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Comprehensive analysis of the prognostic values and immune implication of ESYT3 in lung adenocarcinoma

Xuedong Li, MD[a], Jianlin Chen, MD[b,c], Jie Meng, PhD[a,*]

Abstract

Few studies have reported the association between ESYT3 and tumors. The purpose of this study was to investigate the molecular features and potential roles of ESYT3 in lung adenocarcinoma (LUAD). In the present study, GEPIA, UALCAN, TCGA databases, and KM Plotter were primarily used to study ESYT3 mRNA expression profiles and prognostic values in patients with LUAD. Then we evaluated co-expressed genes of ESYT3 by cBioPortal online tools and performed enrichment analysis using Metascape. Moreover, the relationship between ESYT3 and immune infiltrating cells was explored via TIMER2, and MethSurv database was used to conduct methylation analysis. We found ESYT3 was downregulated in LUAD tissues based on TCGA and GEPIA databases. Low expression of ESYT3 mRNA was observed to be significantly correlated with N classification and stage classification. GEPIA2, UALCAN databases and KM Plotter showed that low expression levels of ESYT3 was associated with poor survival in LUAD patients. The enrichment analysis indicated that co-expressed genes of ESYT3 were highly enriched in cell division. Then, our study showed ESYT3 was correlated with immune infiltration and immune checkpoints. Additionally, hypomethylation was associated with low ESYT3 expression and poor prognosis in LUAD. In conclusion, this study suggested ESYT3 could be a potential prognostic marker and a promising therapeutic target in LUAD.

Abbreviations: ADGRF5 = adhesion G protein-coupled receptor F5, BTBD9 = BTB domain containing 9, CDKL2 = cyclin dependent kinase like 2, E-Syts = extended synaptotagmins, GO = gene ontology, HLF = hepatic leukemia factor, KEGG = Kyoto encyclopedia of genes and genomes, LUAD = lung adenocarcinoma, NK = natural killer, NKX2-1 = NK2 homeobox 1, OS = overall survival, SFTA3 = surfactant associated 3.

Keywords: biomarker, ESYT3, immune implication, LUAD, prognosis

1. Introduction

It is well-known that lung cancer, including small cell lung cancer and non-small cell lung cancer, constitutes as the leading cause of cancer-related deaths around the world.[1] For non-small cell lung cancer, it has 3 subtypes of: lung adenocarcinoma (LUAD), lung squamous cell carcinoma, and large cell carcinoma.[2] Although the effectiveness of therapeutic modalities has improved, the LUAD patient still has a low 5-year survival rate. Therefore, it is urgent to identify a prognostic marker and a therapeutic target for LUAD treatment.

Extended synaptotagmins (E-Syts) are endoplasmic reticulum proteins that bind phospholipids and Ca^{2+} and contain an SMP domain. E-Syts form a contact junction between the endoplasmic reticulum and plasma membrane that facilitates glycerophospholipid exchange.[3] Due to high expression of E-syts in some mammalian tissues, such as in the immune system and lungs, Saheki et al[4] proposed that studies of these systems may help shed light on their physiological impact. Few reports have been published on the functional study of ESYT3. The loss of E-Syt2 and E-Syt3 impacts cell migration and survival under stress in vitro.[3] Tremblay et al showed expression of Orp5 was found to be 1.5 times higher in lung from E-Syt-null mice as compared with wild type mice.[5] In addition, overexpression of Orp5 promoted invasiveness of lung cancer cells.[6] Therefore, it is necessary to investigate the biological significance of ESYT3 (mRNA and protein expression) in LUAD patients.

XL and JC contributed equally to this work.

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2. Materials and methods

2.1. Expression analyses of ESYT3

We assessed gene expression levels of ESYT3 among 33 cancer types using TIMER 2.0 (http://timer.cistrome.org/) based on TCGA database. Then, we compared ESYT3 gene expression levels between tissues with LUAD and normal tissues using TCGA datasets alone or TCGA with GTEx databases (http://gepia.cancer-pku.cn/).[7] The ESYT3 protein level was analyzed by the UALCAN database (https://ualcan.path.uab.edu/analysis-prot.html/).[8] In addition, a total of 510 mRNA and clinical data in the TCGA-LUAD (provisional) dataset were retrieved from the cBioPortal online platform (https://www.cbioportal.org/),[9] but only 340 samples with complete prognosis data on age, gender, tumor (T) stage, neoplasm disease lymph node (N) stage, metastasis (M) stage, neoplasm disease stage, overall survival (OS) and OS status were found to be used in further analyses.

2.2. Association between ESYT3 expression and clinical features

The UALCAN resource is a premier source for exploring TCGA gene expression data. We utilized UALCAN to analyze the relationship between ESYT3 expression and different clinical characteristics in LUAD.

2.3. Survival prognosis analysis in LUAD

The prognostic value of ESYT3 expression was assessed for LUAD patients using the GEPIA (http://gepia.cancer-pku.cn/), Kaplan–Meier Plotter (http://kmplot.com/analysis/) and UALCAN online databases (https://ualcan.path.uab.edu/analysis.html).

2.4. Analyses of genes co-expressed with ESYT3 in LUAD

First, we identified 300 co-expressed genes of ESYT3. Then, the top 6 co-expressed genes of ESYT3 were selected based on the

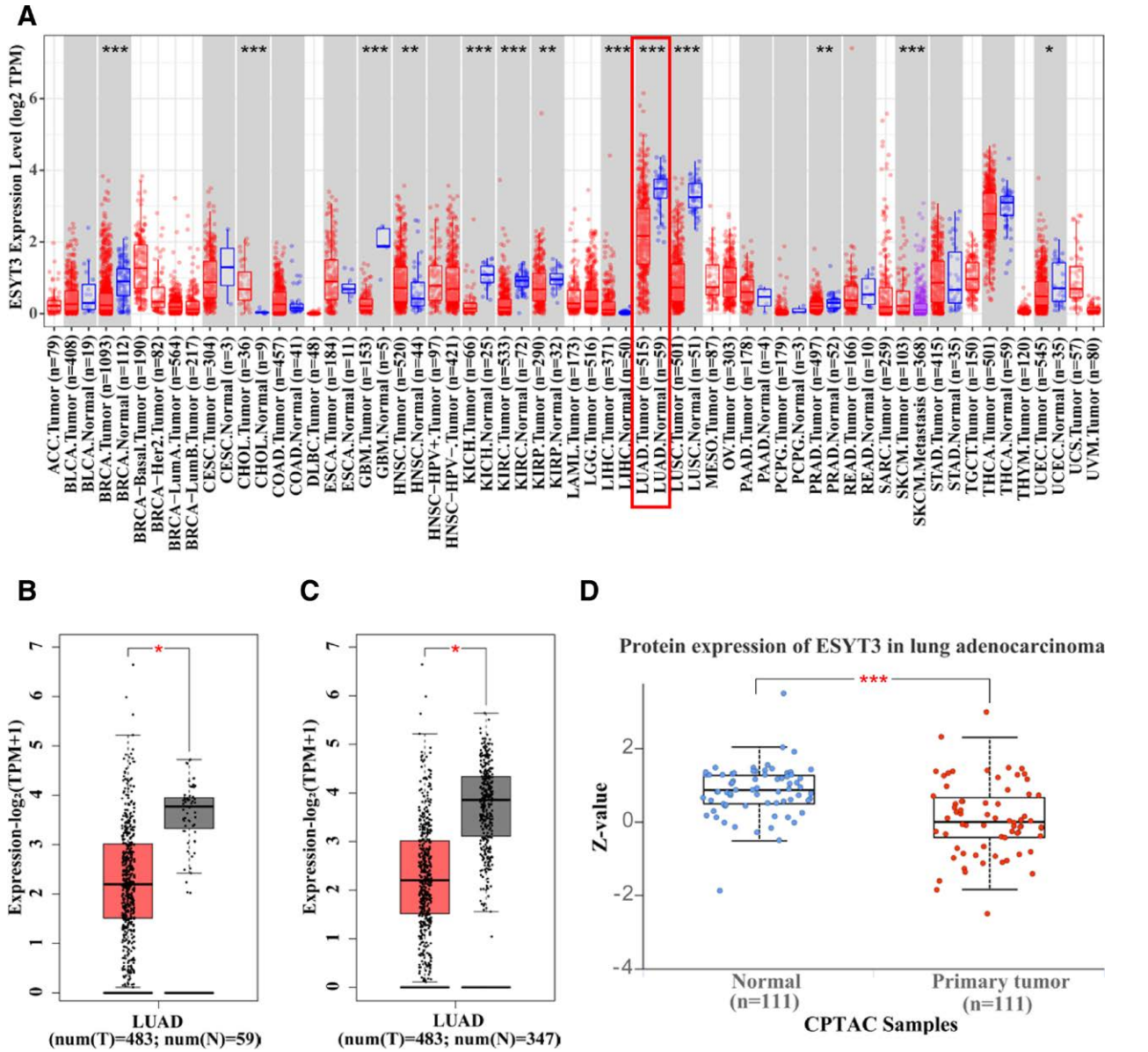

Figure 1. The mRNA and protein level of ESYT3 in LUAD tissue. (A) The mRNA level of ESYT3 in tumor and adjacent normal tissues of different types of cancers based on TCGA database. (B) The mRNA level of ESYT3 in unpaired LUAD tissues and normal tissues based on TCGA. (C) The mRNA level of ESYT3 in normal and tumor tissues based on matching TCGA normal and GTEx data. (D) The protein level of ESYT3 in LUAD tumor and normal tissues by CPTAC dataset. *$P < .05$, ** $P < .01$, ***$P < .001$. LUAD = lung adenocarcinoma.

Spearman rank correlation coefficient, and the correlation analysis was visualized in cBioPortal. Furthermore, the survival analysis of top 6 co-expressed genes was conducted by GEPIA2. Moreover, Metascape database was used to perform enrichment analysis [gene ontology (GO) and Kyoto encyclopedia of genes and genomes (KEGG) pathway] of co-expressed genes of ESYT3.[10]

2.5. Association between ESYT3 and immune cell infiltration in LUAD

A systematic analysis of ESYT3 expression and tumor-infiltrating immune cells was performed using the TIMER2 database. In addition, the "correlation analysis" module in GEPIA2 was used to analyze correlations between ESYT3 expression and TIL gene markers in LUAD.

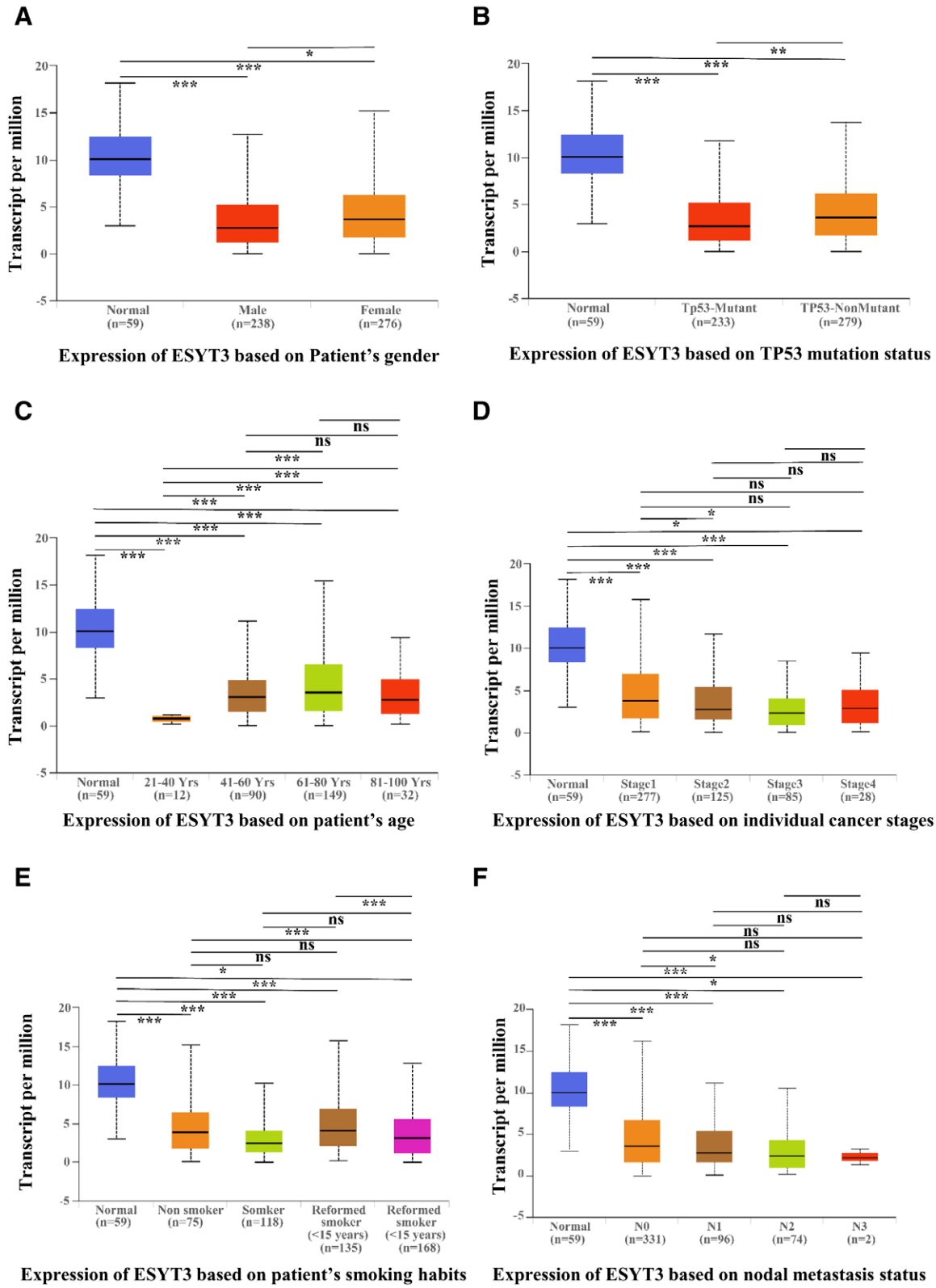

Figure 2. Correlation analysis between ESYT3 expression and clinical pathological characteristics of LUAD patients via TCGA cohort (A) Gender. (B) TP53 mutation status. (C) Age. (D) Cancer stage. (E) Smoking habit. (F) Nodal metastasis status. ns, *$P < .05$, ** $P < .01$, ***$P < .001$. LUAD = lung adenocarcinoma.

2.6. DNA methylation analysis

We analyzed the ESYT3 methylation corresponding to TCGA-LUAD cohorts by the Methsurv tool.[11] Then, the survival analysis was performed based on the methylation status of ESYT3 at multiple sites.

2.7. Statistical analysis

Chi-square tests were used to analyze differences between categorical variables. Potential prognostic factors were screened using single-factor and multivariate cox analyses. Statistical analyses were processed using SPSS 26.0 software (SPSS, Inc., Chicago, IL). For all analyses, $P < .05$ was considered significant.

3. Results

3.1. Down-regulation of ESYT3 mRNA and protein expression in LUAD tissue based on TCGA database

In this study, the expression of ESYT3 mRNA in various cancer types from TCGA was analyzed using TIMER 2.0 database. We found that the expression of ESYT3 mRNA was low in various cancers (Fig. 1A), including LUAD. Compared with unmatched adjacent normal tissues, ESYT3 was expressed remarkably lower in LUAD tissues (Fig. 1B). These results were confirmed in tumor tissues and matched normal tissue (Fig. 1C). Further investigation of the protein expression level of ESYT3 was performed using UALCAN database, and we observed a significant reduction in ESYT3 protein levels in LUAD tissues compared with adjacent normal tissues (Fig. 1D).

3.2. Correlation analysis between ESYT3 expression and clinical pathological characteristics of LUAD patients via TCGA cohort

As the function of ESYT3 in LUAD is still unclear, we further analyzed ESYT3 expression on the basis of different clinical characteristics in LUAD samples from TCGA. In subgroup analyses based on gender, age, smoking habits, individual cancer stages, nodal metastasis, and TP53 mutation status, ESYT3 expression was significantly lower in LUAD patients than the normal control (Fig. 2). Specifically, patients with nodal metastasis had lower ESYT3 mRNA expression than those with non-nodal metastasis (Fig. 2E). Furthermore, patients with TP53 mutations have a lower expression of ESYT3 (Fig. 2F).

A total of 340 patients with LUAD were divided into low and high ESYT3 groups based on cutoff value (the median mRNA expression of ESYT3, Supplemental Digital Content 1, http://links.lww.com/MD/J423). The correlations between ESYT3 expression and clinical characteristics in LUAD were presented in Table 1. Results showed that ESYT3 expression was related to gender ($P = .009$), N stage ($P < .001$), and pathologic stage ($P = .002$) (Table 1).

3.3. Down-regulation of ESYT3 expression was associated with poor prognosis in LUAD

The prognostic value of ESYT3 expression was assessed in LUAD patients using GEPIA, UALCAN databases and Kaplan–Meier Plotter. These results showed that low levels of ESYT3 mRNA expression were significantly related to poor OS among LUAD patients in GEPIA (Log rank $P = 1.3E-05$, HR = 0.51; Fig. 3A), Kaplan–Meier Plotter (Log rank $P = .00013$, HR = 0.56; Fig. 3B) and UALCAN ($P = .0095$; Fig. 3C).

Going further, we conducted univariate and multivariate Cox regression analyses. According to univariate analysis, T stage, N stage, M stage, pathologic stage and low ESYT3 mRNA level were associated with a worse overall survival (Table 2). In multivariate cox analysis, only T stage and N stage were associated with poor OS (Table 2).

3.4. Functional enrichment analysis of co-expressed genes of ESYT3.

A robust method for co-expression gene analysis is used to predict gene function. We applied the cBioPortal web server

Table 1

Relationship between the clinical features and ESYT3 expression in patients with LUAD.

Characteristic	Low expression of ESYT3, n (%)	High expression of ESYT3, n (%)	χ^2	*P* value
N	170	170		
Gender			6.777	**.009**
Female	74	98		
Male	96	72		
Age			0.852	.356
<= 60	60	52		
> 60	110	118		
T stage			5.742	.125
T1	44	58		
T2	97	96		
T3	17	10		
T4	12	6		
N stage			17.207	**<.001**
N0	88	125		
N1	82	45		
M stage			0.778	.378
M0	157	161		
M1	13	9		
Pathologic stage			15.108	**.002**
Stage I	71	103		
Stage II	45	39		
Stage III	41	19		
Stage IV	13	9		

Data in bold indicates $P < .05$.

LUAD = lung adenocarcinoma.

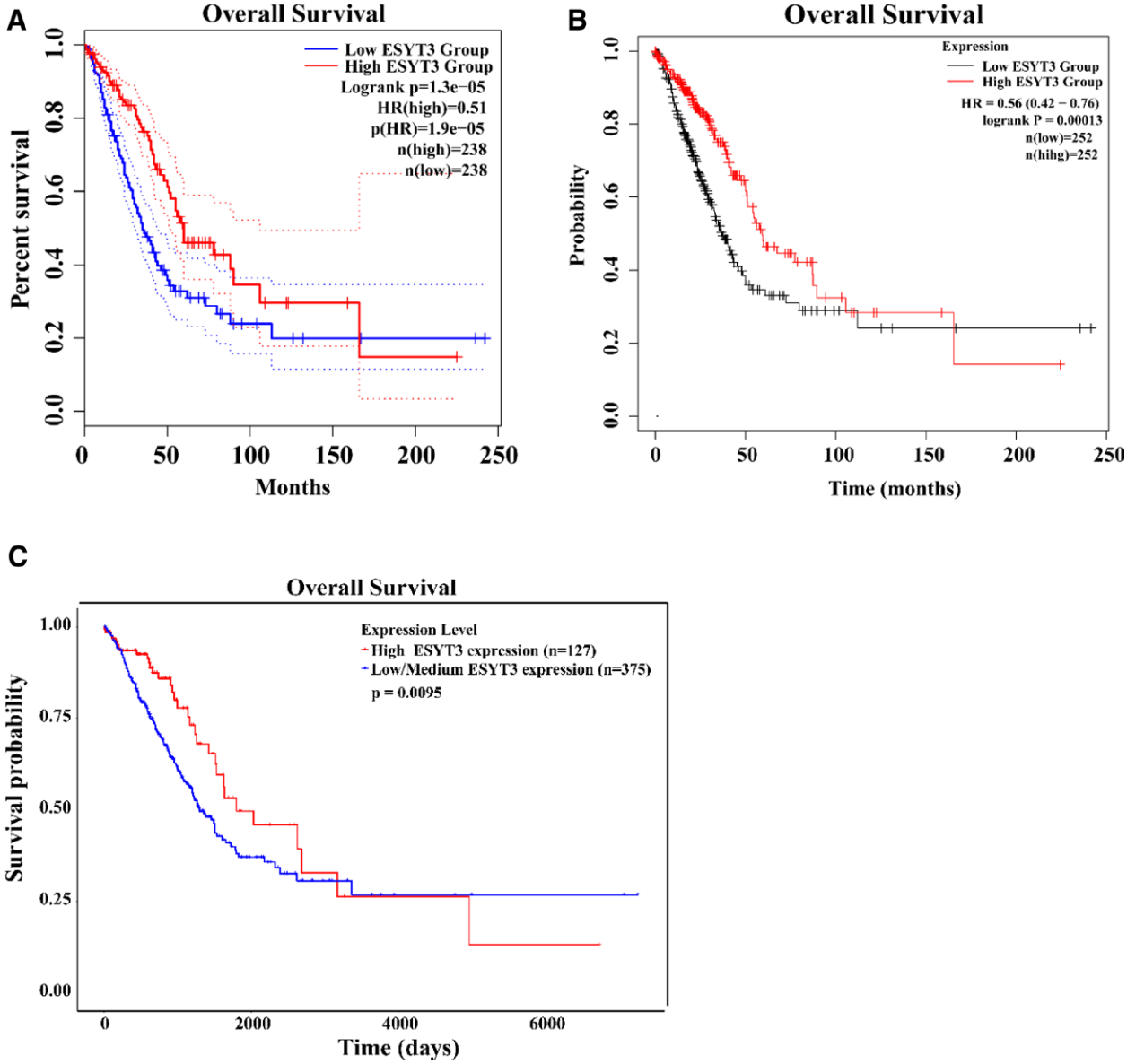

Figure 3. Survival analyses of ESYT3 in LUAD using three databases. (A) OS curves of LUAD from GEPIA2 (group cutoff = median). (B) OS curves of LUAD from K-M-Plotter dataset (group cutoff = median). (D) OS curves of LUAD from UALCAN dataset (group cutoff = quartile). LUAD = lung adenocarcinoma, OS = overall survival.

Table 2

Cox regression analysis for clinical outcomes in LUAD patients.

	Univariate analysis			Multivariate analysis		
Characteristics	**HR**	**95% CI**	***P* value**	**HR**	**95% CI**	***P* value**
Gender	0.893	0.639–1.249	.509			
Age	1.039	0.723–1.492	.838			
Pathologic stage	1.584	1.353–1.854	**<.001**	1.255	0.892–1.764	.192
T stage	1.61	1.321–1.961	**<.001**	1.278	1.016–1.606	**.036**
N stage	2.501	1.783–3.509	**<.001**	1.627	1.002–2.642	**.049**
M stage	1.867	1.052–3.315	**.033**	0.965	0.410–2.272	.935
ESYT3	0.549	0.389–0.775	**.001**	0.728	0.506–1.047	.087

Data in bold indicates $P < .05$.

CI = confidence interval, HR = hazard ratio, LUAD = lung adenocarcinoma.

to identify top 300 co-expressed genes with ESYT3 in 3 different studies from TCGA (TCGA, Firehose Legacy; TCGA, Nature 2014; TCGA, PanCancer Atlas) (Supplemental Digital Content 2, http://links.lww.com/MD/J424). A total of 236 ESYT3 co-expressed genes were identified in 3 TCGA studies, as shown in Figure 4A. To further explore enrichment function

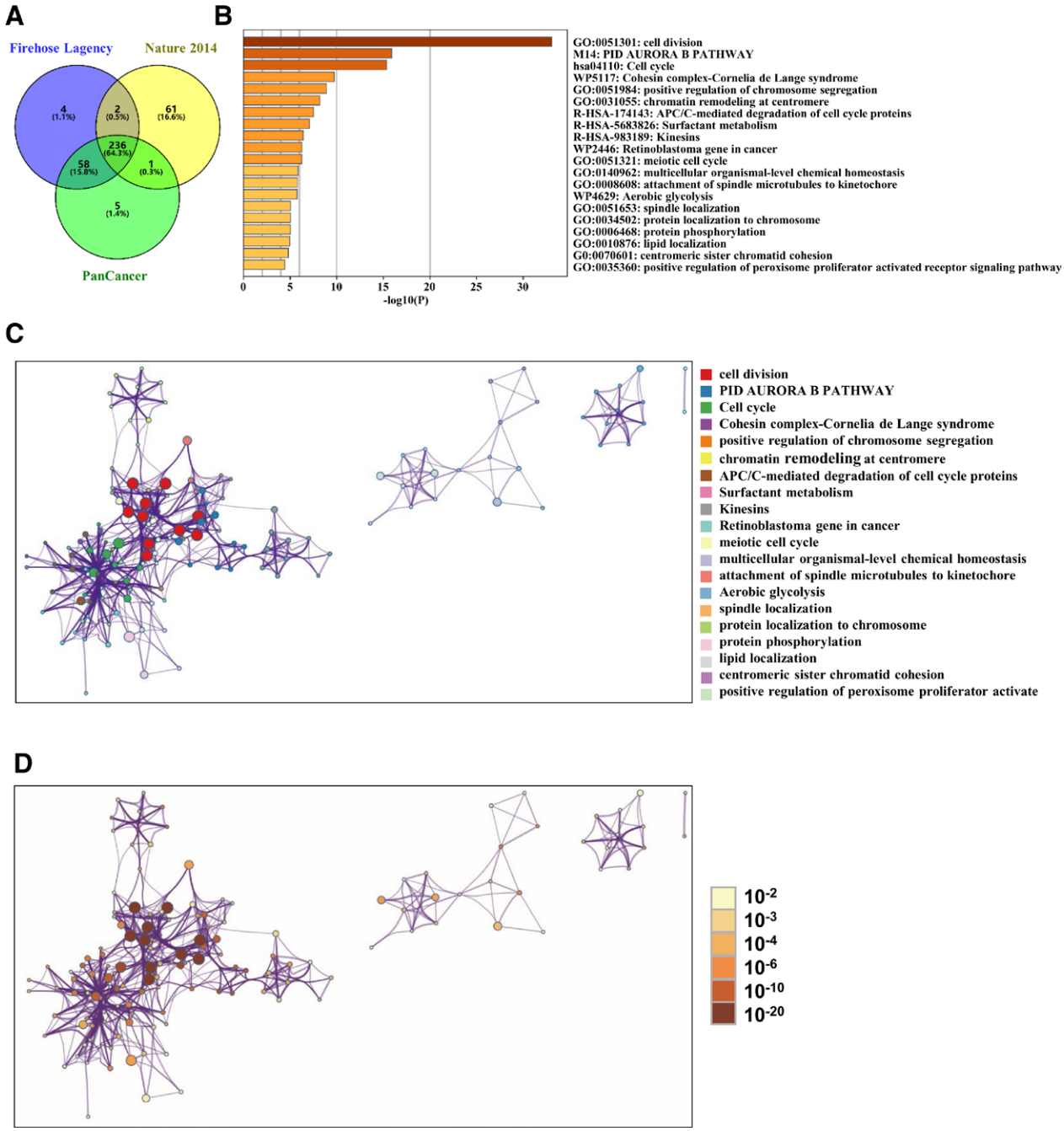

Figure 4. Functional enrichment analysis of co-expressed genes of ESYT3. (A) The Venn diagram of the 236 co-expressed genes in three TCGA studies on LUAD. (B) Using Metascape, a bar graph of enriched terms across co-expressed genes was shown, colored based on the *P* value of each term. (C) The top 20 enriched terms were represented by an interactive network colored by cluster IDs. (D) The top 20 enriched terms were represented by an interactive network colored by *P* values. LUAD = lung adenocarcinoma.

of co-expressed genes of ESYT3, 236 ESYT3 co-expressed genes were selected through Metascape to perform GO and KEGG pathway analyses. In Figure 4B, co-expressed genes of ESYT3 were highly enriched in cell division, spindle elongation, FOXM1 pathway and G2/M Transition. As shown in Figure 4C and D, ESYT3 enrichment terms were displayed by cluster ID and *P* value. Furthermore, the top 6 co-expressed genes of ESYT3 arranged by the values of Spearman rank correlation coefficient were identified. In a correlation analysis, ESYT3 exhibited a strong positive correlation with Surfactant Associated 3 (SFTA3), BTB Domain Containing 9 (BTBD9), hepatic leukemia factor (HLF), NK2 Homeobox 1 (NKX2-1), cyclin dependent kinase like 2 (CDKL2), and adhesion G protein-coupled receptor F5 (ADGRF5) (Fig. 5A–F). Overall survival analysis further confirmed that deregulation of expression levels of SFTA3, BTBD9, HLF, NKX2-1, CDKL2, and ADGRF5 were associated with poor prognosis of LUAD (Fig. 5G–L).

4. ESYT3 expression was associated with tumor-infiltrating immune cells in LUAD

The occurrence and development of lung cancer involves the interaction between immune cells in the local microenvironment. And tumor-infiltrating lymphocytes (including T and B cells, natural killer (NK) cells, infiltrating dendritic cells and

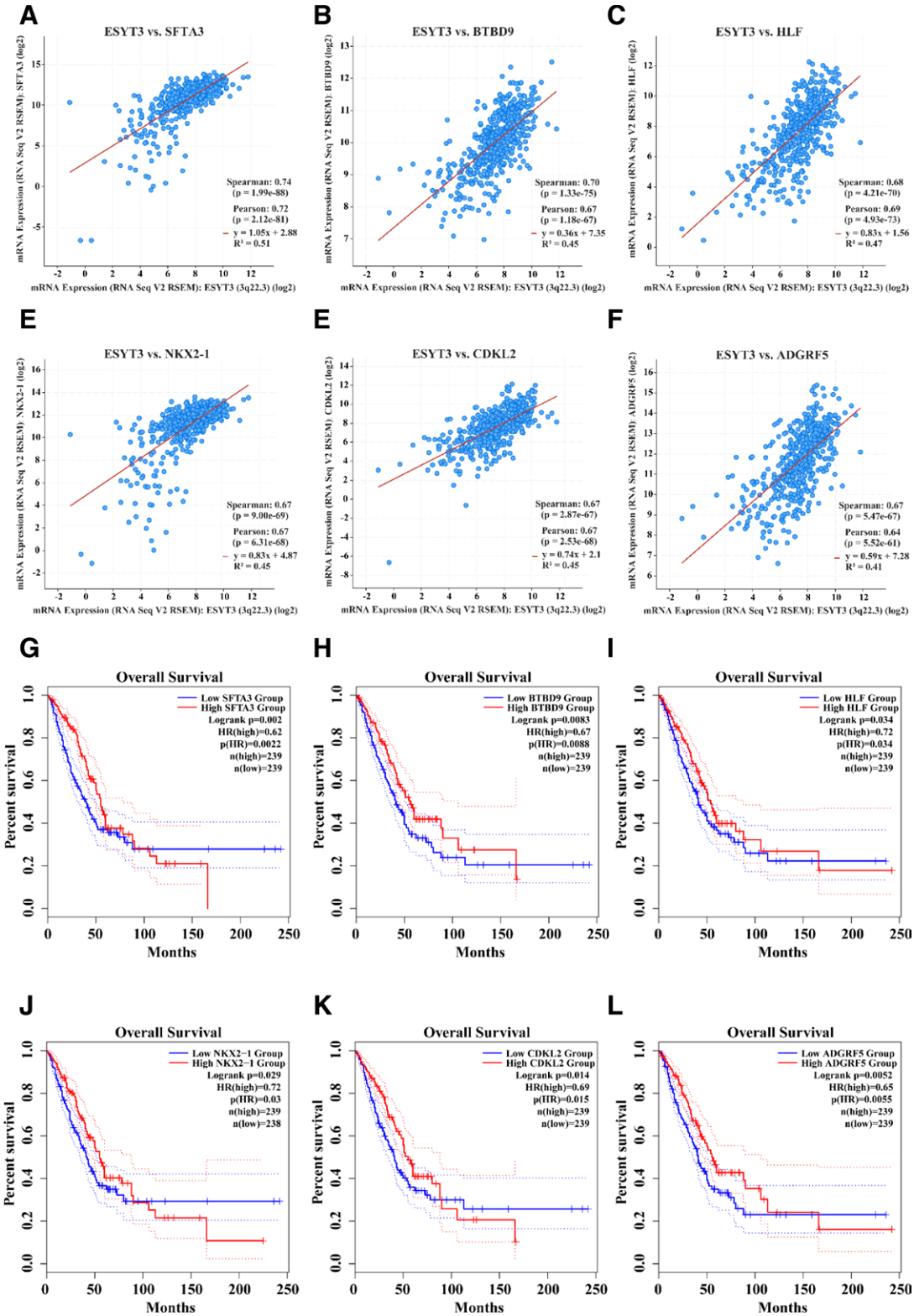

Figure 5. Analyses of genes co-expressed with ESYT3. (A–F) Correlation showing by scatterplot between ESYT3 and its top six co-expressed genes (SFTA3, BTBD9, HLF, NKX2-1, CDKL2, and ADGRF5). (G–L) The K-M (Kaplan–Meier) plots of OS showed the difference between the low and high expression of (G) SFTA3, (H) BTBD9, (I) HLF, (J) NKX2-1, (K) CDKL2, and (L) ADGRF5 in LUAD. ADGRF5 = adhesion G protein-coupled receptor F5, BTBD9 = BTB domain containing 9, CDKL2 = cyclin dependent kinase like 2, HLF = hepatic leukemia factor, LUAD = lung adenocarcinoma, NKX2-1 = NK2 homeobox 1, OS = overall survival, SFTA3 = surfactant associated 3.

macrophages) are involved in the antitumor response within the lung tumor niche.[12] TIMER2 database was used to assess the relationship between ESYT3 expression and immune infiltration in LUAD. There was a positive correlation between ESYT3 expression and CD4 + T cell ($R = 0.134$, $P = 72.81e{-}03$), B cell ($R = 0.214$, $P = 1.66e{-}06$), NK cell ($R = 0.316$, $P = 7.41e{-}13$), neutrophil ($R = 0.257$, $P = 17.38e{-}09$), and monocyte ($R = 0.242$, $P = 5.65e{-}08$) (Fig. 6). Additionally, a

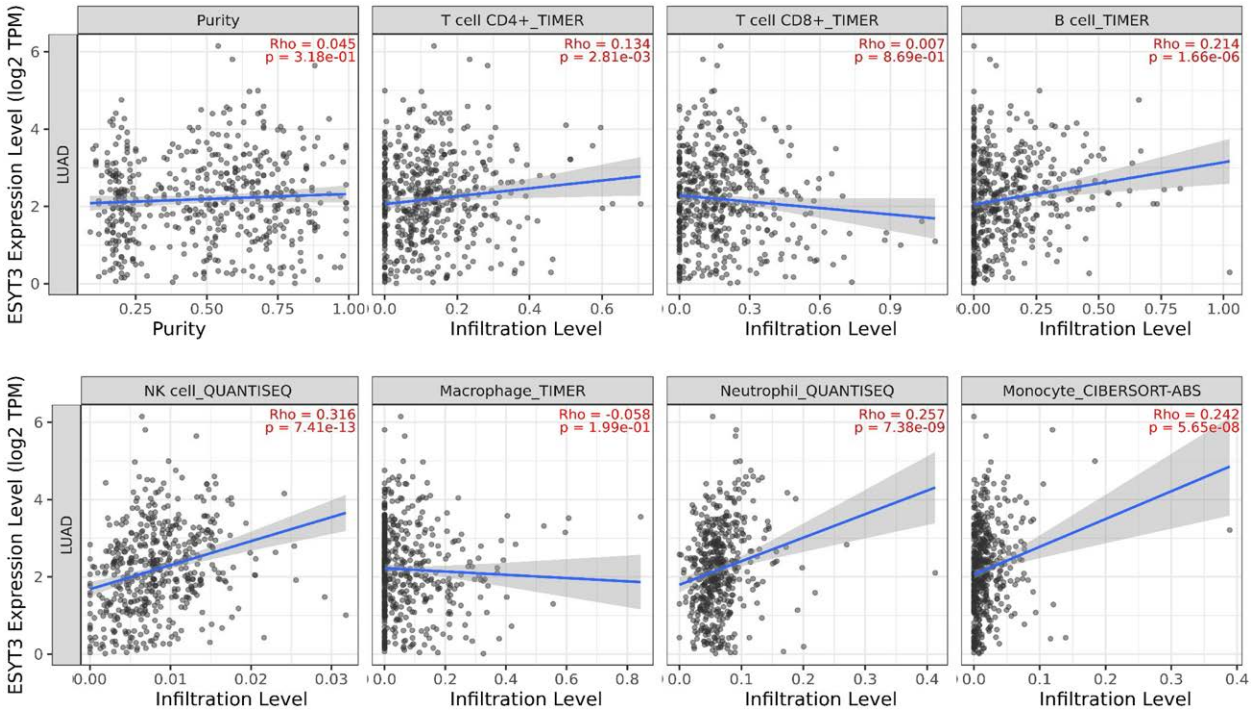

Figure 6. Correlations between immune cell infiltration levels and ESYT3 expression levels in LUAD. The correlation between ESYT3 expression and immune cells was analyzed via TIMER database and evaluated by Spearman correlation. LUAD = lung adenocarcinoma.

number of immune marker genes corresponding to different tumor-infiltrating immune cells (CD8 + T cells, T cells, B cells, monocytes, mast cells, neutrophils, NK cells, Th1, Th2, Tfh, Th17) were also investigated by TIMER and ESYT3 was found to be associated with most of these marker genes (Table 3). Generally, ESYT3 expression were positively correlated with the majority of gene markers of different functional tumor-infiltrating immune cells, which suggested that ESYT3 could influence the recruitment of immune cells in immune microenvironment in LUAD. Then, we analyzed the correlation between ESYT3 expression and immune checkpoint-related genes and the results showed ESYT3 expression was significantly negatively correlated with PDCD1 and LAG3. These results suggested that patients with high levels of ESYT3 expression may benefit from immunotherapy.

4.1. Analysis of ESYT3 methylation in LUAD

A hypermethylated DNA promoter inhibits gene expression while a hypomethylated promoter activates gene expression.[13] To examine whether ESYT3 expression might be influenced by DNA methylation states in LUAD, we used MethSurv tool to visualize the correlation between gene expression and DNA methylation. Using MethSurv tool, ESYT3 DNA methylation sites and the prognostic values of each CpG obtained from TCGA database were analyzed. Differentially methylated CpG sites of ESYT3 gene were identified as shown in Figure 7A. In survival analysis, we found that hypermethylation of cg15579376, cg16802792, cg04810656, and cg01905210 in the ESYT3 gene promoter was associated with poor survival (Fig. 7B). Figure 7C shows that the methylation level of ESYT3 was higher in tumor tissues compared with paired normal tissues.

5. Discussion

Globally, lung cancer is one of the most deadly cancers, and LUAD is the most common histological subtype. A number of advances in targeted therapy and immunotherapy have led to significant progress in treating LUAD. However, the long-term (5-year) survival rate remains unsatisfactory.[14,15] Therefore, identifying new biomarkers and promising immuno-related therapeutic targets for lung cancer is becoming increasingly important in clinical settings. In this study, we found that the expression of ESYT3 was downregulated at both mRNA and protein levels in LUAD tissues. There was a correlation between decreased ESYT3 expression and gender, higher N stage, T53 mutation and advanced pathological stage. The tumor suppressor gene TP53 often mutates in human cancer and abnormal TP53 gene plays an important role in the tumorigenesis of lung epithelial cells.[16] Our study showed that in subgroup analyses based on TP53 mutation status, ESYT3 expression was significantly lower in LUAD patients than the normal control and patients with TP53 mutations have a lower expression of ESYT3. For the purpose of evaluating the prognostic significance of ESYT3 expression in LUAD, various databases and tools were analyzed in this study, including Kaplan–Meier plotters, GEPIA and UALCAN. There were similar results obtained despite the use of different databases and methods. It was found that abnormally decreased ESYT3 expression was associated with poor survival in patients with LUAD, indicating that it may play a significant role in the progression of LUAD. Then, we explored the potential functions and mechanisms of ESYT3 in LUAD based on co-expression analysis. Our study showed ESYT3 was strongly positively correlated with the top 6 co-expressed genes (SFTA3, BTBD9, HLF, NKX2-1, CDKL2, and ADGRF5), and all of these genes played an important role in the survival of LUAD patients ($P < .05$). In order to further elucidate ESYT3's potential molecular mechanisms, co-expressed genes were subjected to GO and KEGG analyses. Cell division cycle-associated genes play an important role in lung carcinogenesis progression.[17] Dysregulation in the process of cell division may lead to malignancy. Co-expressed genes of ESYT3 were highly enriched in cell division, spindle elongation, FOXM1 pathway and G2/M Transition, which may be related to genomic instability and cell cycle progression of lung cancer.

Table 3

The relationship between many immune marker genes corresponding to different tumor-infiltrating immune cells and ESYT3 in LUAD.

		LUAD			
		Tumor		Normal	
Immune cell types	Gene markers	R	*P* value	R	*P* value
B cell	CD19	0.015	.75	−0.028	.84
	CD79A	−0.033	.47	−0.062	.64
T cell (general)	CD2	0.037	.42	−0.021	.87
	CD3E	0.038	.4	0.15	.25
CD8 + T cell	CD8A	−0.097	.033	0.13	.34
	CD8B	−0.1	.025	0.043	.75
CD4 + T cell	CD4	0.17	.00012	0.017	.9
Natural killer cell	KIR2DL1	−0.033	.47	0.25	.052
	KIR2DL3	−0.12	.0065	0.11	.42
	KIR2DL4	−0.32	1.00E-12	−0.08	.55
	KIR3DL1	−0.035	.44	0.17	.2
	KIR3DL2	−0.093	.04	0.18	.17
	KIR3DL3	−0.19	3.00E-05	−0.1	.45
	KIR2DS4	−0.028	.54	0.23	.075
Neutrophils	CD66b	0.4	1.50E-19	−0.13	.33
	CD11b	0.17	.00021	0.12	.35
	CCR7	0.2	1.50E-05	0.08	.55
Th1	T-bet	0.62	.023	0.32	.013
	STAT4	0.12	.011	0.33	.01
	TNF-a	0.074	.11	−0.017	.9
Th2	GATA3	−0.059	.2	0.27	.042
	STAT6	0.39	8.90E−19	0.53	1.70E-05
	STAT5A	0.12	.0083	0.21	.11
	IL13	0.039	.39	0.053	.69
Tfh	BCL6	0.37	1.90E-17	0.54	1.20E-05
Th17	STAT3	0.35	9.70E-16	0.63	6.90E-08
	IL17A	−0.077	.091	0.1	.43
Mast cells	TPSB2	0.23	2.10E-07	−0.052	.7
	TPSAB1	0.28	5.60E-10	−0.089	.5
	CPA3	0.31	2.20E-12	−0.082	.54
	MS4A2	0.4	1.10E-19	0.17	.2
	HDC	0.33	8.40E-14	0.32	.014
Monocytes	CD86	0.029	.52	−0.13	.33
	CX3CR1	0.39	4.40E-19	0.12	.38
	CD14	−0.087	.055	−0.21	.11
T cell exhaustion	PDCD1	−0.091	.047	0.23	.078
	CD274	−0.044	.34	0.38	.0034
	CTLA4	0.019	.67	0.24	.063
	LAG3	−0.18	6.10E-05	0.18	.17
	GZMB	−0.34	1.40E-14	0.044	.74

LUAD = lung adenocarcinoma.

The advent of immunotherapy, such as anti-PD-1/PD-L1 immune checkpoint inhibitor immunotherapy, has revolutionized the treatment of cancer. Immune checkpoints play a key role in controlling immune response and can provide new targets for targeted treatment of tumors. The results of this study demonstrated that ESYT3 regulates immune infiltration levels in LUAD. ESYT3 expression was positively related to the infiltration of immune cells including CD4 + T cells, B cells, NK cells, neutrophil, and monocyte. Additionally, ESYT3 expression was positively correlated with most gene markers of CD8 + T cells, T cells, B cells, monocytes, mast cells, neutrophils, NK cells, Th1, Th2, Tfh, Th17. T cells and B cells have been consistently shown to have positive effects on prognosis.[18–20] This further confirms that ESYT3 expression was associated with favorable prognosis of LUAD patients, which was in accordance with the potential role of diverse immune cells. Furthermore, the study showed there was a strong positive correlation between ESYT3 expression and NK cells ($R = 0.316$, $P = 7.41e{-}13$). Numerous studies have proven that NK cells have powerful antitumor effects, and they are associated with favorable prognosis in many cancers.[21] The cGAS-STING pathway plays an important role in antitumor immunity. Activated cGAS-STING pathway can induce the expression of type I IFN, which activate multiple immune cells such as DC, T cells, and NK cells.[22] Therefore, it is possible that activators of the cGAS-STING pathway can act as immunotherapy for lung adenocarcinoma with low expression of ESYT3. In addition, we found that there was a high expression of immune checkpoint genes in LUAD, which were negatively correlated with ESYT3, including PDCD1, LAG3, and GZMB. These immune checkpoint genes are expressed on TILs, which are involved in the immune escape mechanism.[23] The above relations may indicate that ESYT3 has a potential action to T cells in LUAD. In other words, this study indicated that overexpressed ESYT3 appeared to enhance tumor immunity, promote the infiltration of immune cells into tumors and further enhance the antitumor immune response, and finally inhibit tumor progression.

Epigenetic mechanisms such as DNA methylation play a key role in human health.[24] The hypermethylation of the promoter region of some tumor suppressor genes leads to their inactivation, and many previous studies have shown that DNA methylation silences many genes associated with different cancer types. According to our findings, ESYT3 promoter methylation levels in LUAD was significantly higher than those in normal

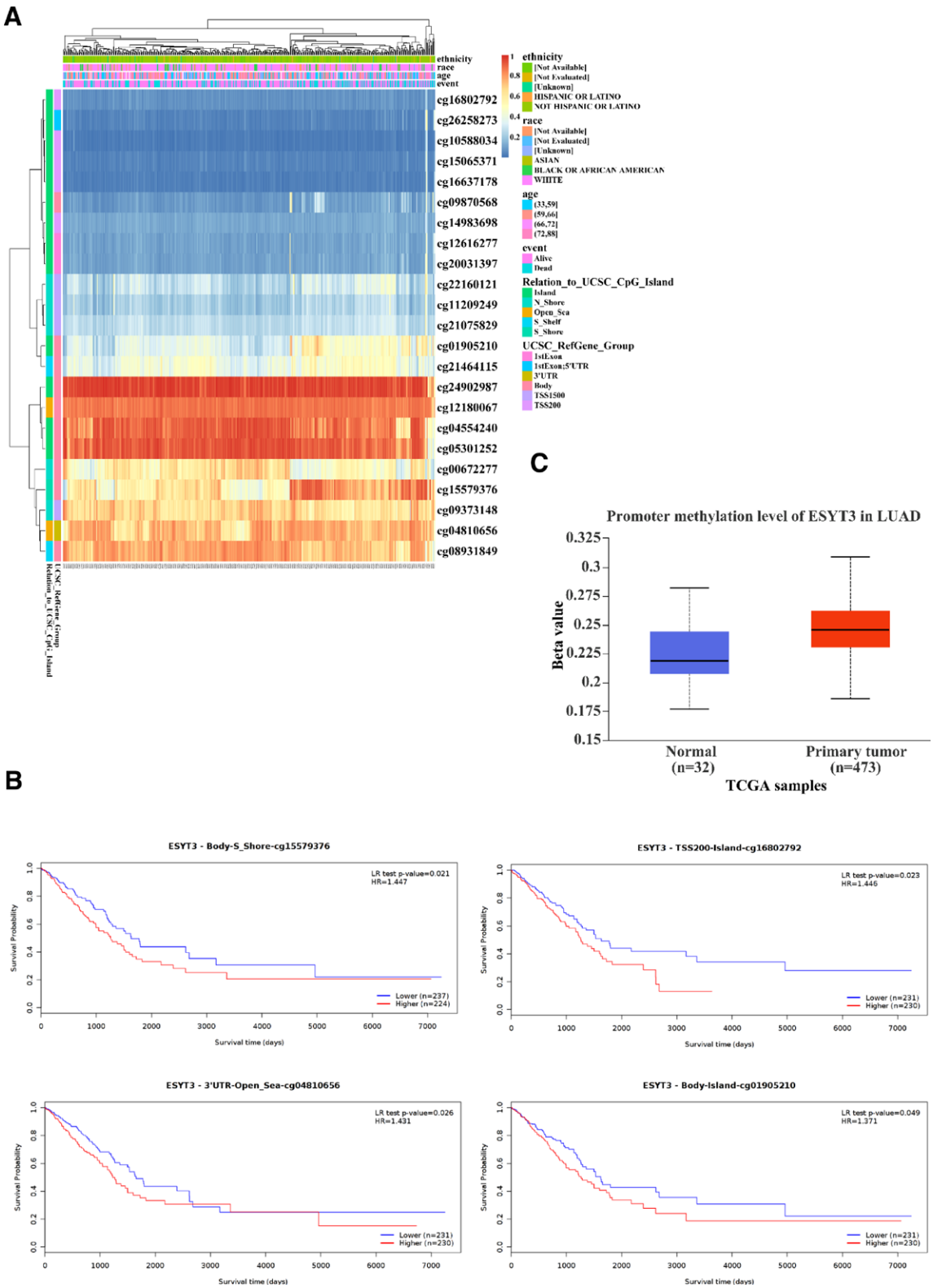

Figure 7. Analysis of ESYT3 methylation in LUAD. (A) Heat map of the methylation level of the ESYT3 gene. The correlations between ESYT3 methylation and its expression were examined. (B) Survival analysis based on methylation at multiple sites. (C) The promoter methylation level of ESYT3 DNA was analyzed in LUAD and normal lung tissue. LUAD = lung adenocarcinoma.

individuals.[25] Therefore, we speculate that DNA methylation may play an important role on the gene expression of ESYT3 in LUAD. And whether demethylation is an effective therapeutic strategy for LUAD patients with low ESYT3 expression still needs more experiments to prove. It is therefore possible that demethylation treatment may be an effective therapeutic strategy for LUAD patients with low ESYT3 expression. However, our current study on ESYT3 still has some limitations. For example, this study used data from only a few databases and did not use clinical samples for validation. In addition, this

study focused only on the expression, diagnosis, and prognosis of ESYT3, and the mechanism of ESYT3 in the progression of LUAD requires further research.

6. Conclusion

In LUAD, ESYT3 can be markedly downregulated and associated with prognosis and the tumor immune microenvironment. In addition, our results demonstrate the importance of epigenetics in downregulating the expression of ESYT3 through its promoter hypermethylation in LUAD. Therefore, we revealed ESYT3 might serve as a potential prognostic marker and promising therapeutic target in LUAD. But further experiments are still needed to uncover the pathogenesis of ESYT3 in the LUAD following this pilot study.

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Author contributions

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