0.01$ for ASO1 and ASO2 at 96 h; Fig. 6C) and PC9 ($p < 0.01$ for ASO1 and ASO2 at 96 h; Fig. 6D) cell lines, compared to the ASO-NC. As depicted in Fig. 6E-F, the knockdown groups exhibited a significantly lower number of colonies compared to the NC group in both H1299 and PC9 cells ($p = 0.049$ for ASO1 and $p = 0.003$ for ASO2 in H1299 cells; $p = 0.036$ for ASO1 and $p = 0.005$ for ASO2 in PC9 cells). EDU assays revealed a markedly reduced EdU-positive rate in the knockdown groups relative to the NC in both H1299 ($p < 0.0001$ for ASO1 and $p < 0.0001$ for ASO2; Fig. 6G) and PC9 ($p = 0.0179$ for ASO1 and $p = 0.0008$ for ASO2; Fig. 6H) cell lines. These findings suggest that downregulation of PRKG1-AS1 expression significantly inhibits the proliferation of LUAD cells.

Knocking down PRKG1-AS1 suppresses LUAD Metastasis

We established PRKG1-AS1 knockdown models in PC9 and H1299 cells through ASO transfection. Wound healing assays demonstrated significantly shorter migration distances in PRKG1-AS1 knockdown groups compared to the NC at the same time point. Statistical analysis revealed a notable reduction in the wound healing rate in PRKG1-AS1 knockdown groups ($p < 0.01$ for ASO1 and $p < 0.01$ for ASO2; Fig. 7A) in H1299 cells. Similar results were observed in PC-9 cells ($p < 0.001$ for ASO1 and $p < 0.01$ for ASO2; Fig. 7B). Transwell cell migration assay further corroborated these findings. In H1299 ($p < 0.001$ for ASO1 and $p < 0.001$ for ASO2; Fig. 7D) and PC-9 cells ($p < 0.01$ for ASO1 and $p < 0.01$ for ASO2; Fig. 7C), the number of cells migrating to the lower chamber significantly decreased in the PRKG1-AS1 knockdown groups compared to the NC group. These results collectively

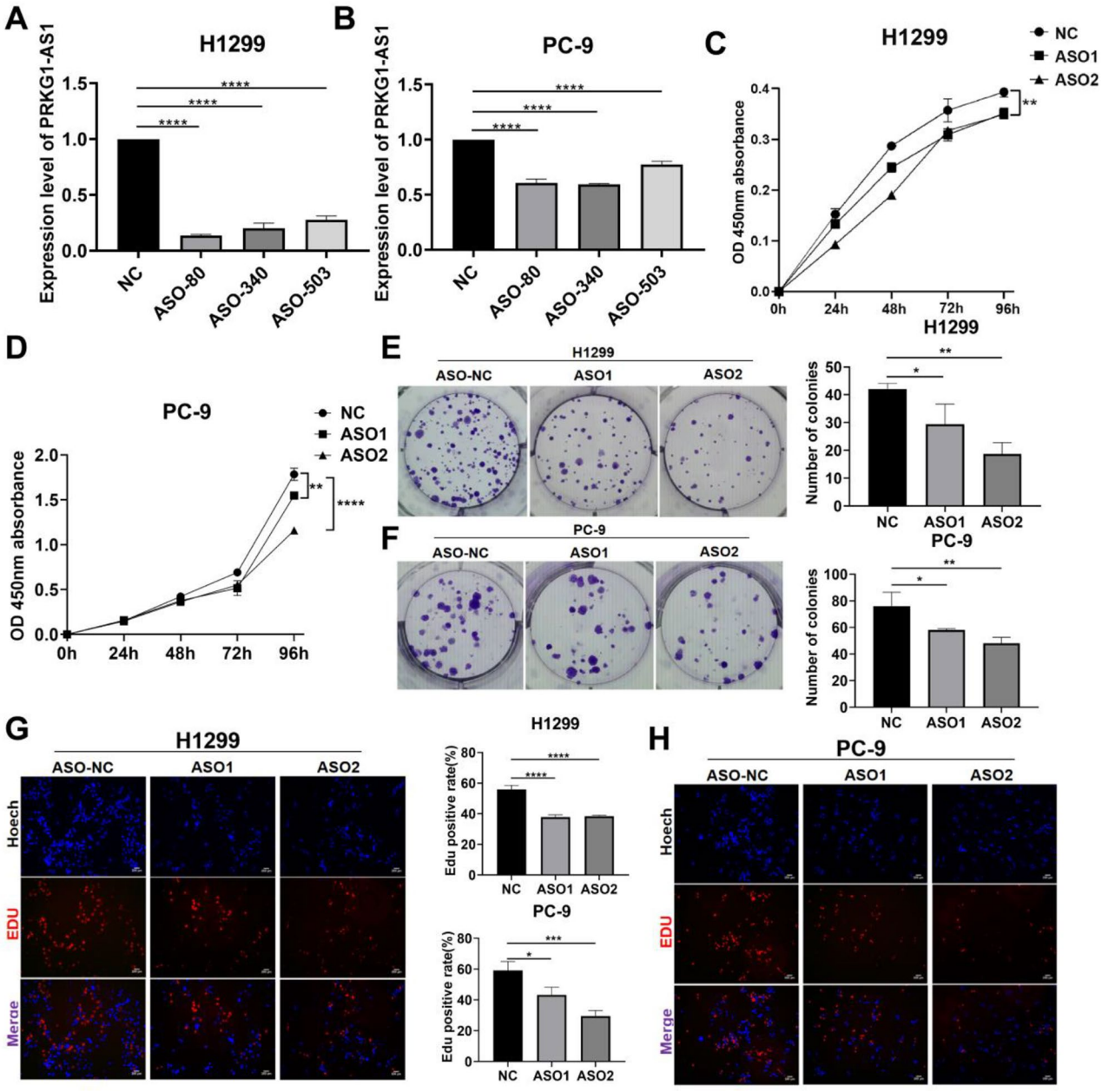

Fig. 6 PRKG-AS1 inhibits cell proliferation. **(A** and **B)** Efficiency of ASO-mediated PRKG1-AS1 knockdown in H1299 cells and PC-9 cells. **(C** and **D)** ASO1- and ASO2-transfected groups showing significant reduced in cell growth rate compared to ASO-NC group at 96 h, in H1299 and PC-9 cells. **(E** and **F)** More number of colonies in the PRKG1-AS1 ASO-transfected groups compared to those in the NC group in H1299 as well as PC-9 cells. **(G** and **H)** Lower EdU-positive rate in ASO1- and ASO2-transfected groups compared to that in NC-group, in H1299 and PC-9 cells

indicate a reduced migratory capacity of LUAD cells following the knockdown of PRKG1-AS1 expression.

PRKG1-AS1 significantly co-expressed with dickkopf-1 (DKK1)

Utilizing RNA sequencing data, we computed the Pearson correlation coefficients between PRKG1-AS1 and individual PCGs. The results revealed a significant co-expression of DKK1 with PRKG1-AS1 (cor = 0.72, $p < 0.0001$, Fig. 8A). Analyzing the TCGA dataset, we found that DKK1 was significantly overexpressed in LUAD tissues (LogFC = 2.44, $p < 0.0001$, Fig. 8B). Following this, qRT-PCR was conducted to assess DKK1 expression in 21 pairs of LUAD tumors and their matched adjacent non-tumor tissues. The findings revealed elevated DKK1 expression in LUAD tumor tissues relative to corresponding non-tumor tissues ($p = 0.009$, Fig. 8C), and a significant co-expression with PRKG1-AS1 was observed (cor = 0.60, $p = 0.004$, Fig. 8D). To validate these findings, we transfected cells with ASOs to knock down

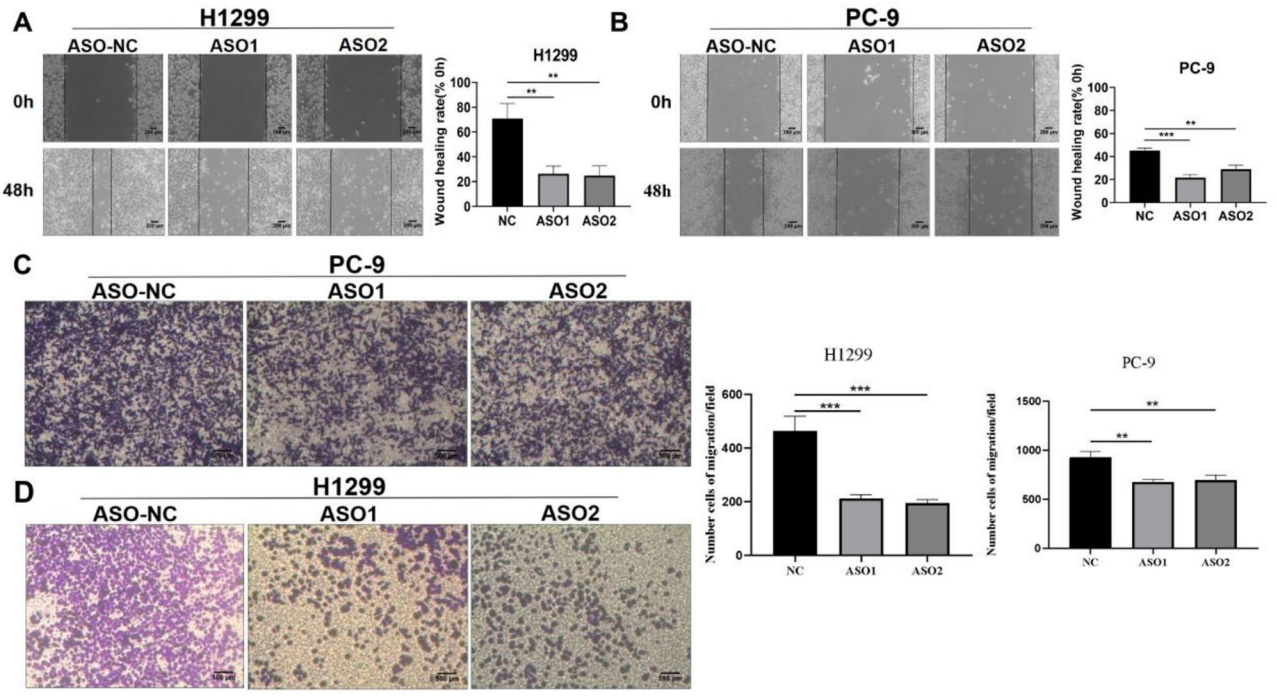

Fig. 7 PRKG-AS1 inhibits cell metastasis. **(A** and **B)** Lower wound healing rates in ASO1- and ASO2-transfected groups compared to that NC-group, in H1299 and PC-9 cells. **(C** and **D)** Fewer number cells of migration/field in the PRKG1-AS1 ASO-transfected groups compared to those in the NC group in H1299 as well as PC-9 cells

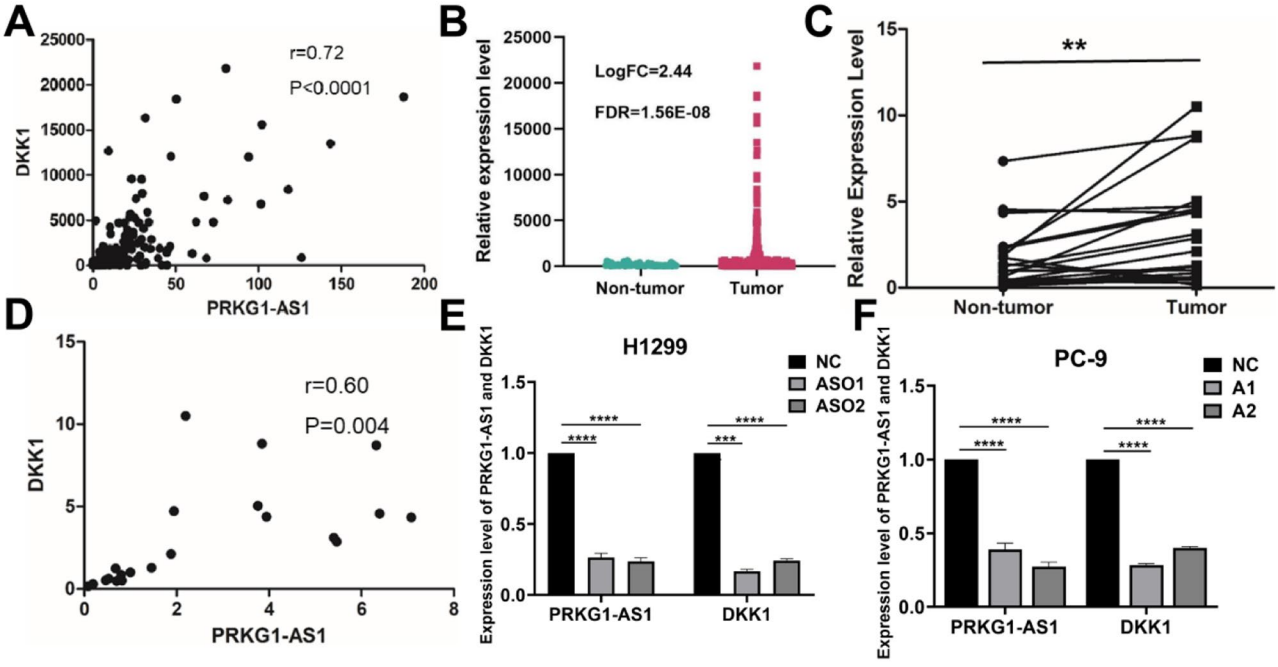

Fig. 8 PRKG1-AS1 regulates the expression of DKK1. **(A)** DKK1 is highly co-expressed with PRKG1-AS1 in TCGA dataset. **(B)** The expression of DKK1 in 535 LUAD tumor and 59 non-tumor tissues in TCGA dataset. **(C)** The expression level of DKK1 in 21 paired LUAD tumor and corresponding para-carcinoma tissues. **(D)** DKK1 is highly co-expressed with PRKG1-AS1 in 21 LUAD tumor tissues. **(E** and **F)** Lower expression level of PRKG1-AS1 and DKK1 in ASO1- and ASO2-transfected groups compared to that NC-group, in H1299 and PC-9 cells. DKK1, dickkopf- 1

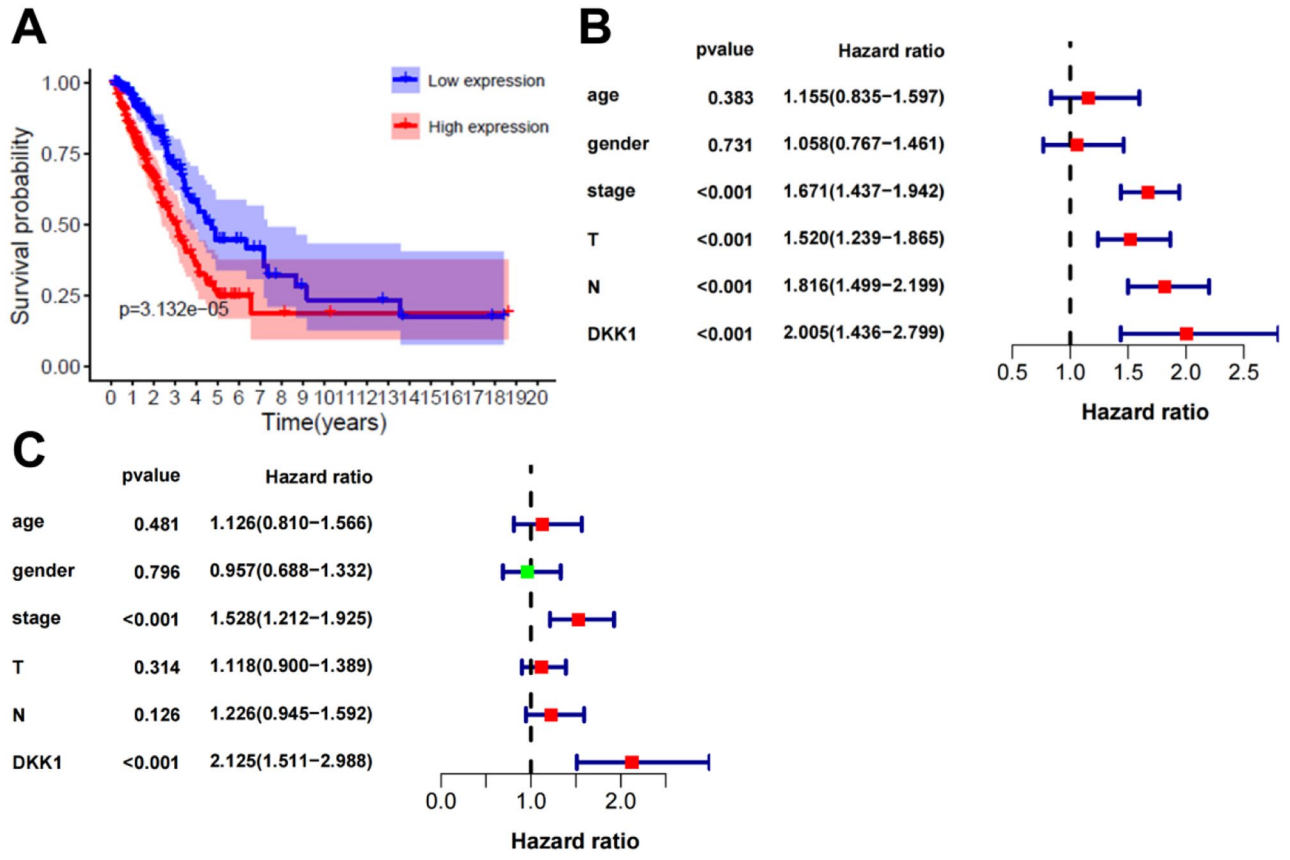

Fig. 9 The prognostic value of DKK1 in LUAD patients in TCGA dataset. **(A)** The Kaplan-Meier survival analysis curve of DKK1 in 448 LUAD patients. **(B)** Univariate Cox regression analysis of clinical factors in 448 LUAD patients. **(C)** Multivariate Cox regression analysis of clinical factors in 448 LUAD patients

PRKG1-AS1 and measured DKK1 expression. The results showed a significant decrease in DKK1 expression levels in H1299 ($p < 0.0001$ for ASO1 and $p < 0.0001$ for ASO2; Fig. 8E) and PC9 ($p < 0.0001$ for ASO1 and $p < 0.0001$ for ASO2; Fig. 8F) cell lines after PRKG1-AS1 knockdown. These results indicate that the expression of PRKG1-AS1 and DKK1 is highly correlated in both tissues and cells. PRKG1-AS1 regulates the expression of DKK1. These findings suggest that DKK1 may serve as a downstream target of PRKG1-AS1.

DKK1 has great prognostic value in LUAD patients

Kaplan-Meier survival analysis unveiled a notable correlation between increased DKK1 expression and adverse outcomes in LUAD patients ($p < 0.001$, Fig. 9A). Univariate Cox regression analysis demonstrated DKK1 as a prognostic risk factor, with its expression level significantly correlating with overall survival ($p < 0.001$, HR = 2.005, Fig. 9B). In order to explore the relationship between DKK1 and prognosis in the presence of other clinical parameters, DKK1 was performed on multivariate Cox regression analysis. The findings indicated DKK1's significant association with overall survival even after adjustment for other clinical factors ($p < 0.001$, HR = 2.125, Fig. 9C). These results suggest that DKK1 has the potential to serve as an independent prognostic biomarker for LUAD patients.

Discussion

In recent years, advancements have been made in lung cancer treatment. However, the survival rate remains low [18]. The potential of lncRNA act as biomarkers has been received attention [19]. Identifying some novel prognostic markers of lung cancer is essential to improve prognosis. In this study, we investigated the expression and clinical value of PRKG1-AS1 in LUAD.

PRKG1-AS1 was highly expressed in LUAD tumor tissues, and it was closely related to prognosis in LUAD. The results indicated that PRKG1-AS1 might promote tumor progression in LUAD. Cox regression analysis revealed a significant association between PRKG1-AS1 expression and patients' prognosis, suggesting its potential as an independent prognostic biomarker.

We investigated the correlation between PRKG1-AS1 and clinical factors. In the TCGA dataset, PRKG1-AS1 demonstrated a close association with TNM stage and N stage. Patients in advanced TNM stages or with lymph node metastasis tended to exhibit higher PRKG1-AS1

expression levels. However, there was no statistical relationship between PRKG1-AS1 and T stage. The results indicated that PRKG1-AS1 closely associated with metastasis rather than proliferation and it may be promote tumor progression through regulating metastasis. Of course, the results may be caused by small sample size.

Additionally, we conducted Gene Set Enrichment Analysis (GSEA) on the TCGA dataset to explore the biological processes and signaling pathways potentially associated with PRKG1-AS1. The results revealed associations of PRKG1-AS1 with several tumor-related biological processes such as "immune response," "NF-kB signaling pathway," "receptor-related signaling pathways," "cell adhesion," "Notch signaling pathway," and "histone acetylation," among others. Notably, two enriched KEGG pathways included "small cell lung cancer" and "P53 signaling pathway." These enriched biological processes and signaling pathways possibly support the hypothesis that PRKG1-AS1 serves as a risk factor in LUAD patients.

Subsequently, we observed a substantial reduction in cell viability, DNA replication activity, and colony formation numbers in LUAD cells following PRKG1-AS1 knockdown. These findings indicate that PRKG1-AS1 enhances tumor cell proliferation in LUAD, consistent with prior observations in head and neck squamous cell carcinoma [14] and oral squamous cell carcinoma [13]. In terms of its effect on tumor migration, knockdown of PRKG1-AS1 resulted in a notable reduction in the migration capacity of LUAD cells. This indicates that PRKG1-AS1 plays a role in promoting LUAD metastasis, aligning with our earlier bioinformatics results.

Research has shown that most lncRNAs participate in various biological processes by interacting with protein-coding genes (PCGs) [20]. Dickkopf-related protein 1 (DKK-1) serves as an inhibitor in the Wnt/β-catenin signaling pathway and primarily regulates cell proliferation, migration, and other characteristics by affecting the Wnt pathway [21]. DKK1 is located downstream of PRKG1-AS1 at 10q21.1. Previous research suggested that DKK1 can act as a biomarker in hepatocellular carcinoma [22], gastric cancer [23], and pancreatic ductal adenocarcinoma [24], implicating its involvement in tumor formation. Literature has already confirmed that DKK1 is upregulated in NSCLC and can influence the migration and invasion abilities of lung cancer cell lines [25, 26], indicating its potential role in the development of lung cancer.

In our investigation, we observed a positive correlation between DKK1 and PRKG1-AS1 expression. Additionally, we created PRKG1-AS1 knockdown cell lines and measured DKK1 mRNA expression levels in LUAD cell lines after knockdown 48 h. The results showed a decrease in DKK1 expression following PRKG1-AS1 knockdown, suggesting that PRKG1-AS1 can regulate DKK1 expression. Subsequently, we assessed DKK1 expression levels and prognostic value in LUAD using public databases and LUAD patient tissue samples. The findings revealed significant upregulation of DKK1 in LUAD, and higher DKK1 levels were positively correlated with shorter survival and poor prognosis in LUAD patients. In summary, DKK1 expression is highly correlated with PRKG1-AS1, and DKK1 is regulated by PRKG1-AS1. Moreover, DKK1 is highly expressed in LUAD and correlates with poor prognosis. Considering the spatial relationship between DKK1 and PRKG1-AS1, along with the aforementioned findings, we propose that DKK1 could be a downstream target gene of PRKG1-AS1. Thus, PRKG1-AS1 may play a role in the initiation and progression of LUAD through the regulation of DKK1 expression.

Conclusion

In our investigation, we noted significant upregulation of PRKG1-AS1 in both LUAD tissues and cells. This elevated expression was linked to poor prognosis among LUAD patients, indicating its potential as an independent prognostic biomarker. Furthermore, PRKG1-AS1 expression showed significant correlations with TNM stage and N stage. Functional enrichment analysis indicated the potential involvement of PRKG1-AS1 in various tumor-related biological processes and KEGG pathways. PRKG1-AS1 primarily localized in the cell nucleus, and downregulation of PRKG1-AS1 markedly suppressed the proliferation and migration of LUAD cells. Additionally, we observed a significant co-expression between PRKG1-AS1 and the oncogene DKK1, with knockdown of PRKG1-AS1 resulting in a substantial decrease in DKK1 expression levels. Notably, DKK1 was found to be markedly overexpressed in LUAD tissues and associated with poor prognosis, suggesting its potential as a target gene of PRKG1-AS1. Overall, our findings offer new insights into the underlying mechanisms of LUAD.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12931-025-03122-y.

Supplementary Material 1

Acknowledgements
Not applicable.

Author contributions
Tong Jiao and Haimei Wen was responsible for the planning and design of the experiment, the implementation of the experiment and the analysis of the data, and the drafting of the article. Lizhong Zeng and Yang Chen were responsible for data collection and analysis of clinical data, prepared Figs. 1, 2 and 3. Jie Shi and Shuanying Yang provided funding support and revised the article. All authors reviewed the manuscript.

Funding
This work was supported by the Natural Science Basic Research Program of Shaanxi Province (2022JM-509).

Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethical approval
The present study was approved by the Ethics Committee of the Second Affiliated Hospital of Xi'an Jiaotong University (Xi'an, China).The ethics number is 2023179. Written informed consent was obtained from all participants.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 11 November 2024 / Accepted: 17 January 2025
Published online: 21 February 2025

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