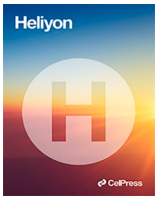
Heliyon 10 (2024) e30642

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

DEP domain containing 1 as a biomarker for poor prognosis in lung adenocarcinoma

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A R T I C L E I N F O

Keywords:
Lung adenocarcinoma
DEP domain containing 1
Immune system
Prognosis
Survival analysis

A B S T R A C T

Objective: The DEP domain-containing 1 (DEPDC1) gene is essential in the development and advancement of different types of cancer. This study is to examine the levels of DEPDC1 in lung adenocarcinoma (LUAD), and to determine its relationship with clinical results and immune response. The goal is to assess its potential as a biomarker and therapeutic target for LUAD.
Methods: By comprehensively utilizing the Cancer Genome Atlas (TCGA), gene Expression Synthesis (GEO), UALCAN, cBioPortal, TISIDB databases and online platforms, we conducted a bioinformatics analysis to investigate DEPDC1 gene survival analysis, prognostic diagnosis, prognostic survival, immune cell infiltration, DNA methylation, and the correlation of genetic mutations in LUAD. The results were validated through cell assay and immunohistochemical staining.
Results: DEPDC1 shows high levels of expression in the majority of tumors, with its expression being notably elevated in LUAD compablue to normal tissues. The expression of DEPDC1 varies based on the clinical characteristics of patients with LUAD. DEPDC1 expression affects the survival prognosis and prognostic model construction of LUAD patients. In addition, the presence of DEPDC1 is linked to immune infiltration. Various chemokines and chemokine receptors, immunoinhibitors and immune-stimulators in LUAD are significantly correlated with DEPDC1 methylation levels. Cell experiments confirmed through qPCR that the mRNA expression of DEPDC1 in LUAD was markedly elevated in comparison to the normal population, and immunohistochemistry showed positive DEPDC1 expression in LUAD pathological sections.
Conclusion: Systematic analysis and experiments have verified that DEPDC1 serves as a biomarker for detecting early, prediction of survival, and evaluation of immune cell infiltration in LUAD.

1. Introduction

Cancer exhibits diversity and complexity, associated with a range of genetic and epigenetic abnormalities [1,2]. Lung cancer is the most common type of cancer worldwide and the main cause of cancer-related fatalities. It is categorized into two principal groups: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) [3]. NSCLC, accounting for approximately 85 % of lung cancer

Abbreviations: LUAD, lung adenocarcinoma; DEPDC1, DEP domain containing 1; TCGA, The Cancer Genome Atlas; PCA, Principal Component Analysis; HR, hazard ratio; IHC, immunohist-ochemistry; PD, progressive disease; SD, stable disease; PR, partial response; CR, complete response; OS, overall survival; DSS, disease-specific survival; PFI, progression-free interval; AUC, Area Under Curve.

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https://doi.org/10.1016/j.heliyon.2024.e30642
Received 7 February 2024; Received in revised form 29 April 2024; Accepted 1 May 2024
Available online 3 May 2024

cases, is the most common type. It is categorized into three subtypes: lung adenocarcinoma (LUAD), squamous cell carcinoma (LUSC), and large cell carcinoma based on histology [4–6]. LUAD originates from type II alveolar epithelial cells [7–9], representing a distinct subtype of lung cancer characterized by specific mutations and unique cellular features. Even with progress made in both fundamental research and the diagnosis of lung cancer, the prognosis for LUAD patients continues to be unfavorable [10,11]. Presently, lung cancer is treated with a combination of targeted therapy and immunotherapy alongside conventional surgery, radiotherapy, and chemotherapy, all of which have significantly enhanced patient survival rates. Nonetheless, a majority of NSCLC patients are typically diagnosed at advanced stages due to diagnostic constraints [12], the survival rate after five years is extremely poor. To enhance patient prognosis and survival rates, it is crucial to identify a novel biomarker, enhance the diagnostic accuracy of lung adenocarcinoma, and pinpoint new drug targets for treatment. Studying the molecular mechanisms of lung cancer development is essential for identifying valuable diagnostic markers and improving treatment outcomes.

DEP (disheveled, EgI-10, plechstrin) domain protein 1 (DEPDC1) is a gene associated with tumors, responsible for coding a protein that includes a DEP domain. Overexpression of this gene was initially identified in bladder cancer in 2007, where it is involved in controlling the growth of cells that are bladder cancerous [13]. The DEPDC1 gene can express a protein containing a DEP domain, which has functions such as regulating GTPase activity, signal transduction, establishment of cell polarity, and cell membrane anchoring [14]. While DEPDC1 is found in the testis, it is not present in any other normal human tissues. Research has shown that DEPDC1 is closely linked to the formation of different types of tumors, including prostate tumor, breast tumor, gastric tumor, colorectal tumor, and hepatocellular carcinoma [15–19]. High levels of expression of DEPDC1 have been observed in these tumors, leading to a strong correlation with a negative clinical outlook. Previous research has demonstrated that DEPDC1 is highly expressed in the majority of tumors, indicating its promise as a biomarker and potential target for therapy. The overexpression of DEPDC1 in lung adenocarcinoma has also been initially confirmed. Several studies have shown that by targeting DEPDC1, the nuclear factor kappa-B (NF–B) signaling pathway can be triggered to stimulate the apoptosis of lung adenocarcinoma cells [20]. The relationship between DEPDC1 and the survival outlook in LUAD, as well as its impact on the immune system, remains uncertain. Therefore, this study aimed to explore whether DEPDC1 can become a biomarker for LUAD and affect the prognosis and survival of LUAD patients. The pan-cancer study utilized multiple databases and bioinformatics analysis to reveal that DEPDC1 exhibited significant up-regulation in 29 out of 33 human cancers examined. In addition, significant differences in DEPDC1 expression were noted among various tumor immune subtypes and molecular subtypes. In various scenarios involving cancer, DEPDC1 was observed to enhance cell cycle advancement, DNA repair, response to DNA damage, and cell growth. Importantly, the presence of DEPDC1 was discovered to have correlations with overall survival, disease-specific survival, and prognosis for progression-free interval across various tumor types. Additionally, ROC analysis demonstrated a high degree of predictive accuracy for diverse cancer scenarios, indicating that DEPDC1 exhibits abnormal expression and contributes to immune-related oncogenesis. These discoveries indicate that DEPDC1 could be a useful indicator for both the detection and management of cancer [21]. Research on gastric adenocarcinoma indicates that DEPDC1 is upregulated in gastric adenocarcinoma compared to adjacent normal gastric tissue. The expression levels of DEPDC1 are closely linked to cancer metastasis and differentiation, and are associated with negative survival results. This highlights the significance of DEPDC1 in gastric adenocarcinoma, suggesting that its upregulation is linked to tumor progression and poor clinical prognosis in patients. Furthermore, DEPDC1 has the potential to be a target for therapeutic interventions in gastric tumor [17].

There have been numerous studies delved into the therapeutic targets of tumors using bioinformatics analysis. This research examines the amount of DEPDC1 expression in LUAD was analyzed through a comprehensive examination of TCGA, GEO, UALCAN, cBioPortal, and TISIDB databases, as well as various online platforms. Through survival analysis, prognostic diagnosis, and correlation with the immune system, the study demonstrated that DEPDC1 can serve as a biomarker for predicting survival results and the degree of immune cell infiltration in individuals diagnosed with LUAD. (Fig. 1). This discovery highlights the important involvement of DEPDC1 in the development and advancement of lung adenocarcinoma, providing fresh perspectives for the clinical identification and management of LUAD.

2. Method

2.1. Data collection

Retrieve TCGA-LUAD transcriptome expression data from the TCGA database, which consists of 539 samples of tumor and 59

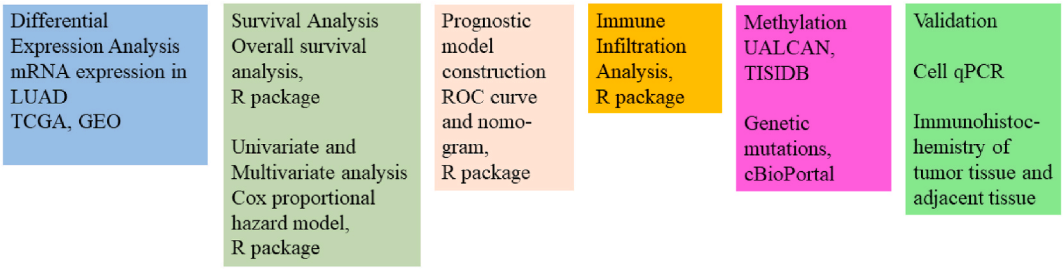

Fig. 1. Method diagram.

samples of normal. Transcriptome data were processed and visualized using R language. An analysis was conducted on the expression discrepancies of DEPDC1 among LUAD tissues and their neighboring tissues. Access the GEO database to download transcriptome data comprising LUAD tumor tissues and normal tissues. Utilize the online analysis tool GEO2R to conduct an investigation the differential DEPDC1 expression in LUAD tissues in comparison to healthy tissues through online analysis. The datasets GSE31210, GSE116959, and GSE140797 consist of varying numbers of samples from patients with LUAD and normal lung tissues. GSE31210 includes 226 samples from patients with LUAD and 20 samples of normal lung tissue, GSE116959 includes 57 samples from patients with LUAD and 11 samples of normal lung tissue, while GSE140797 has 7 samples from patients with LUAD and 7 samples of normal lung tissue.

2.2. Differential expression analysis

The data from GSE31210, GSE116959, and GSE140797 were retrieved from the GEO dat-abase for subsequent analysis. The analysis utilized a filtering condition of $|LogFC|>2$ and $p < 0.05$ for differential expression. Examine the clustering patterns among sample groups using Principal Component Analysis (PCA) plot. Display the results of differentially expressed genes in the two groups using a volcano plot. Additionally, visualize the significantly ex-pressed differential genes with a heat map. Evaluate the quantitative performance by standardizing the data for comparison across different groups. Used R packages "GEOquery", "limma", "ggplot2" "ComplexHeatmap".

2.3. Analysis of survival

Patients diagnosed with LUAD in the TCGA database were divided into two categories based on the expression levels of DEPDC1: high and low expression, using the median expression level as the dividing line. Survival regression was performed using the R "survival" package to test the proportional hazards hypothesis. The data was visualized with the "survminer" and "ggplot2" packages to create Kaplan-Meier plots. The plots were used to assess the relationship between DEPDC1 expression and patient survival in LUAD. The hazard ratio (HR) and P value for the 95 % confidence interval were also calculated through this analysis.

2.4. Establishment of ROC curve and nomogram for LUAD patients

Diagnostic ROC is used to analyze the prediction accuracy performance of variables. Use the "pROC" package to perform ROC analysis on the data. The data is graphically represented using "ggplot2". The area under the ROC curve (AUC) is commonly employed to assess diagnostic tests. A higher AUC value, closer to 1, suggests that the variable has a more effective diagnostic capability in predicting outcomes. Time-dependent ROC, the information is processed with the "timeROC" software package, and the findings are presented visually with "ggplot2". The Prognostic Nomogram utilizes the "survival" package for conducting proportional hazards hypothesis testing and Cox regression analysis. Use the "rms" package to create a nomogram for a correlation model and create a visual representation to demonstrate the connections between each predictor variable in the predictive model. The objective of the research is to assess the one-year, five-year, and ten-year survival rates of individuals diagnosed with LUAD.

2.5. Clinical analysis of DEPDC1 expression

Using the "ggplot2" software, the DEPDC1 expression levels in tumor tissues and normal tissues from the TCGA cohort were assessed. The relationship between DEPDC1 expression and overall survival (OS), disease-specific survival (DSS), as well as progression-free interval (PFI) was also analyzed. Examining the clinical features recorded in the TCGA repository permits an assessment of the association between these features and DEPDC expression levels, with the aim of exploring the link between DEPDC1 and the clinical prognosis of individuals with LUAD.

2.6. Relationship between DEPDC1 and immune cells

To examined the correlation between DEPDC1 and infiltration of 24 different types of immune cells was conducted using the "ggplot2" package. The level of immune cell infiltration was evaluated in connection with the expression of DEPDC1. Individuals with LUAD were categorized into high and low expression groups based on the median DEPDC1 expression, and the disparities in immune infiltration were analyzed. Furthermore, the association between DEPDC1 and immune checkpoint molecules PDCD1, CD274, and CTLA4 was explored through Spearman's rank correlation.

2.7. Correlation between DEPDC1 expression and methylation

The promoter methylation level of DEPDC1 in LUAD was investigated using UALCAN, cBioPortal to study genetic mutations of DEPDC1, TISIDB is used to analyze the correlation between DEPDC1 methylation levels, immune checkpoint gene levels, and chemokines.

2.8. Cell culture and qPCR validation

Human BEAS-2B and A549 cells were provided by Procell Life Science Technology and cultured in MEM medium with 10 % fetal

bovine serum and 1 % cyanin-streptomycin. The cells were maintained by changing the medium every two days and passaging every three to four days. DEPDC1 mRNA expression levels were assessed in BEAS-2B and A549 cells. Total RNA was isolated from the cells in culture, and then converted to cDNA using a reverse transcription kit (Vazyme), starting with around 1 g of total RNA. Following this, a Quantitative SYBR Green PCR Kit (Vazyme) was utilized to perform qRT-PCR on a SLAN-96S Detection System. The procedure started with an initial stage at 95 °C for 30 s, then proceeded with 40 repetitions of 95 °C for 10 s and 60 °C for 30 s. Finally, it finished with a last step at 95 °C for 15 s, 60 °C for 60 s, and a final 95 °C for 15 s. The PCR primers used were following. DEPDC1 forward, 5′-TGCCATGAAGTGCCTAGCAA-3′ and reverse, 5′- TGATGT AGCCACAA ACAACCAAA-3′, GAPDH forward, 5′-CATGTTGCAACCGGG AAGGA-3' and reverse, 5′- GCCCAATACGA CCAAA TCAGAGA -3′. Utilizing GAPDH as the reference, the mRNA levels were determined and normalized through the $2^{-\Delta\Delta Ct}$ technique. Each experiment was conducted three times.

2.9. Tissue specimen collection and immunohistochemical staining

Samples were collected from 27 patients diagnosed with LUAD and 35 individuals with adjacent tissues at the First Affiliated Hospital of Dali University for this research study. The Ethics Committee at Dali University approved it (NO.20230806), and patients granted written Informed Consent. Preoperative adjuvant therapy was not administered to any of the patients, and all tissue samples were fixed in paraffin for immunohistochemistry (IHC) staining. The paraffin blocks were sliced into 4 μm sections using a slicer. These slices underwent dewaxing with xylene and rehydration with a series of ethanol solutions. A solution of hydrogen peroxide at a concentration of 3 % was utilized for an incubation period lasting 20 min in order to block the activity of intrinsic peroxidase. Antigen retrieval was performed using citrate buffer, followed by the addition of Rabbit anti-human polyclonal antibodies DEPDC1 and overnight incubation at 4 °C. The next day, the slices were treated with sheep anti-rabbit secondary antibodies and incubated at room temperature for 1 h. Detection of DEPDC1 expression in LUAD and adjacent tissues was achieved through staining with diaminobenzidine (DAB) followed by dehydration, clearing, and fixation for observation under a light microscope.

Table 1
Clinical features of patients with LUAD.

Characteristics	Low expression of ERBB2	High expression of ERBB2	P value
N	269	270	
Pathologic T stage, n (%)			0.994
T1	88 (16.4 %)	88 (16.4 %)	
T2	145 (27.1 %)	147 (27.4 %)	
T3	24 (4.5 %)	25 (4.7 %)	
T4	10 (1.9 %)	9 (1.7 %)	
Pathologic N stage, n (%)			0.517
N0	180 (34.4 %)	170 (32.5 %)	
N1	47 (9 %)	50 (9.6 %)	
N2	31 (5.9 %)	43 (8.2 %)	
N3	1 (0.2 %)	1 (0.2 %)	
Pathologic M stage, n (%)			0.277
M0	178 (45.6 %)	187 (47.9 %)	
M1	15 (3.8 %)	10 (2.6 %)	
Pathologic stage, n (%)			0.215
Stage I	149 (28.1 %)	147 (27.7 %)	
Stage II	64 (12.1 %)	61 (11.5 %)	
Stage III	34 (6.4 %)	50 (9.4 %)	
Stage IV	16 (3 %)	10 (1.9 %)	
Primary therapy outcome, n (%)			0.127
PD	43 (9.6 %)	28 (6.2 %)	
SD	22 (4.9 %)	16 (3.6 %)	
PR	4 (0.9 %)	2 (0.4 %)	
CR	158 (35.2 %)	176 (39.2 %)	
Gender, n (%)			0.130
Female	153 (28.4 %)	136 (25.2 %)	
Male	116 (21.5 %)	134 (24.9 %)	
Age, n (%)			0.664
≤ 65	129 (24.8 %)	128 (24.6 %)	
>65	127 (24.4 %)	136 (26.2 %)	
Residual tumor, n (%)			0.710
R0	178 (47.6 %)	179 (47.9 %)	
R1	8 (2.1 %)	5 (1.3 %)	
R2	2 (0.5 %)	2 (0.5 %)	
Location, n (%)			0.330
Central Lung	32 (16.8 %)	31 (16.3 %)	
Peripheral Lung	55 (28.9 %)	72 (37.9 %)	
Smoker, n (%)			0.945
No	38 (7.2 %)	39 (7.4 %)	
Yes	223 (42.5 %)	225 (42.9 %)	

PD, progressive disease; SD, stable disease; PR, partial response; CR, complete response. $P < 0.05$, the results were statistically significant.

2.10. Statistical analysis

One approach employed for identifying potential prognostic factors was the use of univariate COX analysis, while multivariate analysis was utilized in conjunction with various clinical variables to investigate the relationship between DEPDC1 expression and survival. The association between DEPDC1 expression and the clinical characteristics of patients with LUAD was assessed through the Wilcoxon test and logistic regression. Additionally, a forest plot was generated utilizing the R package "forestplot". Statistical analyses were conducted utilizing either R software or SPSS software, with statistical significance being determined as a P value less than 0.05.

3. Results

3.1. Clinical features of patients with LUAD

The clinical data of 539 LUAD patients in the TCGA database were analyzed, 176 cases were in pathological stages T1, 292 cases were in stages T2, 49 cases were in stages T3, 19 cases were in stages T4; 350 cases were in pathological stages N0, 97 cases were in stages N1, 74 cases were in stages N2, 2 cases were in stages N3; 365 cases were in pathological stages M0, 25 cases were in stages M1; 250 males, 289 females, 263 patients over 65 years old, and 257 patients 65 years old or younger. The detailed results are shown in Table 1.

3.2. The DEPDC1 gene is highly expressed in LUAD

The analysis of DEPDC1 mRNA expression in tumor and normal tissues from the TCGA database revealed high levels of expression in various types of cancer including bladder, breast, cervical, colon, esophageal, brain, head and neck, kidney, liver, lung, and other tumor, the distinction was statistically meaningful, the results were confirmed through paired analysis, the results are shown in Fig. 2A and B.

The analysis of DEPDC1 mRNA expression levels in LUAD patients was conducted using the TCGA database. The expression of DEPDC1 was found to be statistically distinct between normal and tumor tissues, with tumor tissues showing significantly elevated levels of DEPDC1 compared to normal tissues (Fig. 3A). During OS analysis, it was noted that the level of DEPDC1 expression was greater in the deceased group than in the surviving group. Significantly higher expression levels were identified in the Yes group in comparison to the No group, in the analysis of DSS and PFI, as depicted in Fig. 3B–D. This suggests a correlation between higher DEPDC1 expression levels and increased mortality rates. Furthermore, the expression level of DEPDC1 showed significant correlations with pathological stage, gender, smoking status, and primary therapy outcome. Patients in stage I/II showed significantly higher expression of DEPDC1 compablue to those in stage III/IV. Male patients exhibited higher levels of DEPDC1 expression than female patients. Smokers had higher levels of DEPDC1 expression in comparison to non-smokers. Furthermore, the expression of DEPDC1 increased significantly in patients classified as PD/SD group compared to those in PR/CR group (Fig. 3E–H). Through logistic analysis,

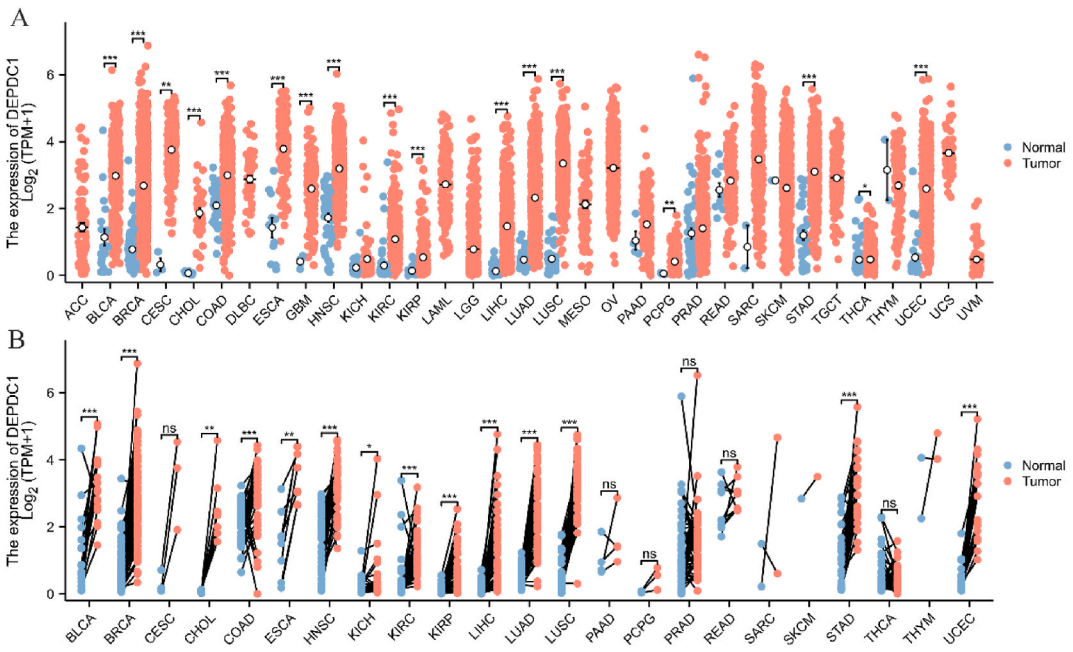

Fig. 2. Levels of DEPDC1 expression in different types of human tumors. A. unpaired analysis; B. paired analysis. *p < 0.05, **p < 0.01, ***p < 0.001.

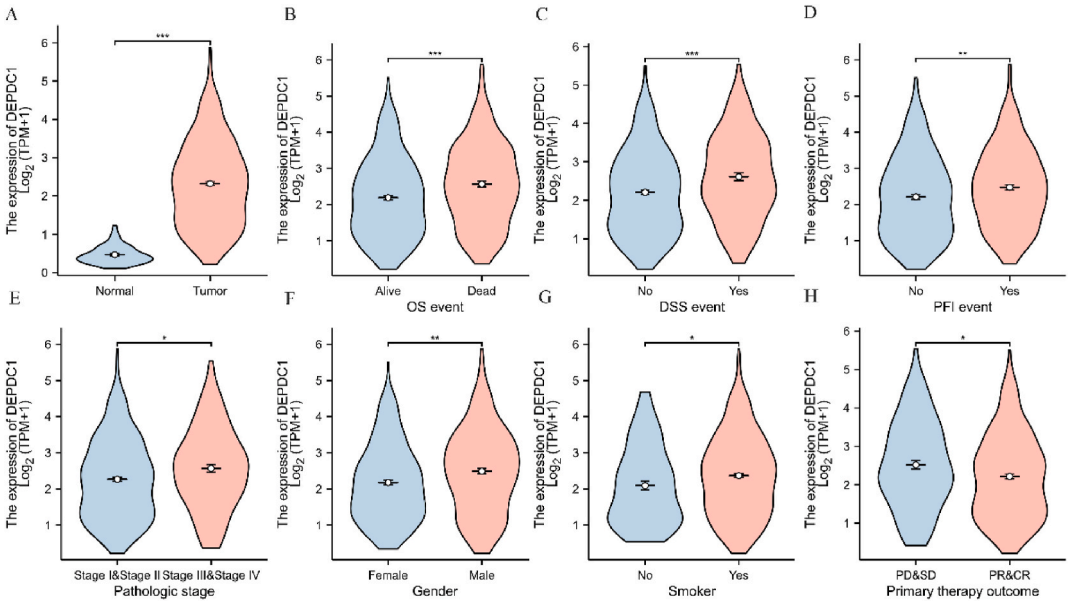

Fig. 3. Analysis of clinical significance of DEPDC1 expression A. The expression of DEPDC1 in both normal and tumor tissues; B–H. Expression of DEPDC1 in different clinical feature groups. *p < 0.05, **p < 0.01, ***p < 0.001.

DEPDC1 was found to be correlated with Pathologic stage, Gender, and Number of pack years smoked in relation to LUAD prognosis, the findings are outlined in Table 2. Further analysis using COX analysis found a link between the expression of DEPDC1 and the clinical features of patients with LUAD. Both univariate and multivariate COX regression analyses showed a relationship between Primary therapy outcome and DEPDC1 expression with LUAD overall survival. These results are presented in Table 3, forest figure results are shown in Fig. 4A and B.

3.3. Validation of DEPDC1 in external data sets

To further validate the previously observed elevated expression of DEPDC1 in LUAD tissues using data from the TCGA database, we utilized three independent external datasets (GSE31210, GSE116959 and GSE140797) as validation sets to evaluate the levels of RNA transcripts of DEPDC1 in cancerous and surrounding tissues. Our research results showed a notable increase in DEPDC1 levels in tumor samples in comparison to adjacent non-cancerous tissues within the three databases analyzed, which was consistent with the TCGA database results (Fig. 5A–C). These results were further validated in paired samples, results illustrated in Fig. 5D–F. The differential genes of the three datasets are illustrated in Fig. 6A, C, and 6E, red denotes up-regulated genes, while blue indicates down-regulated genes. Furthermore, The PCA of GSE31210 showed that the proportion of PC1 and PC2 was 14.6 %, the combined proportion of GSE116959 was 17.3 %, and the combined proportion of GSE140797 was 52.4 % (Fig. 6B, D, 6F), suggesting a wealth of differentially expressed genes across three datasets. Noticeable differences were observed between the tumor and healthy groups within each dataset. Utilizing a heat map, the top 50 genes exhibiting differential expression in the dataset were visually represented (Fig. 7A–C), showcasing the modulation of gene expression in both the tumor and healthy groups.

Table 2
Analysis of the logistic regression model to evaluate the association between the expression of DEPDC1 and the clinical features in individuals with LUAD in the TCGA repository.

Characteristics	Total (N)	OR (95 % CI)	P value
Pathologic T stage (T3&T4 vs. T1&T2)	536	0.927 (0.557–1.542)	0.770
Pathologic N stage (N2&N3 vs. N0&N1)	523	1.568 (0.955–2.575)	0.075
Pathologic M stage (M1 vs. M0)	390	2.068 (0.871–4.910)	0.100
Pathologic stage (Stage III&Stage IV vs. Stage I&Stage II)	531	1.551 (1.014–2.374)	0.043
Primary therapy outcome (PR&CR vs. PD&SD)	449	0.735 (0.476–1.133)	0.163
Gender (Male vs. Female)	539	1.757 (1.248–2.474)	0.001
Age (>65 vs.≤65)	520	0.712 (0.504–1.006)	0.054
Residual tumor (R1&R2 vs. R0)	374	1.630 (0.590–4.502)	0.346
Anatomic neoplasm subdivision (Right vs. Left)	524	1.009 (0.711–1.433)	0.959
Location (Peripheral Lung vs. Central Lung)	190	1.153 (0.629–2.113)	0.646
Number pack years smoked (≥40 vs. < 40)	369	1.790 (1.185–2.705)	0.006
Smoker (Yes vs. No)	525	1.524 (0.934–2.487)	0.092

Table 3
Univariate and multivariate Cox regression analyses investigate the relationship between OS and clinical characteristics.

Characteristics	Total(N)	Univariate analysis		Multivariate analysis	
		Hazard ratio (95 % CI)	P value	Hazard ratio (95 % CI)	P value
Pathologic T stage	527				
T1&T2	461	Reference		Reference	
T3&T4	66	2.352 (1.614–3.426)	<0.001	1.784 (0.991–3.211)	0.054
Pathologic N stage	514				
N0&N1	441	Reference		Reference	
N2&N3	73	2.360 (1.659–3.358)	<0.001	1.957 (0.795–4.817)	0.144
Pathologic M stage	381				
M0	356	Reference		Reference	
M1	25	2.176 (1.272–3.722)	0.005	1.369 (0.501–3.742)	0.541
Pathologic stage	522				
Stage I&Stage II	415	Reference		Reference	
Stage III&Stage IV	107	2.710 (1.994–3.685)	<0.001	1.129 (0.441–2.889)	0.800
Primary therapy outcome	442				
PD&SD	109	Reference		Reference	
PR&CR	333	0.380 (0.270–0.533)	<0.001	0.333 (0.218–0.508)	<0.001
Gender	530				
Female	283	Reference			
Male	247	1.087 (0.816–1.448)	0.569		
DEPDC1	530				
Low	266	Reference		Reference	
High	264	1.589 (1.188–2.125)	0.002	1.754 (1.167–2.638)	0.007

3.4. The correlation between the level of DEPDC1 expression and prognosis for survival

Individuals with LUAD were separated into two categories based on the DEPDC1 expression level: one with high expression and the other with low expression, determined by the median expression level. The Kaplan-Meier survival curve analysis indicated that decreased DEPDC1 expression was linked to OS (HR = 1.59, P = 0.002), DSS (HR = 1.55, P = 0.02), and PFI (HR = 1.44, P = 0.006) (Fig. 8A–C). The group with lower expression levels showed a notably higher rate of survival than the group with higher expression levels. The findings from the subgroup analysis indicated that the OS of pathologic M0, Age<65, anatomic neoplasm subdivision left, smoker, pathologic T2 stage group patients exhibiting low levels of DEPDC1 expression demonstrated markedly superior survival results in contrast to individuals showcasing high levels of expression. The disparity showed statistical significance, showing that decreased levels of DEPDC1 are associated with a more favorable outcome in the survival rates of individuals with LUAD, these results are shown in Fig. 8D–H.

3.5. Relationship between DEPDC1 and immune features

DEPDC1 is associated with immune cells, in Fig. 9A. There is a direct connection with Th2 cells and Tgd immune cells, but an inverse relationship with Mast cells and iDC immune cells. The correlations are Th2 cells (R = 0.799, P < 0.001), Tgd (R = 0.336, P < 0.001), T helper cells (R = 0.180, P < 0.001), NK CD56dim cells (R = 0.153, P < 0.001), Mast cells (R = −0.483, P < 0.001), iDC (R = −0.393, P < 0.001), Eosinophils (R = −0.385, P < 0.001), Th17 cells (R = −0.385, P < 0.001), the results are shown in Fig. 9B–I. DEPDC1 has been categorized based on its median expression value into two groups: one with high expression levels and one with low expression levels. The expression of high and low groups is different in various immune cells, the high-level DEPDC1 group has higher expression in Tgd cells and lower expression in B cells, CD8 T cells, DC, iDC, Eosinophils, Mast cells, pDC, NK cells, and TFH cells, the difference is statistically significant, these results are shown in Fig. 10A–J. Analyzing the correlation between DEPDC1 and immune checkpoints, it was found that it was positively correlated with PDCD1, CD274, and CTLA4, the results are shown in Fig. 11A–C. The results suggest a robust association between DEPDC1 and immune suppression and infiltration within the tumor microenvironment of LUAD. In essence, the expression of DEPDC1 is linked to the immune system and significantly impacts immune prognosis.

3.6. The diagnostic importance of DEPDC1 expression in LUAD

A curved ROC was created for the assessment of DEPDC1 expression's diagnostic importance in individuals diagnosed with LUAD. With an AUC of 0.971, it is evident that DEPDC1 shows significant diagnostic potential for prognosis assessment, in Fig. 12A. To forecast the survival rates of patients with LUAD, time-specific ROC curves were employed for the 1-year, 5-year, and 10-year intervals. With an AUC of 0.635 at year 1, 0.599 at year 5, and 0.629 at year 10, the data indicates the DEPDC1 gene's credibility as a prognostic marker for LUAD, in Fig. 12B. Through the nomogram analysis, the predictive efficacy of patient survival rates at 1, 5, and 10 years is determined by aligning the DEPDC1 expression levels with various clinical characteristics, in Fig. 12C. The nomogram indicated that LUAD samples exhibiting high expression levels of DEPDC1 were associated with elevated individual total scores and consequently lower survival rates at 1, 5, and 10 years. Overexpressing DEPDC1 could be a valuable prognostic marker for individuals diagnosed with LUAD.

A

Characteristics	Total(N)	HR(95% CI) Univariate analysis	P value Univariate analysis
Pathologic T stage	527		
T1&T2	461	Reference	
T3&T4	66	2.352 (1.614 - 3.426)	< 0.001
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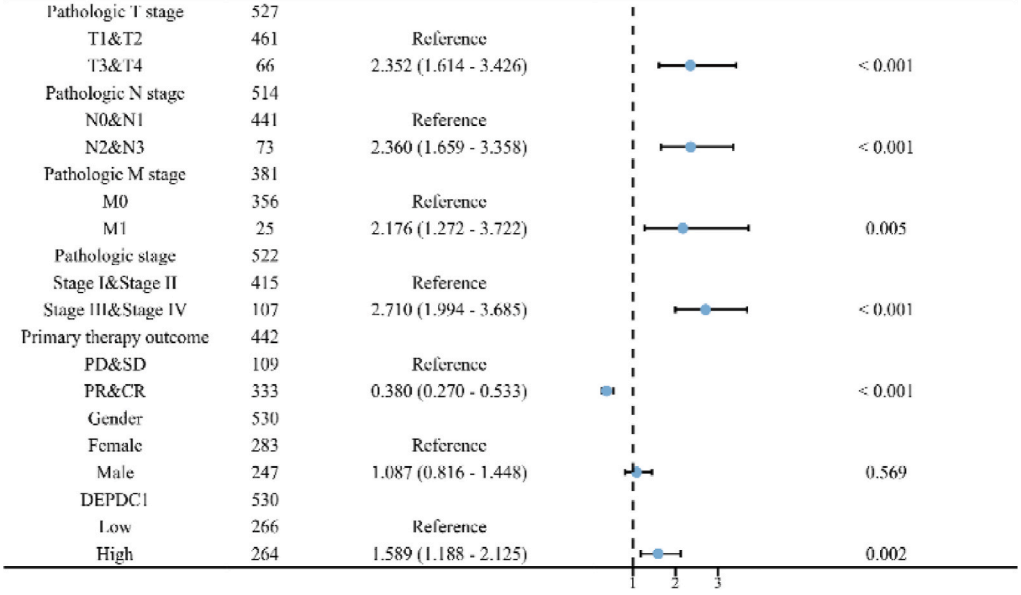
Low	266	Reference	
High	264	1.589 (1.188 - 2.125)	0.002



B

Characteristics	Total(N)	HR(95% CI) Multivariate analysis	P value Multivariate analysis
Pathologic T stage	527		
T1&T2	461	Reference	
T3&T4	66	1.784 (0.991 - 3.211)	0.054
Pathologic N stage	514		
N0&N1	441	Reference	
N2&N3	73	1.957 (0.795 - 4.817)	0.144
Pathologic M stage	381		
M0	356	Reference	
M1	25	1.369 (0.501 - 3.742)	0.541
Pathologic stage	522		
Stage I&Stage II	415	Reference	
Stage III&Stage IV	107	1.129 (0.441 - 2.889)	0.8
Primary therapy outcome	442		
PD&SD	109	Reference	
PR&CR	333	0.333 (0.218 - 0.508)	< 0.001
Gender	530		
Female	283		
Male	247		
DEPDC1	530		
Low	266	Reference	
High	264	1.754 (1.167 - 2.638)	0.007

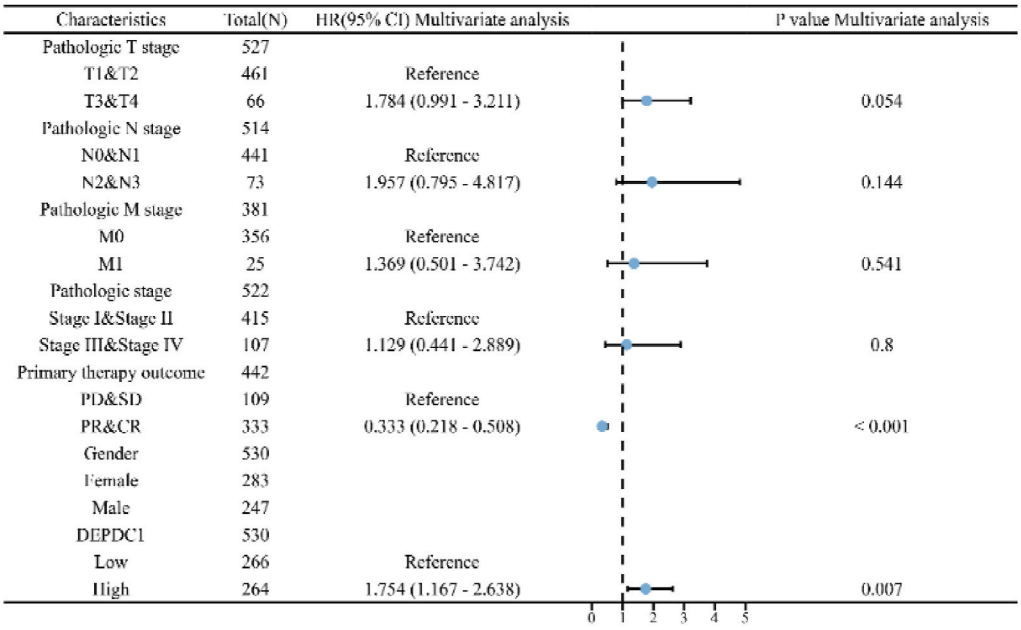


Fig. 4. Plot of the forest showing the Cox regression analyses outcomes in individuals with LUAD. A. univariate Cox regression analyze; B. multivariate Cox regression analyze.

3.7. The relationship between DEPDC1 expression and DNA methylation

The UALCAN platform was employed to examine the levels of DEPDC1 promoter methylation in individuals with LUAD tumors and those without cancer. Results from the research indicated a small rise in the methylation levels of the DEPDC1 promoter in tumor specimens when in comparision to the non-cancer cohort, see Fig. 13A. The TISIDB platform examined how the methylation level of DEPDC1 in LUAD is linked to immune cell chemokines and chemokine receptors, as well as its correlation with immunoinhibitors and immunostimulators. The results showed that a variety of chemokines and chemokines receptors, immunoinhibitors and immunostimulators are significantly correlated with DEPDC1 methylation levels in LUAD, the results are shown in Fig. 13B–E. The mutation status of DEPDC1 gene in LUAD tumor tissue was analyzed through the cBioPortal platform, the results are shown in Fig. 14. The figure

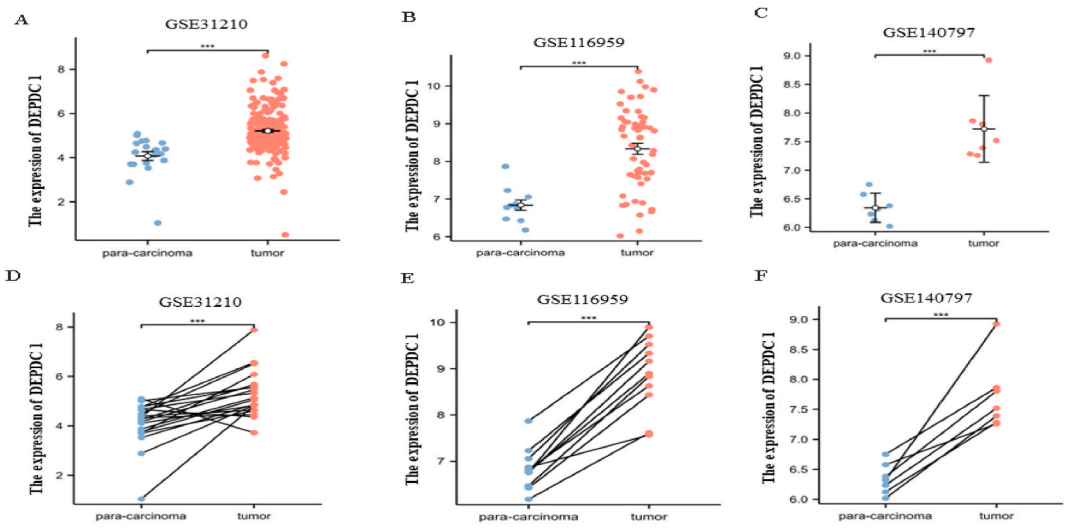


Fig. 5. GEO database validates the high expression of DEPDC1 in LUAD. (A–C) Expression of DEPDC1 in tumors and unpaired adjacent tissues in GSE31210, GSE116959 and GSE140797 data sets. (D–F) DEPDC1 expression in tumors and paired para-tumor tissues in the GSE31210, GSE116959 and GSE140797 datasets. ***$p < 0.001$.

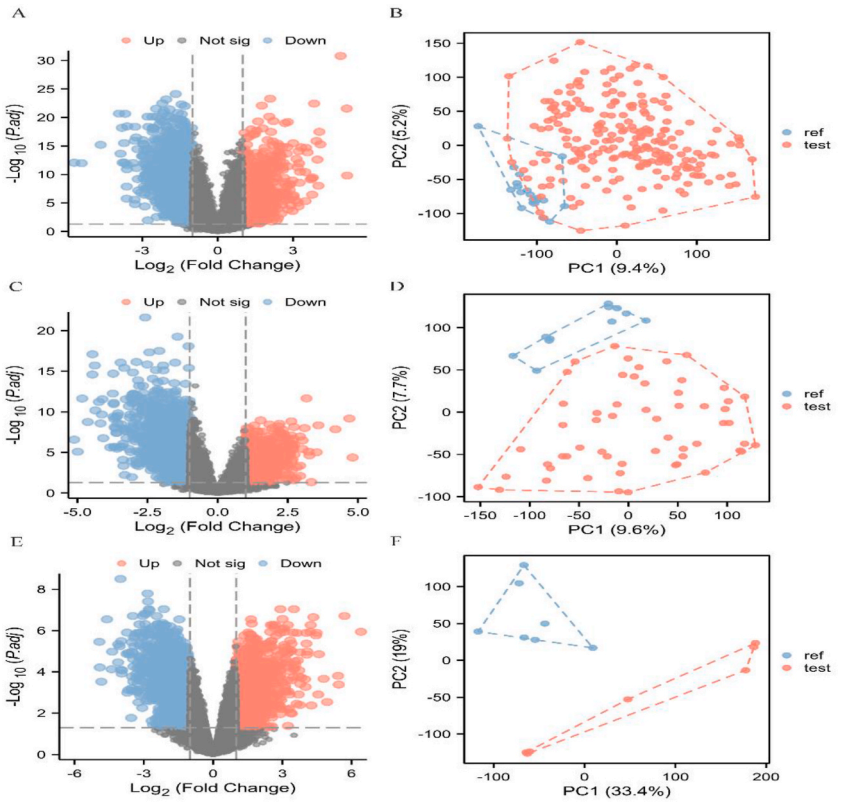


Fig. 6. Visualizations of volcano plot and PCA plot from an external database. (A–B) Volcano plot and PCA plot of GSE31210 data set; (C–D) Volcano plot and PCA plot of GSE116959 data set; (E–F) Volcano plot and PCA plot of GSE140797 data set. In the volcano plot, red signifies genes that are up-regulated, while blue indicates genes that are down-regulated. In the PCA plot, red denotes the tumor group, and blue represents the normal control group.

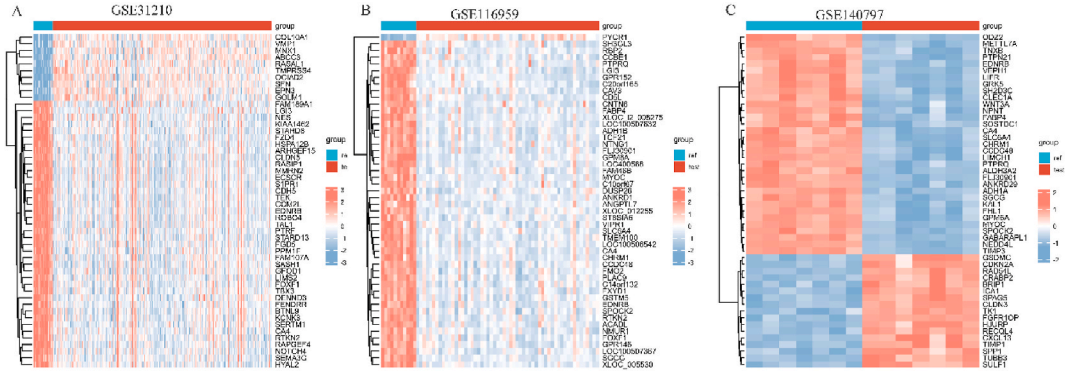


Fig. 7. Heat map. A. GSE31210 data set; B. GSE116959 data set; C. GSE140797 data set. Ref: normal tissue, test: tumor tissue.

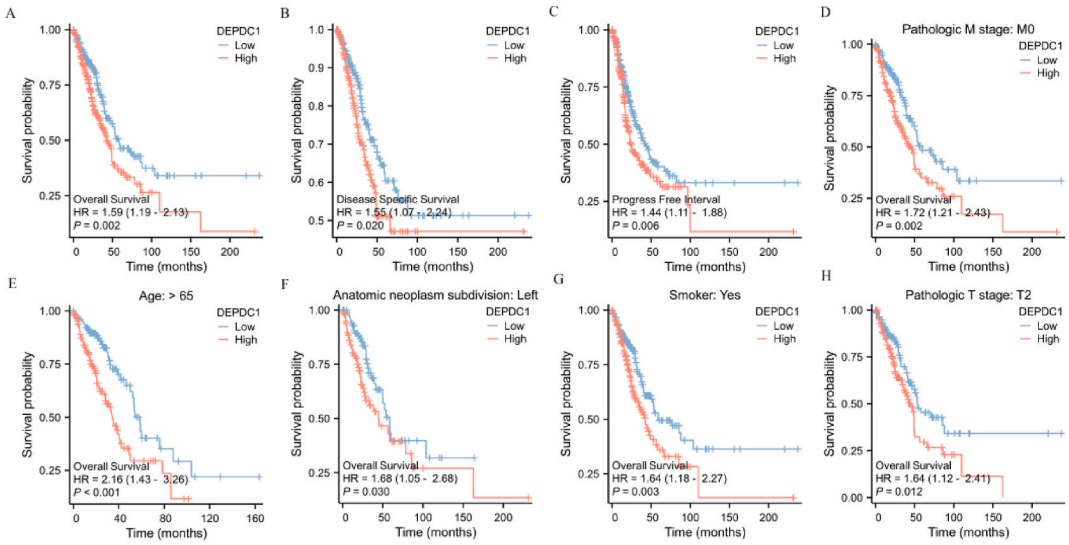


Fig. 8. Prognostic survival analysis of DEPDC1 expression. A-C. Kaplan-Meier survival curve analysis of OS, DSS, PFI; D-H. Kaplan-Meier curves of pathologic M0, Age<65, anatomic neoplasm subdivision left, smoker, pathologic T2 stage subgroups respectively.

shows that 1.7 % of patients have DEPDC1 gene mutations, and missense mutation and amplification are the main forms of changes in the DEPDC1 gene.

B–C. Correlation between DEPDC1 methylation level and immune cell chemokines and chemokine in LUAD; D-E. Correlation between DEPDC1 methylation level and immunoinhibitors and immunostimulators in LUAD. In 13B-13E, graphical representations often use the color red to signify a positive correlation, while the color blue is typically employed to denote a negative correlation.

3.8. Cell and sample validation

The expression of mRNA from the DEPDC1 gene was evaluated in A549 and BEAS-2B cells. Results indicated a significant increase in DEPDC1 mRNA levels in A549 cells compared to BEAS-2B cells, implying a potential role of DEPDC1 in the pathogenesis of LUAD, results are shown in Fig. 15. Immunohistochemical staining was performed on pathological sections of 27 cases of LUAD tumor tissues and 35 cases of para-cancerous tissues. The expression of DEPDC1 in the tumor tissue sections of 27 cases was positive, and the expression of DEPDC1 in the pathological sections of 35 cases of para-cancerous tissues was negative. Statistically significant findings arose from the analysis ($P < 0.001$), with immunohistochemical staining results indicating high expression of DEPDC1 in LUAD, immunohistochemistry of DEPDC1 in tumor tissues and normal tissues is shown in Fig. 16A and B.

4. Discussion

Globally, lung cancer causes the highest number of deaths among all types of cancer, representing around one-third of all cancer-related fatalities. Over the past thirty years, China has seen a notable increase in both the frequency and death rates of lung cancer,

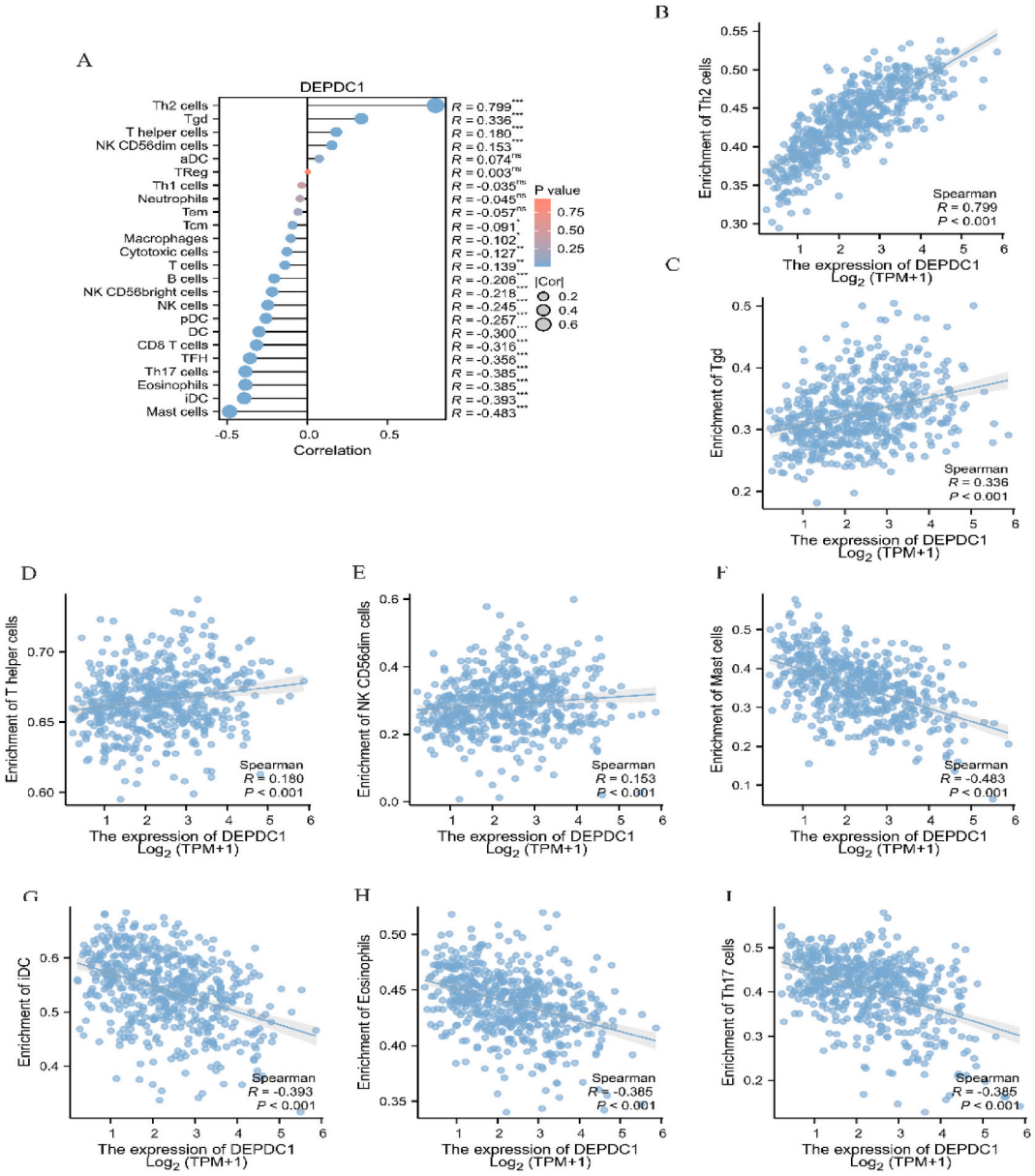


Fig. 9. Investigation into the correlation between DEPDC1 and immune cells utilizing correlation analysis. A. Analyzing the correlation between DEPDC1 and 24 various types of immune cells; B-E. Demonstrating a positive correlation between the expression of DEPDC1 and immune cells; F–I. Identifying a negative correlation between the expression of DEPDC1 and immune cells.

solidifying its position as the most rapidly growing and destructive form of malignant tumor in the nation [22]. During the early stages of lung cancer in clinical patients, cough and sputum are commonly observed as the main symptoms, often leading to misdiagnosis. Early detection and precise diagnostic examinations are vital for the successful management of LUAD [23]. Early detection of LUAD presents challenges due to the lack of specificity in current biomarkers like carcinoembryonic antigen, cancer antigen 125 [24]. While these markers are commonly used for clinical diagnosis, the search for a more specific LUAD marker is crucial for improving early detection accuracy in clinical practice.

DEPDC1, a molecule with oncogenic properties, is crucially involved in the development of numerous types of cancerous tumors. DEPDC1 within prostate cancer cells collaborates with E2F1 to boost the transcriptional capacity of E2F1, leading to the upregulation of target genes and the promotion of cellular growth [15]. The expression of DEPDC1 in malignant liver cells could potentially enhance the quantities of chemokine ligand 20 (CCL20) and chemokine receptor 6 (CCR6), ultimately stimulating cellular proliferation and movement through the CCL20/CCR6 signaling cascade [25]. Studies have shown that DEPDC1 is capable of activating the PI3K/AKT/mTOR pathway in breast cancer [16]. In breast cancer cells, DEPDC1 can enhance the expression of forkhead box M1

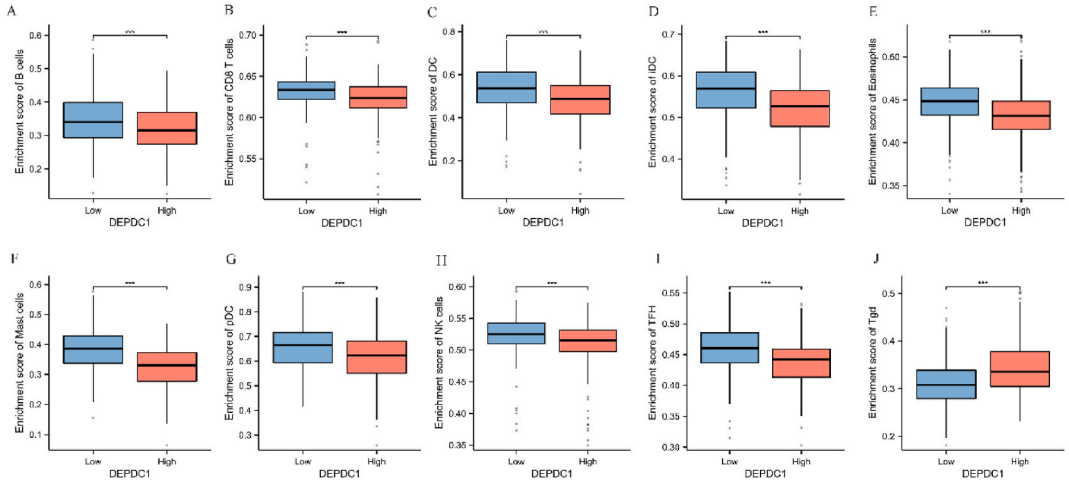


Fig. 10. A–J Analysis of different expression levels of DEPDC1 in immune cells. ***p < 0.001.

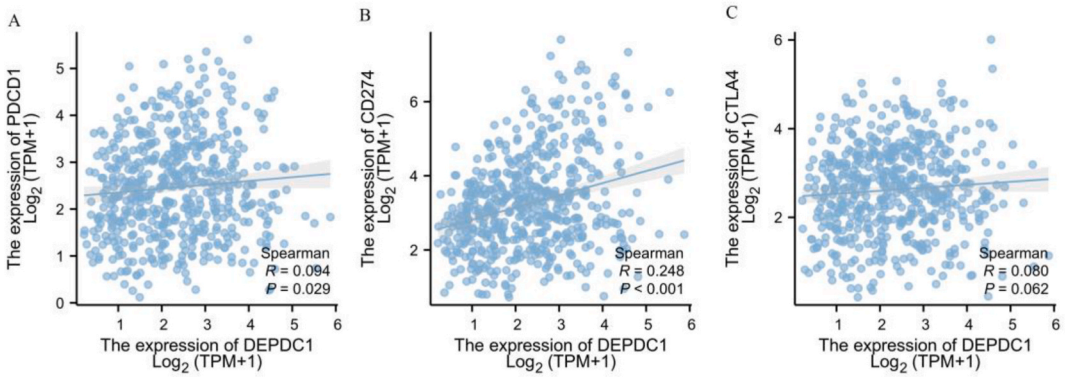


Fig. 11. Relationship between DEPDC1 and immune checkpoints. A. PDCD1; B. CD274; C. CTLA4.

(FoxM1) and promote cell proliferation [14]. Research has demonstrated that the role of DEPDC1 in the advancement and growth of various types of cancer has been established. DEPDC1 has been found to be markedly elevated in diverse tumor types. Research conducted by Kanehira has demonstrated that DEPDC1 is notably overexpressed in both bladder and testicular tissues of individuals with bladder cancer. Furthermore, patients with bladder cancer who exhibit overexpression of DEPDC1 tend to have a less favorable prognosis [13]. Overexpression of DEPDC1 was discovered in breast cancer tissue, with its mRNA levels strongly linked to unfavorable prognosis and recurrence among patients [16]. Additionally, studies show that levels of DEPDC1 mRNA and protein expression are notably increased in liver cancer tissues compared to neighboring tissues. Higher expression levels are associated with a poorer prognosis for individuals [26]. An exploration into the potential role of DEPDC1 expression in LUAD was conducted by investigating various online public databases. The findings suggest that the DEPDC1 gene plays a crucial role in the progression and growth of LUAD tumors. The study revealed a significant up-regulation of DEPDC1 expression in LUAD, with high expression levels correlating with poor prognosis. These results align with previous studies highlighting the role of DEPDC1 in various tumor types. The data analysis in this study reveals that the DEPDC1 gene exhibits elevated levels of expression in LUAD tissues and low levels of expression in healthy tissues. Furthermore, a correlation is observed between higher DEPDC1 expression and poorer survival prognosis among LUAD patients, the finding that aligns with similar research outcomes in other types of tumors.

Numerous studies have shown that cancer development and advancement are closely linked to the tumor microenvironment [27]. The tumor microenvironment encompasses immune cells, extracellular matrix, and inflammatory mediators, which synergistically facilitate tumor cell proliferation, survival, and metastasis [28,29]. Past research has shown that the outlook for patients may be impacted by the level of immune cell infiltration within tumors. Furthermore, the existence of lymphocytes that infiltrate tumors is regarded as a separate prognostic indicator for individuals with tumors [30,31]. DEPDC1 was found to have varying levels of expression in different immune cell types, indicating a potential correlation between DEPDC1 and immune system function. The low expression group of DEPDC1 demonstrated high levels of expression in most immune cells, whereas the high expression group was predominantly found in tumor tissues, while normal tissues displayed lower levels of DEPDC1 expression. These findings suggest a compromised immune system in a majority of tumor patients. Immune checkpoint molecules are a group of immunomodulatory

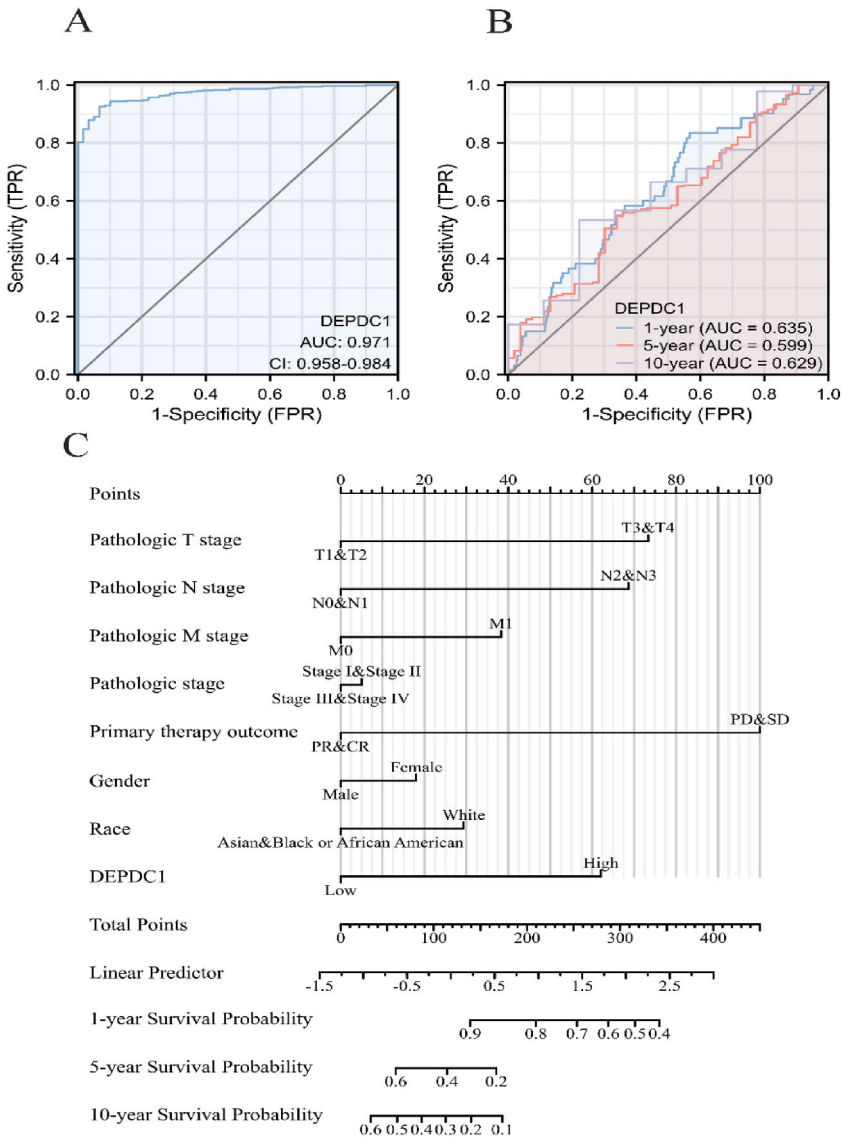


Fig. 12. Importance of DEPDC1 expression in detecting LUAD. A. Analysis of ROC curves; B. Predicting survival rates with time-dependent curves; C. Examining nomograms.

receptors/ligands. Under typical physiological circumstances, these checkpoints serve to prevent overstimulation of the immune system by controlling the activation of T cells. They are essential for preserving the equilibrium of the body's immune response [32,33]. Immune checkpoints are crucial in facilitating tumor immune evasion. A significant tactic employed by tumors to evade immune responses is by modulating the expression of immune checkpoints [33,34], DEPDC1 is positively correlated with common immune checkpoints PDCD1, CD274, and CTLA4. The above results suggest that LUAD patients may obtain better results through immunotherapy.

The construction of the prognostic model shows that DEPDC1 has a high diagnostic value in prognosis and has high prediction credibility. The study on DNA methylation found a notably elevated methylation level in the DEPDC1 promoter compared to the control group. Smith provide a comprehensive analysis of various potential mechanisms through which promoter DNA hypermethylation can result in contradictory gene activation. These mechanisms include binding to transcription inhibitors, interaction with distal regulatory elements, and induction of alternative promoter activation [35]. Chemokines and receptors play a vital role in controlling the precise migration of immune cells. The TISIDB tool was employed to explore the correlation between DEPDC1 methylation levels and the immune cell tumor expression of chemokines and chemokine receptors. Positive correlations were observed between DEPDC1 methylation levels and the expression of CCL5 and CCR2, as well as with the expression of immunoinhibitors CD40 and BTLA and immunostimulators. The results suggest that methylation of DEPDC1 could potentially impede the infiltration of immune cells into the tumor microenvironment. Chemokine signaling via the CCL2-CCR2 pathway is essential for enhancing tumor cell

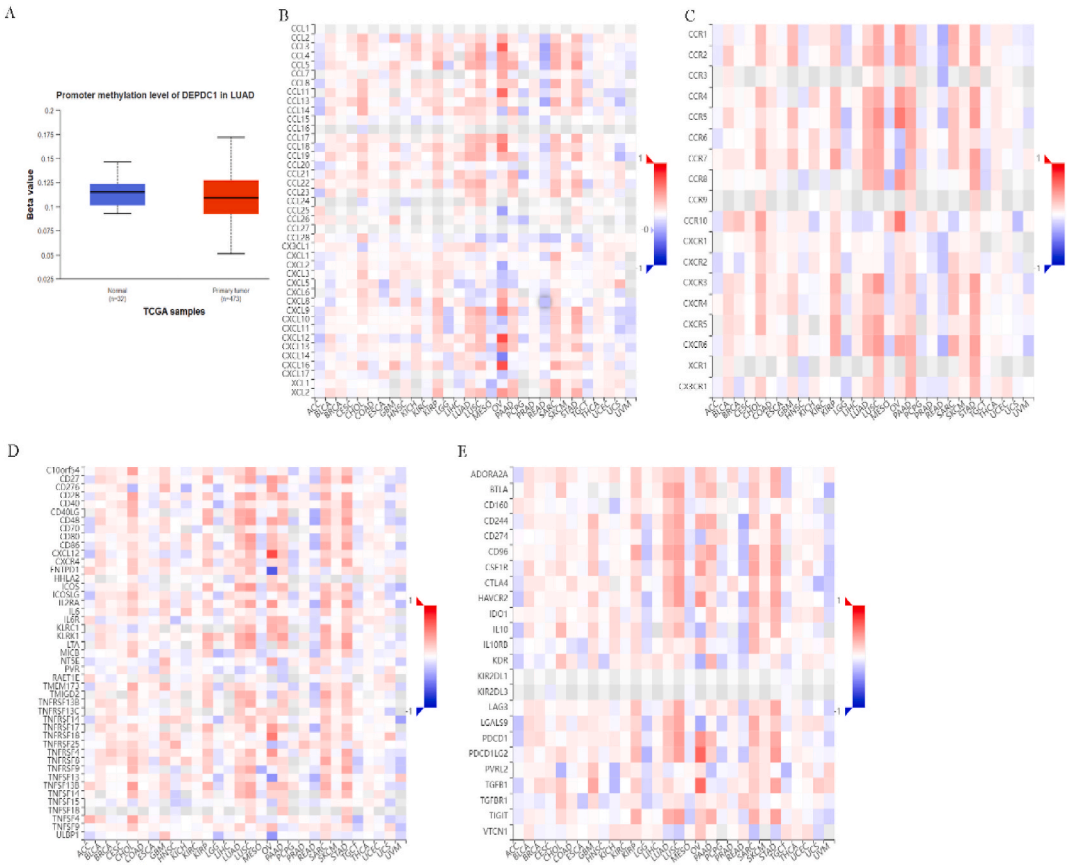


Fig. 13. DEPDC1 methylation analysis A. DEPDC1 promoter methylation level in LUAD.

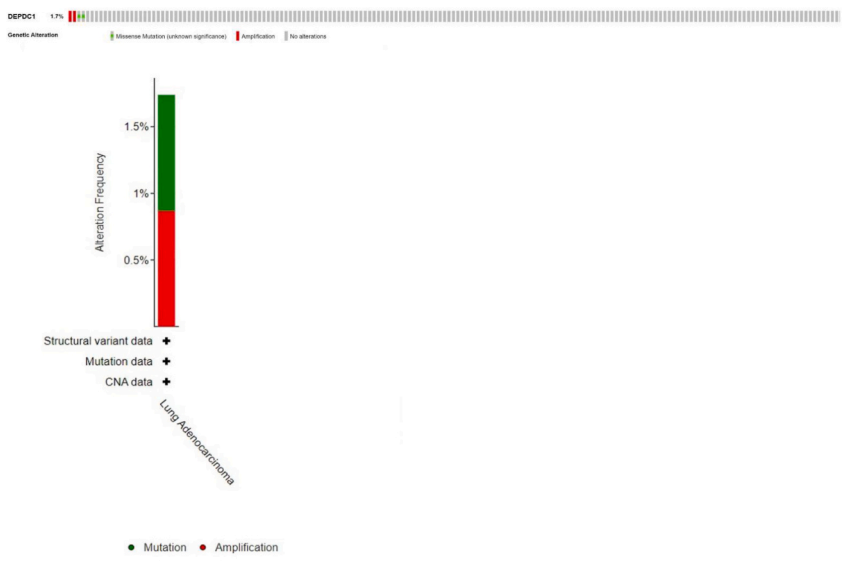


Fig. 14. DEPDC1 mutation.

proliferation and invasiveness as tumors advance. Furthermore, it plays a crucial role in shaping the tumor microenvironment by promoting the formation of new blood vessels and recruiting immune cells that suppress the body's natural immune response against the tumor [36–38]. Therefore, DEPDC1 may have an impact on the progression of LUAD through its effects on the immune system.

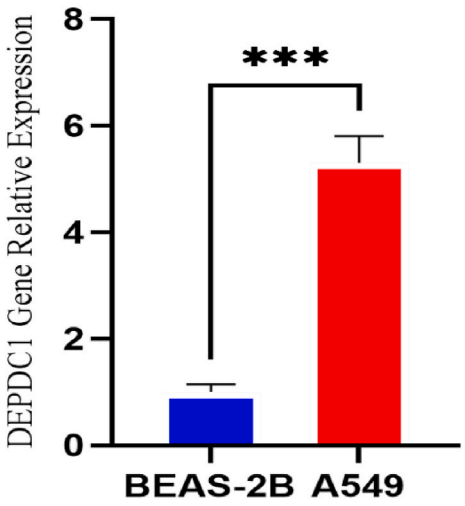


Fig. 15. Verification of DEPDC1 in qPCR.

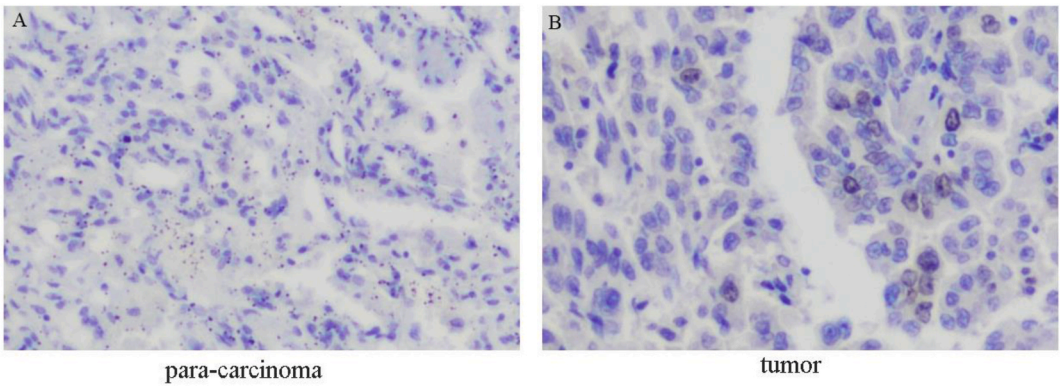


Fig. 16. Immunohistochemical staining of DEPDC1. A. adjacent tissue; B. tumor tissue.

There was notably higher DEPDC1 mRNA expression in A549 cells compared to BEAS-2B cells. Immunohistochemical staining of clinical and pathological sections also confirmed the presence of DEPDC1 expression in tumor tissue and absence of expression in normal tissue. These verification results are consistent with comprehensive information data analysis, indicating that DEPDC1 is a risk factor for LUAD, contributing to its occurrence and development. The DEPDC1 marker is closely connected to the clinicopathological characteristics and long-term outlook of individuals diagnosed with LUAD. Early and timely detection of abnormal expression levels in clinical could potentially extend the survival time of LUAD patients by decreasing DEPDC1 expression. This research emphasizes the importance of DEPDC1 in predicting survival outcomes and immune infiltration in LUAD, indicating its promise as a way to detect LUAD early. However, the study has some limitations: (1) In cell experiments, qPCR confirmed that tumor cells exhibit a higher DEPDC1 expression compared to normal cells. However, additional validation is required; (2) The sample size in the study is limited, warranting larger sample sizes in future research; (3) Future studies will include animal experiments to elucidate the specific mechanism of DEPDC1 in LUAD.

5. In conclusion

In conclusion, our research has shown a notable increase in DEPDC1 expression in LUAD, which is strongly linked to patient prognosis. The presence of DEPDC1 holds crucial implications for the diagnosis and prognosis of LUAD. Moreover, DEPDC1 could impact the development and advancement of LUAD by influencing immune infiltration, making it a possible new marker for individuals with the condition.

Ethics approval and consent to participate

Approval was granted by the Ethics Committee of Dali University for the research (NO.20230806), with patients giving written

Informed Consent.

Data availability

All data and materials are available. TCGA database (https://portal.gdc.cancer.gov/), GEO database (https://www.ncbi.nlm.nih.gov/geo/), UALCAN (http://ualcan. path.uab.edu/), BioPortal (http://www.cbioportal.org/) TISIDB (http://cis.hkuhk/TISIDB/).

Funding

This study was supported by Research Foundation for the Doctoral Program of Dali University (DFY20220302).

CRediT authorship contribution statement

Cuixian Li: Writing – original draft, Conceptualization. **Xiaoling Zhu:** Writing – review & editing, Funding acquisition, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] Y. He, W. Luo, Y. Liu, Y. Wang, C. Ma, Q. Wu, P. Tian, D. He, Z. Jia, X. Lv, et al., IL-20RB mediates tumoral response to osteoclastic niches and promotes bone metastasis of lung cancer, J. Clin. Invest. 132 (20) (2022).
[2] R.L. Siegel, K.D. Miller, H.E. Fuchs, A. Jemal, Cancer statistics, 2022, CA Cancer J. Clin. 72 (1) (2022) 7–33.
[3] Q. Song, J. Shang, Z. Yang, L. Zhang, C. Zhang, J. Chen, X. Wu, Identification of an immune signature predicting prognosis risk of patients in lung adenocarcinoma, J. Transl. Med. 17 (1) (2019) 70.
[4] L. Huang, S. Peng, C. Sun, L. Chen, Q. Chu, S. Thapa, V. Chummun, L. Zhang, P. Zhang, E.L. Chen, et al., Impact of marital status on survival in patients with stage 1A NSCLC, Aging (Albany NY) 14 (2) (2022) 770–779.
[5] C.C. Chang, M.S. Hsieh, M.W. Lin, Y.H. Lee, Y.J. Hsiao, K.Y. Su, T.J. Su, S.L. Yu, J.S. Chen, Novel genetic prognostic signature for lung adenocarcinoma identified by differences in gene expression profiles of low- and high-grade histological subtypes, Biomolecules 12 (2) (2022).
[6] A.D. Tahanovich, N.N. Kauhanka, V.I. Prohorova, D.I. Murashka, O.V. Gotko, [Predicting the risk of tumor progression in patients with early stages of adenocarcinoma and squamous cell lung carcinoma based on laboratory parameters], Biomed. Khim. 67 (6) (2021) 507–517.
[7] T.V. Denisenko, I.N. Budkevich, B. Zhivotovsky, Cell death-based treatment of lung adenocarcinoma, Cell Death Dis. 9 (2) (2018) 117.
[8] J. Huang, J. Li, S. Zheng, Z. Lu, Y. Che, S. Mao, Y. Lei, R. Zang, C. Liu, X. Wang, et al., Tumor microenvironment characterization identifies two lung adenocarcinoma subtypes with specific immune and metabolic state, Cancer Sci. 111 (6) (2020) 1876–1886.
[9] J. Zhang, X. Han, L. Lin, J. Chen, F. Wang, Q. Ding, L. Hao, L. Wang, J. Wei, Y. Wang, et al., Unraveling the expression patterns of immune checkpoints identifies new subtypes and emerging therapeutic indicators in lung adenocarcinoma, Oxid. Med. Cell. Longev. 2022 (2022) 3583985.
[10] Y. Zhou, M. Zhao, Y. Du, Y. Liu, G. Zhao, L. Ye, Q. Li, H. Li, X. Wang, X. Liu, et al., MicroRNA-195 suppresses the progression of lung adenocarcinoma by directly targeting apelin, Thorac. Cancer 10 (6) (2019) 1419–1430.
[11] Y. Liao, D. He, F. Wen, Analyzing the characteristics of immune cell infiltration in lung adenocarcinoma via bioinformatics to predict the effect of immunotherapy, Immunogenetics 73 (5) (2021) 369–380.
[12] C. Zappa, S.A. Mousa, Non-small cell lung cancer: current treatment and future advances, Transl. Lung Cancer Res. 5 (3) (2016) 288–300.
[13] M. Kanehira, Y. Harada, R. Takata, T. Shuin, T. Miki, T. Fujioka, Y. Nakamura, T. Katagiri, Involvement of upregulation of DEPDC1 (DEP domain containing 1) in bladder carcinogenesis, Oncogene 26 (44) (2007) 6448–6455.
[14] L. Zhang, Y. Du, S. Xu, Y. Jiang, C. Yuan, L. Zhou, X. Ma, Y. Bai, J. Lu, J. Ma, DEPDC1, negatively regulated by miR-26b, facilitates cell proliferation via the up-regulation of FOXM1 expression in TNBC, Cancer Lett. 442 (2019) 242–251.
[15] L. Huang, K. Chen, Z.P. Cai, F.C. Chen, H.Y. Shen, W.H. Zhao, S.J. Yang, X.B. Chen, G.X. Tang, X. Lin, DEPDC1 promotes cell proliferation and tumor growth via activation of E2F signaling in prostate cancer, Biochem. Biophys. Res. Commun. 490 (3) (2017) 707–712.
[16] H. Zhao, M. Yu, L. Sui, B. Gong, B. Zhou, J. Chen, Z. Gong, C. Hao, High expression of DEPDC1 promotes malignant phenotypes of breast cancer cells and predicts poor prognosis in patients with breast cancer, Front. Oncol. 9 (2019) 262.
[17] Z. Gong, H. Chu, J. Chen, L. Jiang, B. Gong, P. Zhu, C. Zhang, Z. Wang, W. Zhang, J. Wang, et al., DEPDC1 upregulation promotes cell proliferation and predicts poor prognosis in patients with gastric cancer, Cancer Biomark. 30 (3) (2021) 299–307.
[18] Q. Wang, S. Jiang, J. Liu, G. Ma, J. Zheng, Y. Zhang, DEP domain containing 1 promotes proliferation, invasion, and epithelial-mesenchymal transition in colorectal cancer by enhancing expression of suppressor of zest 12, Cancer Biother. Radiopharm. 36 (1) (2021) 36–44.
[19] M. Amisaki, T. Yagyu, E.I. Uchinaka, M. Morimoto, T. Hanaki, J. Watanabe, N. Tokuyasu, T. Sakamoto, S. Honjo, Y. Fujiwara, Prognostic value of DEPDC1 expression in tumor and non-tumor tissue of patients with hepatocellular carcinoma, Anticancer Res. 39 (8) (2019) 4423–4430.
[20] Q. Wang, A. Li, J. Jin, G. Huang, Targeted interfering DEP domain containing 1 protein induces apoptosis in A549 lung adenocarcinoma cells through the NF-κB signaling pathway, OncoTargets Ther. 10 (2017) 4443–4454.
[21] B. Jia, J. Liu, X. Hu, L. Xia, Y. Han, Pan-cancer analysis of DEPDC1 as a candidate prognostic biomarker and associated with immune infiltration, Ann. Transl. Med. 10 (24) (2022) 1355.
[22] R.S. Zheng, S.W. Zhang, K.X. Sun, R. Chen, S.M. Wang, L. Li, H.M. Zeng, W.W. Wei, J. He, [Cancer statistics in China, 2016], Zhonghua Zhongliu Zazhi 45 (3) (2023) 212–220.
[23] H. Liljedahl, A. Karlsson, G.N. Oskarsdottir, A. Salomonsson, H. Brunnström, G. Erlingsdottir, M. Jönsson, S. Isaksson, E. Arbajian, C. Ortiz-Villalón, et al., A gene expression-based single sample predictor of lung adenocarcinoma molecular subtype and prognosis, Int. J. Cancer 148 (1) (2021) 238–251.
[24] Y. Zhang, B. Sun, M. Hu, Y. Lou, J. Lu, X. Zhang, H. Wang, J. Qian, T. Chu, B. Han, CXCL9 as a prognostic inflammatory marker in early-stage lung adenocarcinoma patients, Front. Oncol. 10 (2020) 1049.
[25] W. Guo, H. Li, H. Liu, X. Ma, S. Yang, Z. Wang, DEPDC1 drives hepatocellular carcinoma cell proliferation, invasion and angiogenesis by regulating the CCL20/CCR6 signaling pathway, Oncol. Rep. 42 (3) (2019) 1075–1089.
[26] D. Qu, F. Cui, D. Lu, Y. Yang, Y. Xu, DEP domain containing 1 predicts prognosis of hepatocellular carcinoma patients and regulates tumor proliferation and metastasis, Cancer Sci. 110 (1) (2019) 157–165.

[27] D.F. Quail, J.A. Joyce, Microenvironmental regulation of tumor progression and metastasis, Nat. Med. 19 (11) (2013) 1423–1437.
[28] R. Baghban, L. Roshangar, R. Jahanban-Esfahlan, K. Seidi, A. Ebrahimi-Kalan, M. Jaymand, S. Kolahian, T. Javaheri, P. Zare, Tumor microenvironment complexity and therapeutic implications at a glance, Cell Commun. Signal. 18 (1) (2020) 59.
[29] Y. Yuan, Y.C. Jiang, C.K. Sun, Q.M. Chen, Role of the tumor microenvironment in tumor progression and the clinical applications, Oncol. Rep. 35 (5) (2016) 2499–2515 (Review).
[30] Q. Fu, N. Chen, C. Ge, R. Li, Z. Li, B. Zeng, C. Li, Y. Wang, Y. Xue, X. Song, et al., Prognostic value of tumor-infiltrating lymphocytes in melanoma: a systematic review and meta-analysis, OncoImmunology 8 (7) (2019) 1593806.
[31] E. García-Martínez, G.L. Gil, A.C. Benito, E. González-Billalabeitia, M.A. Conesa, T. García García, E. García-Garre, V. Vicente, Ayala de la Peña F: Tumor-infiltrating immune cell profiles and their change after neoadjuvant chemotherapy predict response and prognosis of breast cancer, Breast Cancer Res. 16 (6) (2014) 488.
[32] M. Wagner, M. Jasek, L. Karabon, Immune checkpoint molecules-inherited variations as markers for cancer risk, Front. Immunol. 11 (2020) 606721.
[33] G. Morad, B.A. Helmink, P. Sharma, J.A. Wargo, Hallmarks of response, resistance, and toxicity to immune checkpoint blockade, Cell 184 (21) (2021) 5309–5337.
[34] M. Khan, S. Arooj, H. Wang, NK cell-based immune checkpoint inhibition, Front. Immunol. 11 (2020) 167.
[35] J. Smith, S. Sen, R.J. Weeks, M.R. Eccles, A. Chatterjee, Promoter DNA hypermethylation and paradoxical gene activation, Trends Cancer 6 (5) (2020) 392–406.
[36] S. Kadomoto, K. Izumi, A. Mizokami, Roles of CCL2-CCR2 Axis in the tumor microenvironment, Int. J. Mol. Sci. 22 (16) (2021).
[37] T. O'Connor, M. Heikenwalder, CCL2 in the tumor microenvironment, Adv. Exp. Med. Biol. 1302 (2021) 1–14.
[38] H. Yang, Q. Zhang, M. Xu, L. Wang, X. Chen, Y. Feng, Y. Li, X. Zhang, W. Cui, X. Jia, CCL2-CCR2 axis recruits tumor associated macrophages to induce immune evasion through PD-1 signaling in esophageal carcinogenesis, Mol. Cancer 19 (1) (2020) 41.