

High DSCC1 Level Predicts Poor Prognosis of Lung Adenocarcinoma

Sisi Chang¹Yahui Zhu²Yutan Xi²Fuyan Gao²Juanjuan Lu²Liang Dong¹Chunzheng Ma¹Honglin Li¹

¹Department of Oncology, Henan Provincial Hospital of Traditional Chinese Medicine (The Second Affiliated Hospital of Henan University of Traditional Chinese Medicine), Zhengzhou, Henan Province, People's Republic of China;

²Department of Oncology, Henan University of Traditional Chinese Medicine, Zhengzhou, Henan Province, People's Republic of China

Purpose: To evaluate the role of DSCC1 in LUAD.

Patients and Methods: Based on TCGA and GTEx, the Wilcoxon rank-sum test was used to compare the expression differences of DSCC1 between the normal samples of GTEx combined TCGA and the unpaired tumor samples of TCGA, and to compare DSCC1 expression values between tumor tissues and paired normal LUAD tissues in the TCGA cohort. Kruskal–Wallis rank-sum test, Wilcoxon rank-sum test, and logistics regression were used to compare the relationship between the expression of DSCC1 and the clinicopathological parameters. The biological function of DSCC1 was annotated by GSEA and ssGSEA, while Kaplan–Meier and Cox regression analysis were used to evaluate the prognostic value of DSCC1. Furthermore, the time-dependent ROC curve was used to analyze the diagnostic efficacy of DSCC1 in LUAD.

Results: We downloaded the RNA-Seq data of 513 LUAD cases. The expression of DSCC1 was significantly correlated with T stage (OR = 1.04(1.02–1.07), P = 0.002), pathological stage (OR=1.03 (1.01–1.05), P = 0.008) and TP53 status (OR=1.10 (1.07–1.14), P < 0.001). The high expression of DSCC1 was significantly correlated with DSS (HR=1.56 (1.07–2.26), P = 0.021) and OS (HR=1.53 (1.14–2.05), P = 0.004). Moreover, ROC curve analysis (AUC=0.845, CI (0.820–0.870)) indicated DSCC1 as a potential diagnostic molecule for LUAD. In the group with high DSCC1 expression phenotype, down-regulation of EGFR signal, reduction of IL-6 deprivation, cell cycle, and p53 signal pathway were significantly abundant. Spearman correlation analysis showed that the expression of DSCC1 was positively correlated with the infiltration of Th2 cells, T Helper cells.

Conclusion: Our results suggest that DSCC1 may be an important biomarker for the treatment of LUAD.

Keywords: DSCC1, LUAD, GSEA, prognosis, biomarker

Introduction

Lung cancer is one of the most common cancers and the main cause of cancer-related death.¹ It is divided into two main subtypes: SCLC and NSCLC, accounting for 15 and 85% of all cases, respectively.² LUAD is the most common histological type of NSCLC, found in more than 40% of patients.^{3,4} The incidence of LUAD has been steadily increasing.⁵ Over recent years, comprehensive treatments such as chemotherapy and targeted therapy have brought more possibilities to improve the prognosis of patients with LUAD.⁴ Conventional chemotherapy is effective in most cases; yet, it has serious side effects and can seriously affect patients' quality of life. In addition, the effectiveness of molecular targeted therapeutic drugs is limited, and new targeted drugs tend to fail due

Correspondence: Chunzheng Ma; Honglin Li

Henan Provincial Hospital of Traditional Chinese Medicine (The Second Affiliated Hospital of Henan University of Traditional Chinese Medicine), Zhengzhou, Henan Province, People's Republic of China
Tel +86-18801611544
Email mchzh666@126.com; 46991701@qq.com

to the development of drug resistance.⁶ For patients at advanced stages and those who cannot use targeted drugs, the survival rate is greatly reduced. At present, the treatment of advanced NSCLC is mainly based on immunotherapy of PD-1 or PD-L1 antibody, which can be used alone or in combination with chemotherapy;⁷ however, the remission rate is very low. Therefore, it is urgent to improve the prognosis, diagnosis, and treatment of patients with LUAD. Moreover, there is no effective biomarker to predict the adverse prognosis and treatment of LUAD.

DSCC1, also known as DCC1, is a component of selective replication factor c complex (RFC),⁸ which participates in the S phase of the cell cycle. In all eukaryotes, RFC is a complex composed of 1–5 subunits of RFC, ie, a large subunit, Rfc1, and four small subunits, Rfc2 to Rfc5. Previous studies have reported that RFC can load PCNA onto completely duplex DNA^{9,10} and unload PCNA.^{11,12} The alignment marker of chromosome transmission fidelity protein 18 (Ctf18) is RFC1.^{13,14} Sister chromatid cohesion requires using the RFC complex, in which the RFC1 subunit is substituted to form isoheptamer Ctf18-RFC complex.^{15,16} Its loader complex Ctf18-RFC contains Ctf18-DSCC1-Ctf8 modules, in which two non-RFC subunits include unique DSCC1 and CTF8 binding.¹⁷ Complex Ctf18-RFC, targeting PCNA, contributes to the activation of sister chromatid cohesion and replication of stress checkpoints.⁸ A previous study showed that Ctf18-RFC and double-stranded DNA bind to the same winglike helix domain on DSCC1.¹⁸ In addition, it had been reported that DSCC1 contains mitotic genes and could directly participate in the cell cycle.¹⁹ These processes suggested that DSCC1 had a critical role in DNA replication, which led us to think about its role in tumor proliferation. In breast cancer, colorectal cancer, and hepatocellular carcinoma, DSCC1 was highly expressed in tumor tissues and promoted the proliferation of tumor cells.^{8,13} However, the role of DSCC1 in LUAD still remains unclear.

In this study, we analyzed the expression of DSCC1 in LUAD based on TCGA data. The possible signal transduction pathways of DSCC1 in LUAD were analyzed by GSEA. The relationship between the expression of DSCC1 and immune cell infiltration in LUAD was analyzed by the ssGSEA method. Subsequently, we further analyzed the value of DSCC1 expression in evaluating the prognosis of LUAD.

Materials and Methods

Data Source and Preprocessing

We downloaded RNAseq data and clinical information in level 3HT Seq-FPKM format from the lung adenocarcinoma project in TCGA database (<https://cancergenome.nih.gov>). RNAseq data from cases with no or incomplete clinical information and from those follow-up for <30 days were discarded. The design and process of our study are shown in the flowchart (Figure 1), included data selection and exclusion. Among them, the RNAseq data in FPKM format were converted into TPM format for expression comparison between samples. In addition, we downloaded RNAseq data in TCGA and GTEx TPM format from UCSC XENA (<https://xenabrowser.net/datapages/>), which has been uniformly processed by the Toil process.²⁰ The median of the expression level of DSCC1 was used as the cut-off value, and the tumor samples were divided into high expression group and low expression group. In this study, the clinical data of 513 patients with LUAD were collected, including T stage, N stage, M stage, pathological stage, primary treatment outcome, sex, race, smoking status, tumor status, TP53 status, and KARS status. Data on 57 cases of LUAD with paired normal lung tissues were also included.

Different Expression Gene in TCGA and GTEx

Based on TCGA and GTEx, we compared the expression of DSCC1 in different types of human cancers. In addition, the expression level of DSCC1 was compared between LUAD in TCGA and normal samples in GTEx combined with TCGA. We also compared the DSCC1 expression levels in LUAD and matched normal lung tissues in the TCGA cohort.

Gene Set Enrichment Analysis

Based on the gene expression matrix, GSEA was divided into high expression and low expression groups according to the median level. GSEA can analyze the difference in signal pathway enrichment between the two groups and infer phenotypes and signaling pathways that might be related to genes. In this study, we used the R package clusterProfiler for GSEA analysis and visualization. We analyzed the functions and signal pathways with significant differences between the high and low expression groups. Based on the default weighted enrichment statistical method, the procedure was repeated 1000 times for

each analysis. $FDR < 0.25$ and a family-wise error rate < 0.05 were set for gene sets. Significant differences were demonstrated in the rich set of MSigDB (C2.all.v7.0.symbols.GMT) collection.

Immune Infiltration Analysis

According to the gene markers of 24 immune cells, ie, DCs, iDCs, aDCs, eosinophils, mast cells, macrophages, NKs, NK CD56^{dim} cells, NK CD56^{bright} cells, neutrophils, B cells, T cells, T helper cells, Th1, Th2, T $\gamma\delta$, CD8+ T, Tem, Tem, Tfh cells, Th17 cells, Treg cells, and cytotoxic cells, mentioned in the literature,²¹ the infiltration of 24 immune cells in the tumor was evaluated by ssGSEA method in GSVA software package,²² and the correlation between DSCC1 and relative infiltration abundance of immune cells was analyzed by Spearman correlation. Wilcoxon rank-sum test was used to analyze the differences in immunocyte infiltration between the DSCC1 high expression group and low expression. P -value < 0.05 was considered statistically significant.

Statistical Analysis

R (v4.0.0) software was used for statistical analysis. Wilcoxon rank-sum test was used to compare the expression of DSCC1 normal samples of GTEX combined with TCGA and the expression of DSCC1 in tumor samples of TCGA. Wilcoxon rank-sum test also was used to compare values in DSCC1 expression between tumor tissues and paired normal LUAD tissues in the TCGA cohort. Then, we used the Kruskal–Wallis rank-sum test, Wilcoxon rank-sum test, and logistics regression methods to compare the relationship between the expression of DSCC1 and the clinicopathological parameters. Furthermore, we used Cox regression to analyze the prognostic factors of LUAD. The variables with $P < 0.1$ in the single-factor Cox regression were included in the multi-factor Cox regression. We also used the Kaplan–Meier method to evaluate the prognostic value of DSCC1 in LUAD. The primary endpoints of prognostic analysis was OS, and DSS. The primary endpoints of prognostic analysis was OS, and the secondary end point was DSS. Finally, ROC curve, AUC, and Uno's C-index were identified to evaluate the predictive value of the gene for overall survival using the R package “survival ROC.”²³ $P < 0.05$ was considered statistically significant.

Results

Clinical Characteristics

As shown in Table 1, data on 513 primary tumors with clinical and gene expression data were downloaded from

TCGA, including TNM stage, pathological stage, primary treatment outcome, sex, race, smoking status, tumor status, TP53 status, KARS status, and smoking age. In the subsequent correlation analysis, the expression level of DSCC1 was significantly correlated with T stage ($P=0.002$), N stage ($P=0.005$), M stage ($P=0.048$), pathological stage ($P<0.001$), primary treatment outcome ($P=0.027$), sex ($P=0.004$), smoking status ($P=0.007$) and TP53 status ($P<0.001$).

Relationship Between DSCC1 Expression and Clinicopathological Parameters of LUAD

Wilcoxon signed-rank test was used to evaluate the expression effect of DSCC1 in tumor patients. The expression of DSCC1 in GTEX combined with TCGA was compared with that in unpaired TCGA tumor tissues (Figure 2A). It was finally concluded that the expression of DSCC1 in BLCA, BRCA, CESC, CHOL, COAD, DLBC, ESCA, GBM, HNSC, KICH, KIRC, KIRP, LAML, LGG, LIHC, LUAD, LSC, OV, PAAD, PCPG, PRAD, READ, SARC, SKCM, STAD, TGCT, THCA, THYM, UCEC, and UCS was remarkably high ($P<0.05$). Also, it was also found that DSCC1 was highly expressed in LUAD, and the results were statistically significant ($P<0.001$) (Figure 2B). Moreover, DSCC1 expression values in 57 tumor tissues were significantly higher than that in 57 paired normal LUAD tissues in the TCGA cohort ($P<0.001$) (Figure 2C). The above results showed that the expression of DSCC1 was significantly higher in LUAD samples.

Relationship Between DSCC1 Expression and Clinical Variables

As shown in Figure 3, Wilcoxon rank-sum test showed that high expression of DSCC1 was significantly correlated with T stage (Figure 3A, $P < 0.001$), N stage (Figure 3B, $P = 0.002$), M stage (Figure 3C, $P = 0.043$), pathological stage (Figure 3D, $P < 0.001$), tumor status (Figure 3E, $P = 0.007$), primary treatment outcome (Figure 3F, $P = 0.010$), TP53 status (Figure 3G, $P < 0.001$), smoking (Figure 3H, $P = 0.011$), sex (Figure 3I, $P = 0.008$) and so on. The relationship between the clinicopathologic features of LUAD and DSCC1 expression was analyzed using logistics regression. Logistic regression univariate analysis showed that the expression of DSCC1 as a classification dependent variable was associated with the clinicopathological features of poor prognosis (Table 2). The

Table I The Characteristics of LUAD Patients in TCGA

Characters	Level	Low Expression of DSCC1 (n=257)	High Expression of DSCC1 (n=256)	P
T stage (%)	T1	103 (40.4%)	65 (25.5%)	0.002 ^b
	T2	118 (46.3%)	158 (62.0%)	
	T3	25 (9.8%)	22 (8.6%)	
	T4	9 (3.5%)	10 (3.9%)	
N stage (%)	N0	180 (72.6%)	150 (59.3%)	0.005 ^a
	N1	41 (16.5%)	54 (21.3%)	
	N2	27 (10.9%)	47 (18.6%)	
	N3	0 (0.0%)	2 (0.8%)	
M stage (%)	M0	174 (96.1%)	170 (90.4%)	0.048 ^b
	M1	7 (3.9%)	18 (9.6%)	
Pathologic stage (%)	Stage I	159 (63.3%)	115 (45.3%)	<0.001 ^{b*}
	Stage II	52 (20.7%)	69 (27.2%)	
	Stage III	32 (12.7%)	52 (20.5%)	
	Stage IV	8 (3.2%)	18 (7.1%)	
Primary therapy outcome (%)	CR	169 (77.2%)	146 (70.5%)	0.027 ^a
	PD	25 (11.4%)	43 (20.8%)	
	PR	5 (2.3%)	1 (0.5%)	
	SD	20 (9.1%)	17 (8.2%)	
Gender (%)	Female	155 (60.3%)	121 (47.3%)	0.004 ^b
	Male	102 (39.7%)	135 (52.7%)	
Race (%)	Asian	4 (1.7%)	3 (1.4%)	1.000 ^a
	Black or African American	27 (11.7%)	25 (11.6%)	
	White	200 (86.6%)	187 (87.0%)	
Smoker (%)	No	48 (19.4%)	26 (10.4%)	0.007 ^b
	Yes	200 (80.6%)	225 (89.6%)	
Tumor status (%)	Tumor free	154 (66.4%)	134 (59.3%)	0.141 ^b
	With tumor	78 (33.6%)	92 (40.7%)	
TP53 status (%)	Mut	74 (29.0%)	167 (66.0%)	<0.001 ^{b*}
	WT	181 (71.0%)	86 (34.0%)	
KRAS status (%)	Mut	73 (28.6%)	66 (26.1%)	0.587 ^b
	WT	182 (71.4%)	187 (73.9%)	
Age (median [IQR])		67.00 [60.00,74.00]	64.00 [58.00,71.00]	0.003 ^c
Number pack years smoked (median [IQR])		35.00 [20.00,51.50]	40.00 [25.00,50.00]	0.115 ^c

Notes: ^aFisher's exact test; ^bChi-squared test; ^cWilcoxon rank-sum test; *Statistically significant.

results suggested that high expression of DSCC1 was significantly correlated with T stage (OR = 1.04(1.02–1.07), $P=0.002$) and pathological stage (OR = 1.03 (1.01–1.05), $P=0.008$). In addition, it was also significantly related to

TP53 status (OR = 1.10 (1.07–1.14), $P<0.001$). These results suggest that LUAD with high expression of DSCC1 may be more likely to progress to the advanced stage than those with low expression of DSCC1.

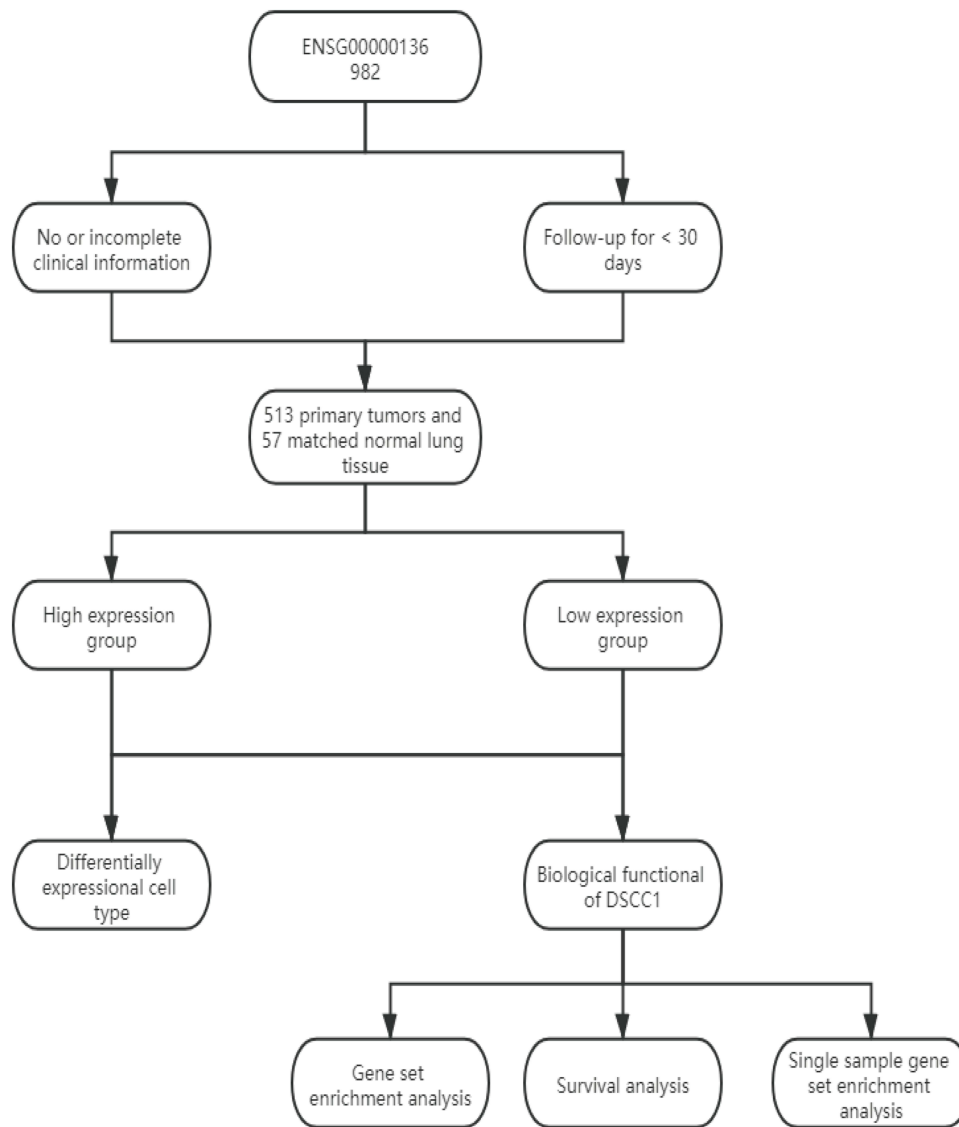


Figure 1 Flowchart showed the analysis strategy. The design and process of our study are shown in the flowchart, included data selection and exclusion.

Univariate and Multivariate Analyses of Survival

Univariate analysis showed that high expression of DSCC1 was significantly correlated with poor OS in patients with LUAD (HR: 1.531; CI: 1.142–2.054), $P=0.004$) (Table 3). We found OS could reach 43.4 months and DSS 50 months, the median follow-up time reached 43.4 months (Figure 4A and B). Other clinicopathological variables related to poor survival included T stage (HR: 1.668 (1.184–2.349), $P=0.003$), N stage (HR: 2.606 (1.939–3.503), $P < 0.001$), M stage (HR: 2.111 (1.232–3.616), $P=0.007$), primary therapy outcome (HR: 2.818 (2.004–3.963), $P < 0.001$) and tumor status (HR: 6.211 (4.258–9.059), $P < 0.001$). In

multivariate analysis (Table 3), primary therapy outcome (HR: 1.774 (1.136–2.771), $P=0.012$) and DSCC1 (HR = 1.683 (1.071–2.643), $P=0.024$), tumor status (HR: 6.064 (3.596–10.226), $P<0.001$) were independent prognostic factors affecting OS in patients with LUAD. As shown in Figure 4, the high expression of DSCC1 was significantly correlated with shorter DSS (Figure 4B, HR = 1.56 (1.07–2.26), $P=0.021$) and OS (Figure 4A, HR=1.53 (1.14–2.05), $P=0.004$) when compared with the low expression of DSCC1. We also analyzed the diagnostic efficacy of DSCC1 for LUAD by ROC. Our results suggested that DSCC1 might be a potential diagnostic molecule (AUC=0.845, CI (0.820–0.870)) (Figure 4C).

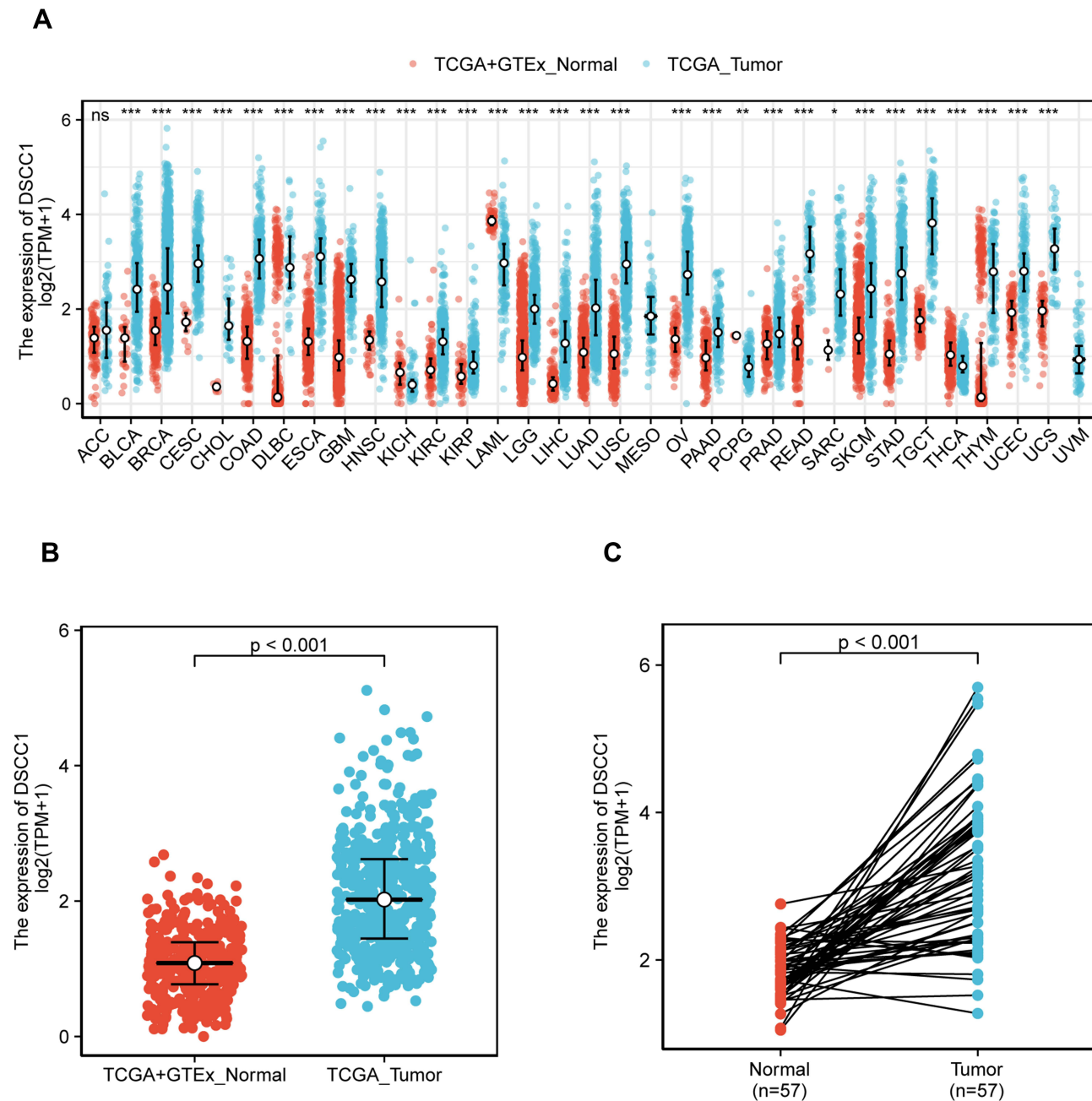


Figure 2 DSCC1 expression levels in different types of human cancers (A), ns, $P \geq 0.05$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. The higher expression level of DSCC1 is shown in LUAD compared with normal samples ($P < 0.001$) (B). DSCC1 expression levels in LUAD and matched normal lung tissues in TCGA cohort ($P < 0.001$) (C).

Abbreviations: ACC, adrenocortical carcinoma; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CESC, cervical and endocervical cancers; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; DLBC, lymphoid neoplasm diffuse large B-cell lymphoma; ESCA, esophageal carcinoma; GBM, glioblastoma multiforme; HNSC, head and neck squamous cell carcinoma; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LAML, acute myeloid leukemia; LGG, brain lower grade glioma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; MESO, mesothelioma; OV, ovarian serous cystadenocarcinoma; PAAD, pancreatic adenocarcinoma; PCPG, pheochromocytoma and paraganglioma; PRAD, prostate adenocarcinoma; READ, rectum adenocarcinoma; SARC, sarcoma; SKCM, skin cutaneous melanoma; STAD, stomach adenocarcinoma; TGCT, testicular germ cell tumors; THCA, thyroid carcinoma; THYM, thymoma; UCEC, uterine corpus endometrial carcinoma; UCS, uterine carcinosarcoma; UVM, uveal melanoma.

GSEA Identifies DSCC1-Related Signaling Pathways

To identify differentially activated signaling pathways in LUAD, GSEA was used to find the functional and biological

pathways between low and high expression of DSCC1 on TCGA expression datasets. Significant differences ($FDR < 0.25$ and $P_{\text{adjust}} < 0.05$) were found in the rich set of MSigDB (C2.all.v7.0.symbols.GMT) collection. We

Table 2 Relationship Between Clinicopathologic Features and Gene TPM in LUAD

Characteristics	Total(N)	Odds Ratio in DSCC1 Expression	P value
T stage (T2&T3&T4 vs T1)	510	1.04 (1.02–1.07)	0.002
N stage (N1&N2&N3 vs N0)	501	1.02 (1.00–1.04)	0.081
M stage (M1 vs M0)	369	1.03 (0.99–1.06)	0.113
Pathologic stage (Stage II&Stage III&Stage IV vs Stage I)	505	1.03 (1.01–1.05)	0.008
Primary therapy outcome (PD&SD&PR vs CR)	426	1.02 (0.99–1.04)	0.143
Race (Asian&Black or African American vs White)	446	0.99 (0.96–1.02)	0.748
Tumor status (With tumor vs Tumor free)	458	1.02 (0.99–1.04)	0.184
TP53 status (Mut vs WT)	508	1.10 (1.07–1.14)	<0.001
KRAS status (Mut vs WT)	508	0.98 (0.95–1.00)	0.066

Abbreviations: PD, Progressive Disease; SD, Stable Disease; PR, Partial Response; CR, Complete Response; Mut, Mutant; WT, Wide Type; TPM, transcriptspermillionreads.

selected the most significant signal pathway according to the NES (Table 4). This analysis showed that the high expression phenotype of DSCC1 was composed of many key pathways and was related to tumorigenesis.

Among the high expression phenotypes of DSCC1, DSCC1 showed different degrees of enrichment, such as poor lung cancer survival, down-regulation of EGFR signal, reduction of IL-6 deprivation, cell cycle, and p53 signal pathway. The pathways of DSCC1 with low expression phenotype were also identified, including linoleic acid metabolism, VEGF signal pathway, and B cell receptor signal pathway.

The Relationship Between the Expression of DSCC1 and the Infiltration of Immune Cells

The tumor microenvironment is mainly composed of immune cells, extracellular matrix, tumor-associated fibroblasts, various growth factors, inflammatory factors, and cancer cells. Many studies have proved that there are complex interactions among various cells in the tumor microenvironment, and there are special cell infiltration patterns, which affect the diagnosis, survival, prognosis, and clinical efficacy of the tumor. We used ssGSEA to analyze the relationship between the expression of DSCC1 and immune cell infiltration in the microenvironment of LUAD, as shown in Figure 5A. Spearman correlation analysis showed that the expression of DSCC1 was positively correlated with the infiltration of Th2 cells (Figure 5B, $R = 0.750$, $P < 0.001$) and T Helper cells (Figure 5C, $R = 0.174$, $P < 0.001$). Wilcoxon rank-sum revealed that the infiltration level of the immune cells in the high DSCC1 expression group was significantly increased compared with the DSCC1 low-expression

group, and the differences were statistically significant (Figure 5D, $P < 0.001$, Figure 5E, $P = 0.003$).

Discussion

Over recent years, it has been reported that DSCC1 was highly expressed in colorectal cancer cells²⁴ and hepatocellular carcinoma cells.²⁵ Compared with non-tumor tissues, DSCC1 was also highly expressed in breast cancer.⁸ Experiments have shown that DSCC1 enhances the vitality, invasiveness, and vitality of breast cancer cells.⁸ Similar to the above results, we used bioinformatics tools to observe the high expression of DSCC1 in LUAD tissues from the public database. It is worth noting that, consistent with the findings of DSCC1 in hepatocellular carcinoma and breast cancer, DSCC1 is an independent prognostic factor for patients with poor prognoses.^{8,25} In this study, the results showed that the expression of DSCC1 in LUAD tumor tissues was significantly higher than that in paracancerous tissues. The high expression of DSCC1 in LUAD was related to T stage, pathological stage, TP53 status, shorter survival time, and worse prognosis, thus supporting the theory that the expression of DSCC1 is closely related to the development of LUAD.

In order to further study the role of DSCC1 in LUAD, we used TCGA data for GSEA analysis. GSEA results showed that lung cancer had a low survival rate, down-regulation of EGFR signal, reduction of IL-6 deprivation, cell cycle, p53 signal pathway, and small cell lung cancer were significantly abundant in the DSCC1 high expression phenotype group. Clinically, EGFR-TKI has been accepted as a common strategy for targeted therapy in patients with non-small cell lung cancer.^{26,27} Moreover, these are among few targeted treatments available.^{28,29} We speculate that the down-regulation of the EGFR signal could reduce the

Table 3 Univariate and Multivariate COX Proportional Hazards Analysis of OS for Patients

Characteristics	Total(N)	Univariate Analysis HR(95% CI)	P value	Multivariate Analysis HR(95% CI)	P value
Tumor status (With tumor vs Tumor free)	450	6.211 (4.258–9.059)	< 0.001 ^a	6.064 (3.596–10.226)	<0.001 ^a
Primary therapy outcome (PD&SD&PR vs CR)	419	2.818 (2.004–3.963)	< 0.001 ^a	1.774 (1.136–2.771)	0.012
DSCC1 (High vs Low)	504	1.531 (1.142–2.054)	0.004	1.683 (1.071–2.643)	0.024
T stage (T2&T3&T4 vs T1)	501	1.668 (1.184–2.349)	0.003	1.237 (0.714–2.143)	0.448
N stage (N1&N2&N3 vs N0)	492	2.606 (1.939–3.503)	< 0.001 ^a	1.631 (0.792–3.360)	0.184
M stage (M1 vs M0)	360	2.111 (1.232–3.616)	0.007	0.852 (0.355–2.048)	0.721
Pathologic stage (Stage II & Stage III & Stage IV vs Stage I)	496	2.975 (2.188–4.045)	< 0.001 ^a	0.935 (0.424–2.062)	0.867
Gender (Male vs Female)	504	1.060 (0.792–1.418)	0.694		
Age (>65 vs ≤65)	494	1.228 (0.915–1.649)	0.171		
Race (White vs Asian & Black or African American)	446	1.422 (0.869–2.327)	0.162		
Anatomic neoplasm subdivision (Right vs Left)	490	1.024 (0.758–1.383)	0.878		
Anatomic neoplasm subdivision2 (Peripheral Lung vs Central Lung)	182	0.913 (0.570–1.463)	0.706		
Number pack years smoked (≥40 vs <40)	345	1.038 (0.723–1.490)	0.840		
Smoker (Yes vs No)	490	0.887 (0.587–1.339)	0.568		
TP53 status (Mut vs WT)	499	1.254 (0.936–1.680)	0.130		
KRAS status (Mut vs WT)	499	1.087 (0.779–1.517)	0.623		

Note: ^aStatistically significant.

Abbreviations: PD, Progressive Disease; SD, Stable Disease; PR, Partial Response; CR, Complete Response; Mut, Mutant; WT, Wild Type; TPM, transcriptspermillionreads; CI, confidence interval; HR, hazard ratio.

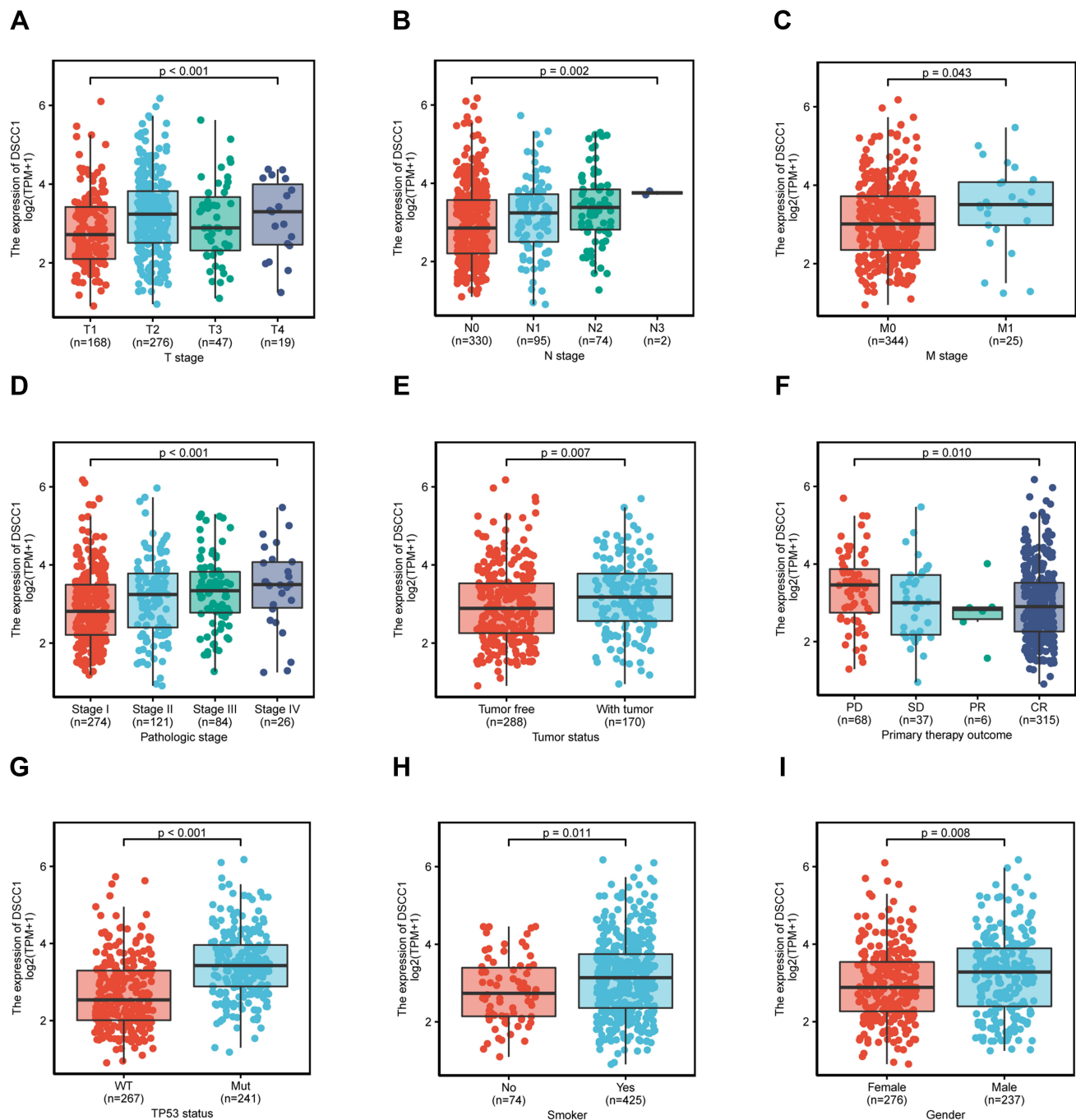


Figure 3 Association between DSCC1 expression and clinicopathologic characteristics, including T stage (A), N stage (B), M stage (C), pathological stage (D), tumor status (E), primary treatment outcome (F), and TP53 status (G), smoking (H), and sex (I).

Abbreviations: CR, complete response; Mut, mutant; WT, wild type; PD, progressive disease; PR, partial response; SD, stable disease.

number of people using targeted drugs, which may further shorten the survival time of patients.

Many studies have shown that the hallmark of cancer is tumor-promoting inflammation.³⁰ Based on the results of our research, the decrease of IL-6 deprivation leads to

a relative increase in the amount of IL in serum. Previous reports revealed a relatively poor prognosis^{31,32} in LUAD patients with increased inflammatory cytokine IL-6^{32,33} and a high number of inflammatory cells in TME. Previously, researchers have found that blocking IL-6 can

Table 4 Gene Sets Enriched in Phenotype High

Gene Set Name	NES	P. adjust	FDR
SHEDDEN_LUNG_CANCER_POOR_SURVIVAL_A6	2.904	0.018	0.014
KOBAYASHI_EGFR_SIGNALING_24HR_DN	2.872	0.018	0.014
CROONQUIST_IL6_DEPRIVATION_DN	2.703	0.018	0.014
KEGG_CELL_CYCLE	2.4	0.018	0.014
KEGG_P53_SIGNALING_PATHWAY	1.652	0.051	0.038
KEGG_B_CELL_RECEPTOR_SIGNALING_PATHWAY	-1.399	0.112	0.083
KEGG_VEGF_SIGNALING_PATHWAY	-1.433	0.087	0.065
KEGG_LINOLEIC_ACID_METABOLISM	-2.079	0.024	0.018

Notes: Gene sets with P. adjust < 0.05 and FDR < 0.25 are considered as significant.

Abbreviations: NES, normalized enrichment score; FDR, false discovery rate; KEGG, Kyoto Encyclopedia of Genes and Genomes.

significantly inhibit lung cancer promotion in mouse models with KRAS mutations. Also, it can inhibit the activation of tumor STAT3 and the proliferation of tumor cells. Among them, STAT3 is an IL-6 reactive transcription factor whose activation can induce tumor to promote the reverse pathway of inflammation.^{34,35}

According to one experimental study, the p53 signal pathway has an important role in the occurrence and development of many kinds of malignant tumors. In one study, up-regulation of central genes (CCNB1, CCNB2, CDK1, CDKN2A, and CHEK1) in the p53 signaling pathway confirmed the p53 signaling pathway as a risk factor for tumorigenesis and survival in LUAD.³⁶ In addition, the

abnormality of the cell cycle pathway accelerates the occurrence of LUAD. Some scholars have found that the p53 gene has an important regulatory role in the cell cycle, apoptosis, and genomic stability.^{37,38} Other studies have found that p53 triggers DNA damage and oncogene activation.^{39,40} Therefore, we speculated that the p53 signal pathway in LUAD may produce more interaction networks with other signal pathways through molecules such as DSCC1 or may promote tumorigenesis by changing the cell cycle.

Immunotherapy represented by immune checkpoint inhibitor has changed the traditional treatment of LUAD, and it is beneficial to patients clinically.^{41,42} However, the survival

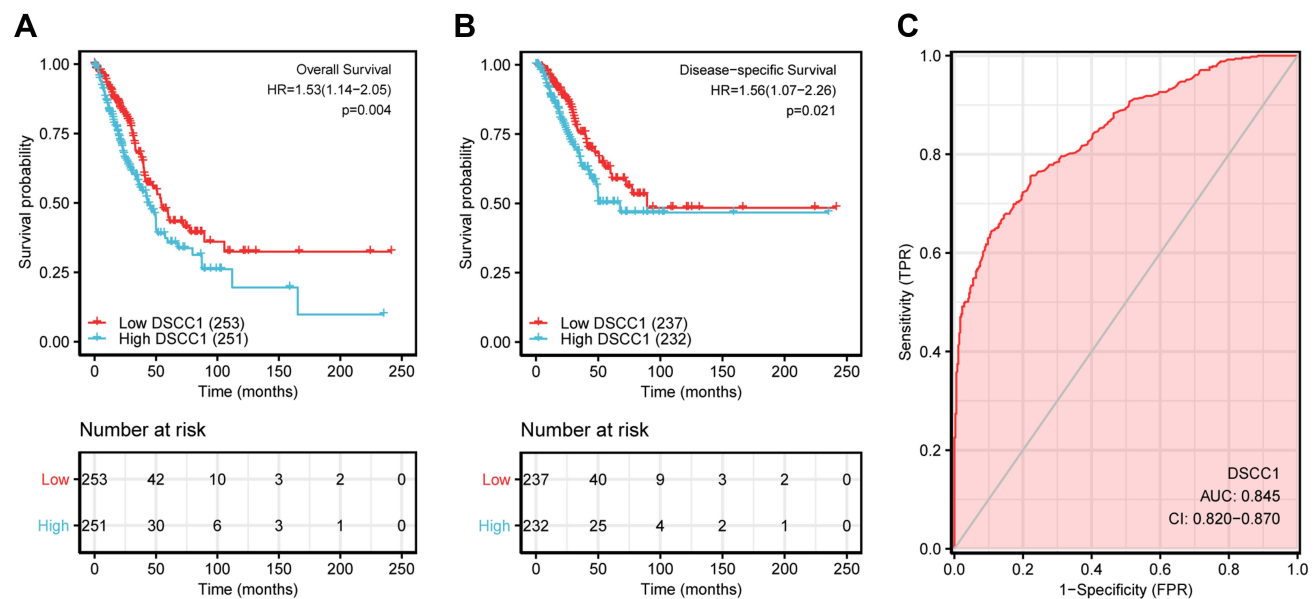


Figure 4 Impact of DSCC1 expression on overall survival (A), disease-specific survival (B) in patients with LUAD in TCGA (The Cancer Genome Atlas) cohort. Receiver operating characteristic is used to evaluate the diagnostic efficacy of DSCC1 for LUAD (C).

Abbreviations: AUC, area under the curve; CI, confidence interval; FPR, false-positive rate; HR, hazard ratio.

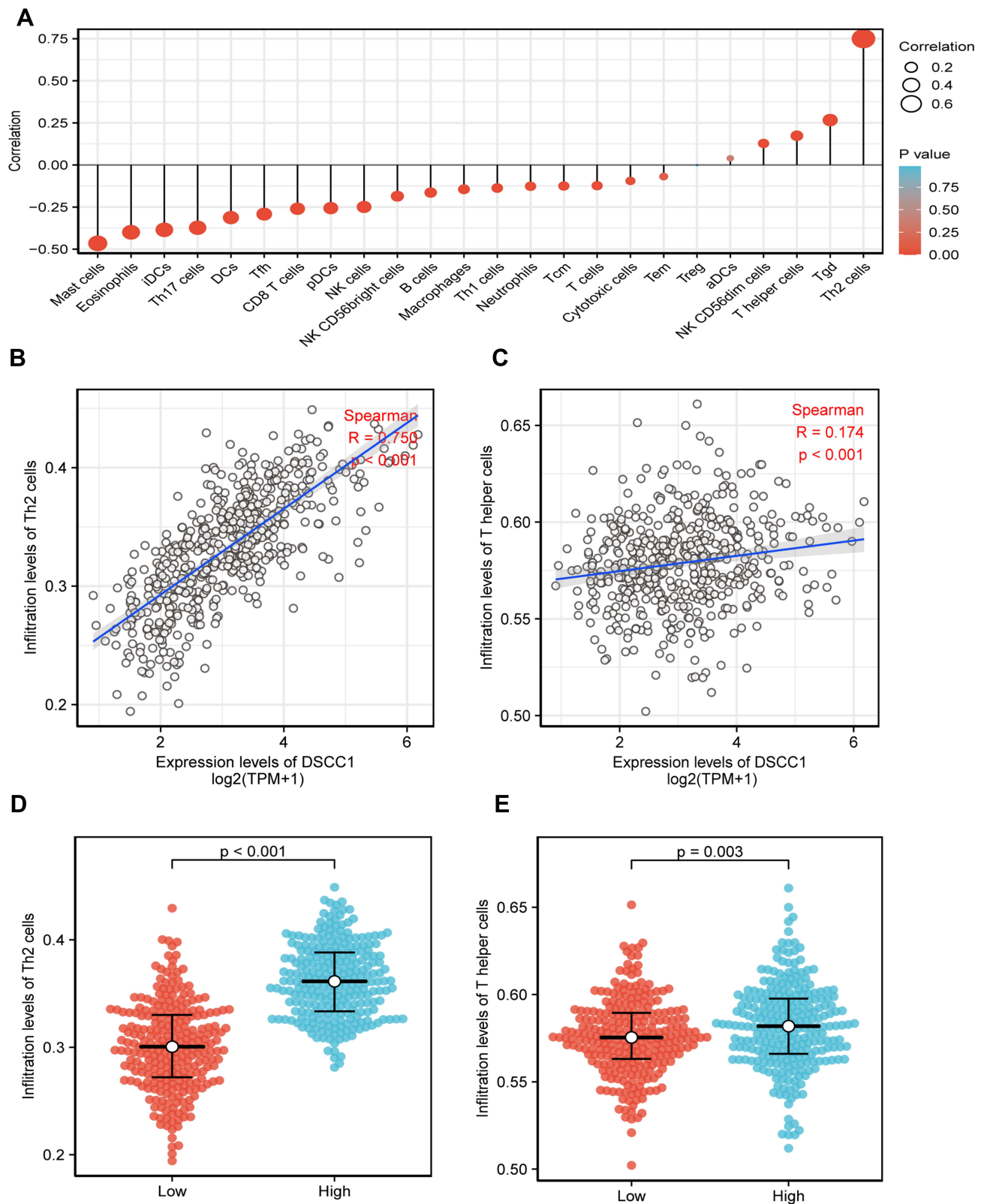


Figure 5 The expression level of DSCC1 was associated with the immune infiltration in the tumor microenvironment. The forest plot shows the correlation between DSCC1 expression and immune cell subsets (A). We used Spearman correlation to analyze the correlation between DSCC1 expression and Th2 cells (B) and T helper cells (C). The Wilcoxon rank-sum test was used to analyze the differences in the infiltration level of Th2 cells (D) and T helper cells (E) between the high and low DSCC1 expression groups.

Abbreviations: DCs, dendritic cells; pDCs, plasmacytoid dendritic cells; aDCs, activated dendritic cells; iDCs, immature dendritic cells; Tcm, central memory T lymphocyte; Tem, effector memory T lymphocyte; Tfh, follicular helper T lymphocyte; Tgd, gamma delta T lymphocyte.

benefit for LUAD patients is still not ideal, and the vast majority of patients have little or no benefit at all. Consequently, we analyzed the relationship between the expression level of DSCC1 in the LUAD microenvironment and immune infiltration. Our results showed that the expression of DSCC1 was negatively correlated with macrophages, NK cells, and T cells. Also, the expression of DSCC1 was low in these immune cells that could kill tumor cells. Compared with the low expression group of DSCC1, the infiltration of DSCC1 in Th2 cells, helper T cells, NKCD56^{dim} cells, and other immune cells was significantly increased. Immune cells in the tumor microenvironment may affect the occurrence and development of malignant tumors.^{43,44} The proportion of immune cell infiltration is also related to tumor metastasis and drug resistance mechanisms.⁴⁵ Previous studies⁴⁶ have shown that different immune cell infiltration may differently affect the clinical outcomes of patients. Among them, T-cell infiltrating tumors may respond to treatments aimed at the mechanism of immune system suppression. Non-T cell infiltrating tumors may require additional interventions to promote inflammation and innate immune activation in the tumor microenvironment. The infiltration of macrophages, dendritic cells, and helper T cells in the center and edge of tumor invasion is related to the resistance to radiotherapy and chemotherapy and the shortening of survival time.⁴⁷ In a study on the characteristics of immune cell infiltration in the overall tumor microenvironment mediated by molecules,⁴⁸ the p53 signal transduction pathway and cancer pathway were activated compared with the low-risk group, which may be related to the short survival time of patients. Moreover, the infiltration of regulatory T cells in high-risk groups significantly increased. These results suggest that DSCC1 may be involved in regulating the infiltration of immune cells in the microenvironment of LUAD, thus affecting the prognosis of patients with LUAD.

Despite these new insights, the number of patients with drug resistance and relapse after treatment is still very high. The targeted therapy of the above molecular markers may be effective, but the final treatment effect is disappointing. Therefore, we should try to find new molecular markers. From the ROC curve, it was found that DSCC1 may be a potential diagnostic molecule.

Although we founded its predictive value in LUAD clinical outcomes, there were some limitations. On the one hand, we could not evaluate a specific role for DSCC1 in LUAD treatment due to the incomplete information about details on treatments. On the other hand, the

corresponding responses was different and unknown. What is more, we were unable to provide information about the relative protein levels. Therefore, comprehensive studies are needed to confirm our findings and thus promote the clinical utility of DSCC1 in the evaluation of LUAD prognosis.

Conclusion

DSCC1 has an important role in the occurrence and development of LUAD. This report revealed that the expression of DSCC1 was up-regulated in LUAD. Also, the high expression of DSCC1 led to a poor prognosis. In addition, DSCC1 may be a potential diagnostic molecule, which may improve patients' prognosis.

Abbreviations

DSCC1, DNA replication and sister chromatid cohesion 1; DCC1, DNA replication and sister chromatid cohesion 1; SCLC, small cell lung carcinoma; NSCLC, non-small-cell lung carcinoma; LUAD, lung adenocarcinoma; TCGA, the cancer genome atlas; GTE_x, genotype tissue expression; GSEA, gene set enrichment analysis; ssGSEA, single sample gene set enrichment analysis; KEGG, Kyoto Encyclopedia of Genes and Genomes; ROC, receiver operating characteristic; CI, confidence interval; HR, hazard ratio; PD, Progressive Disease; SD, Stable Disease; PR, Partial Response; CR, Complete Response; Mut, Mutant; WT, Wide Type; DSS, disease free survival; OS, overall survival; FDR, false discovery rate; NES, normalized enrichment fraction; ROC, the time-dependent receiver operating characteristic curve; AUC, the area under the curve; PD-1, programmed cell death protein 1; PD-L1, programmed cell death-Ligand 1; RFC, replication factor c complex; PCNA, proliferating cell nuclear antigen; Ctf18, chromosome transmission fidelity protein 18; FPKM, FragmentsPerKilobaseperMillion; TPM, transcriptspermillionreads; TP53, tumor protein p53; KARS, V-Ki-ras2 Kirsten ratsarcoma viral oncogene homolog; EGFR, epidermal growth factor receptor; IL-6, interleukin-6; p53, tumor protein 53; VEGF, vascular endothelial growth factor; EGFR TKI, epidermal growth factor receptor tyrosine kinase inhibitor; STAT3, signal transducers and activators of transduction-3; CCNB1, cyclin B1; CCNB2, cyclin B2; CDK1, cyclin dependent kinase 1; CDKN2A, cyclin-dependent kinase inhibitor 2A; CHEK1, checkpoint kinase 1; TME, tumor microenvironment mediated; DCs, dendritic cells; iDCs, immature DCs; aDCs, activated DCs; NKs, natural killer cells; Th1,

T helper 1; Th2, T helper 2; T $\gamma\delta$, T gamma delta; Tcm, T central memory; Tem, T effector memory; Tfh, T follicular helper cells; Th17, T helper 17 cells; Treg, regulatory T cells; ACC, adrenocortical carcinoma; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CESC, cervical and endocervical cancers; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; DLBC, lymphoid neoplasm diffuse large B-cell lymphoma; ESCA, esophageal carcinoma; GBM, glioblastoma multiforme; HNSC, head and neck squamous cell carcinoma; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LAML, acute myeloid leukemia; LGG, brain lower grade glioma; LIHC, liver hepatocellular carcinoma; LUSC, lung squamous cell carcinoma; MESO, mesothelioma; OV, ovarian serous cystadenocarcinoma; PAAD, pancreatic adenocarcinoma; PCPG, pheochromocytoma and paraganglioma; PRAD, prostate adenocarcinoma; READ, rectum adenocarcinoma; SARC, sarcoma; SKCM, skin cutaneous melanoma; STAD, stomach adenocarcinoma; TGCT, testicular germ cell tumors; THCA, thyroid carcinoma; THYM, thymoma; UCEC, uterine corpus endometrial carcinoma; UCS, uterine carcinosarcoma; UVM, uveal melanoma.

Disclosure

The authors report no conflicts of interest in this work.

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