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Research article

Mitochondrial ribosomal protein S24 is associated with immunosuppressive microenvironment and cold tumor in lung adenocarcinoma

Yanni Gao [a,1], Yilin Yu [b,1], Haixia Wu [c,1], Zhenzhou Xiao [a,*], Jiancheng Li [b,**]

[a] Department of Clinical Laboratory, Clinical Oncology School of Fujian Medical University, Fujian Cancer Hospital, Fuzhou, China
[b] Department of Radiation Oncology, Clinical Oncology School of Fujian Medical University, Fujian Cancer Hospital, Fuzhou, China
[c] Shengli Clinical Medical College of Fujian Medical University, Fuzhou, Fujian, China

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ABSTRACT

Objective: *MRPS24* (Mitochondrial Ribosomal Protein S24) belongs to the mitochondrial ribosomal protein family, which participates in the protein synthesis of the mitochondrion. However, the relationship of *MRPS24* with lung adenocarcinoma (LUAD) remained unknown. We aimed to identify its immunological and functional mechanisms in LUAD.

Methods: The analysis of *MRPS24* expression, clinical features, diagnosis, prognosis, function analysis, genetic alteration, copy number variations, methylation, and tumor microenvironment was investigated by the TCGA, UCSC Xena, GEO, HPA, GEPIA, cBioPortal, MethSurv, TIMER, TIMER2.0, and TISIDB databases.

Results: *MRPS24* was found to be more abundant in LUAD tumor tissue than in normal tissue. High levels of *MRPS24* expression were found to be an independent prognostic factor by multivariate analysis. Functional analysis revealed that *MRPS24* expression was associated with the immune, cell cycle and methylation. *MRPS24* methylation level was inversely linked with its expression ($p < 0.001$). Patients with low *MRPS24* methylation had a worse prognosis than those with high methylation ($p < 0.05$). In addition, the result revealed that the *MRPS24* expression was inversely linked to the immune cell infiltration in LUAD. Finally, the validations of the expression level, prognosis, and immune cell infiltration of *MRPS24* were in accordance with our previous results.

Conclusions: This study systematically explored that *MRPS24* expression was significantly correlated with prognosis, tumorigenesis, genetic alteration, copy number variations, methylation, and immune cell infiltration in LUAD. *MRPS24* might be a potential immune-related biomarker in the development and treatment of LUAD, thereby acting as a promising predictor of immunotherapy response in LUAD.

* Corresponding author.
** Corresponding author.
E-mail addresses: zhenzhouxiao@yeah.net (Z. Xiao), jianchengli_jack@fjmu.edu.cn (J. Li).
[1] Yanni Gao, Yilin Yu, and Haixia Wu contributed equally as first authors to this manuscript.

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1. Introduction

Cancer is now the leading cause of death across the globe, making it an important concern for public health. Lung cancer is a tumor with a high rate of mortality and morbidity [1]. The most common histological subtype of lung cancer is lung adenocarcinoma (LUAD). In recent years, the 5-year survival rate of LUAD has been around 20% [2]. In the last ten years, many medical technology advancements have significantly improved the therapeutic methods for LUAD [3]. The early symptoms of LUAD are mild and patients are often diagnosed at an advanced stage, thus missing the best time for therapy [4]. At this point, the role of immunotherapy in such patients is very important and has been demonstrated in several studies [5–7]. Despite the important promise of immunotherapy in patients with LUAD, clinical outcomes and prognosis have been disappointing. There is evidence from some studies that not all patients respond well to immunotherapy [8]. Different responses to immunotherapy among patients may be explained by variations in tumor-infiltrating immune cells and tumor mutational load [9]. Therefore, in order to accurately accomplish individualized decision making for immunotherapy, there is a need to find some prognostic biomarkers to assess the prognosis of LUAD patients and to predict the sensitivity of immunotherapy.

Recent studies have shown that the tumor microenvironment (TME) plays a key role in the occurrence and development of tumors. Despite the rapid development of immunotherapy, the mechanisms regulating immune resistance in LUAD remain to be elucidated due to the limited response to these therapies, emphasizing the importance of identifying novel key markers in LUAD and their relationship to immunity. Mitochondrial Ribosomal Protein S24 (*MRPS24*) is a protein coding gene. Mitochondrial ribosomal proteins in mammalian cells are encoded by nuclear genes and contribute to the process of protein synthesis within the mitochondria. Mitoribosomes, also known as mitochondrial ribosomes, are made up of two subunits: a small 28S subunit and a large 39S subunit. Mitochondrial ribosomes have an approximated 75% protein to rRNA composition compared with prokaryotic ribosomes, where this ratio is opposite. Another difference between prokaryotic ribosomes and mammalian mitoribosomes is that the former contains a 5S rRNA. The proteins that make up the mitoribosomes vary greatly between species in terms of their sequence and occasionally their biochemical characteristics, making it difficult to identify them by sequence homology. This gene encodes a 28S subunit protein. On chromosome 11, a pseudogene that corresponds to this gene is found. There is read-through transcription that occurs between this gene and the upstream gene that controls the upregulation of cell proliferation. (https://www.ncbi.nlm.nih.gov/gene/64951). Nonetheless, the molecular mechanism of *MRPS24* in tumors remains obscure. Furthermore, there have been no studies reported on the relationship between *MRPS24* and immunity to LUAD so far. This is the first study to demonstrate *MRPS24* in LUAD with respect to immunological and functional mechanisms.

This study aimed to explore the prognostic significance, functional mechanisms and immunological function of *MRPS24* in LUAD. We analyzed the expression of *MRPS24* from The Cancer Genome Atlas (TCGA) and various public databases. To further investigate the potential roles of *MRPS24*, the analyses of biological functions and pathways were performed using gene set enrichment analysis (GSEA). In addition, the relationship between *MRPS24* and genetic alteration, copy number variations (CNVs), methylation, and single-sample Gene Set Enrichment Analysis was carried out. Finally, we analyzed the probable correlation between *MRPS24* and tumor-infiltrating immune cells by database and validated by various databases. Our findings provide a new therapeutic strategy for immunotherapy of LUAD, that is, by targeting *MRPS24* might be used to predict the effect of immunotherapy in LUAD.

2. Materials and methods

2.1. Data acquisition

Both TCGA and Gene Expression Omnibus (GEO) datasets were used in our analysis. The transcriptional profiles of *MRPS24* in normal/tumors tissues and clinicopathological results derived from the UCSC Xena website (https://xenabrowser.net/datapages/). Cases with insufficient or missing data were discarded from further processing. GSE11969 and GSE13213 databases were analyzed to verify the prognostic analysis. All of the information used in the study complied with the database's publication standards. Ethical review board approval and written consent were unnecessary in this study.

2.2. Over-expression of MRPS24 in LUAD patients

The receiver operating characteristic (ROC) curve was carried out to investigate the *MRPS24* diagnostics value in LUAD using the pROC package. Besides, we explored the protein expression level of *MRPS24* in LUAD using the Human Protein Atlas database [10]. Immunohistochemistry staining of *MRPS24* was performed using the antibody HPA073947. TIMER database provided the pan-cancer RNA-seq data for *MRPS24* [11,12].

2.3. Validation of the expression level and prognosis of MRPS24

The GEPIA online tool was performed to verify the *MRPS24* expression levels in LUAD [13]. Furthermore, the TISIDB database was explored to validate the correlation between *MRPS24* expression, tumor stage, and prognosis in human tumors [14].

2.4. Construction and evaluation of the nomogram and prognostic model

A nomogram was generated using the rms R package. The Hmisc R package was used to construct the C-index and calibration curve.

2.5. Functional enrichment analysis

Differentially expressed genes were identified by comparing expression profiles between high and low *MRPS24* expression groups using the DESeq2 R package. In order to clarify the significant Gene ontology (GO) function differences between the groups with low and high *MRPS24*, GSEA [15,16] was performed using the R package clusterProfiler. Additionally, GSEA Kyoto Encyclopedia of Genes and Genomes (KEGG) was performed to reveal the statistically significant pathway difference using the Molecular Signatures Database Collection (c2.all.v7.0.entrez.gmt). Significant enrichment was defined as a normalized enrichment score ($|NES|) > 1$, a false discovery rate (FDR) < 0.25, and an adjusted p-value <0.001.

2.6. Analysis of MRPS24 genetic alteration, copy number variations, methylation, and prognosis

The cBioPortal web platform was utilized in order to retrieve the information regarding the genetic alteration of *MRPS24* [17]. The mutation alterations included deep/shallow deletion, diploid, gain, and amplification. With a z-score threshold of ± 1.4, we examined the genomic profiles of *MRPS24* in the study. To determine the prognostic value of *MRPS24*, genetic alteration and their correlation

Table 1
Clinicopathological characteristics of patients with LUAD from TCGA.

Clinical characteristics		Total (497)	Percentage (%)
Gender			
	male	228	45.9
	female	269	54.1
Age			
	≤70 years old	327	65.8
	>70 years old	160	32.2
Number pack years smoked			
	<40	167	33.6
	≥40	174	35
Race			
	white	384	77.3
	other	113	22.7
Tumor site			
	upper lobe	291	58.6
	other	206	41.4
EGFR status			
	mut	79	15.9
	wt	190	38.2
ALK status			
	mut	33	6.6
	wt	206	41.4
KRAS status			
	mut	61	12.3
	wt	244	49.1
T stage			
	T1	166	33.4
	T2	267	53.7
	T3	43	8.7
	T4	18	3.6
N stage			
	N0	321	64.6
	N1	94	18.9
	N2	69	13.9
	N3	2	0.4
M stage			
	M0	331	66.6
	M1	24	4.8
TNM stage			
	stageI	267	53.7
	stageII	118	23.7
	stageIII	80	16.1
	stageIV	25	5
Vital status			
	dead	180	36.2
	alive	317	63.8
MRPS24 expression			
	low	249	50.1
	high	248	49.9

LUAD, lung adenocarcinoma; TCGA, The Cancer Genome Atlas; EGFR, epithelial growth factor receptor; Mut, mutation type; Wt, wild type; ALK, anaplastic lymphoma kinase; KRAS, kirsten rat sarcoma viral oncogene; *MRPS24*, Mitochondrial Ribosomal Protein S24.

with prognosis were studied. In groups with different *MRPS24* copy number variations, the various *MRPS24* gene expressions were compared. An investigation into the relationship between the amount of methylation of the *MRPS24* gene and its expression was carried out. The MethSurv web platform was utilized in order to perform an analysis of the prognostic value of the *MRPS24* methylation level in LUAD [18].

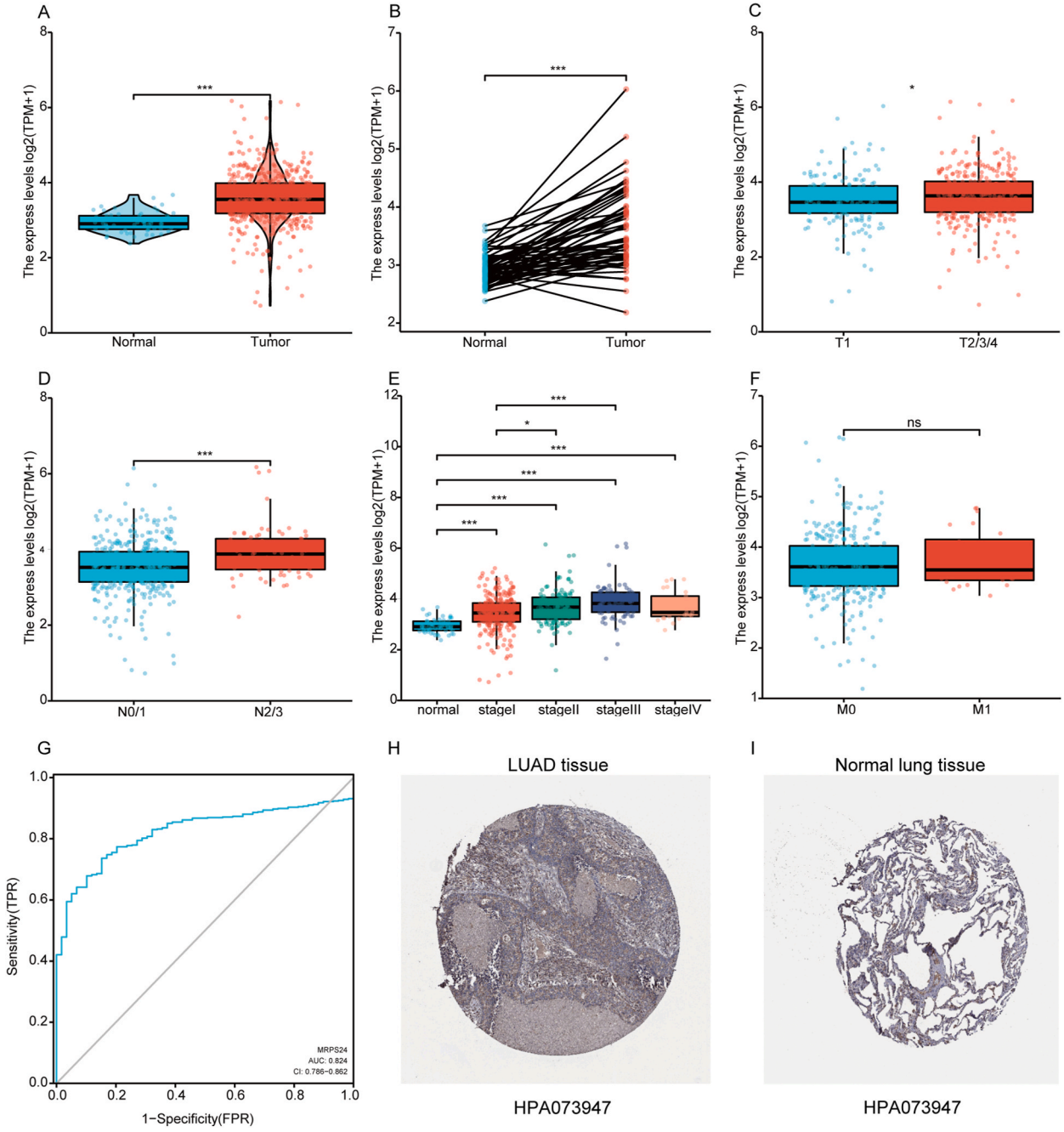

Fig. 1. Expression of *MRPS24* in LUAD and other human cancers based on data from TCGA.(A) *MRPS24* expression levels in LUAD tissue and normal tissue; (B) *MRPS24* expression levels in LUAD tissue and its paired adjacent tissue; (C) The relationship between the T stage and *MRPS24* expression in LUAD; (D) The relationship between the N stage and *MRPS24* expression in LUAD; (E) The relationship between the pathologic stage and *MRPS24* expression in LUAD; (F) The relationship between the M stage and *MRPS24* expression in LUAD; (G) Analysis of the *MRPS24*'s receiver operating characteristics (ROC) in LUAD; (H–I) The protein expression of *MRPS24* in LUAD.

2.7. Single-cell functional analysis of MRPS24

We examined the functional status of *MRPS24* in LUAD and other cancer types using CancerSEA. With 14 tumor-related cellular functions of 900 cancer cells from 25 cancers, the CancerSEA is a tool for analyzing the cancer cell functions at the single-cell level [19]. As a result, the CancerSEA was conducted to investigate the functional relationship of the *MRPS24* with LUAD. A p-value of less than 0.05 and a correlation of great than 0.15 were used as filtering criteria for the correlation between *MRPS24* and the functional state of distinct single-cell datasets.

2.8. Analysis of immune infiltration and its correlation with MRPS24 expression

The single-sample Gene Set Enrichment Analysis (ssGSEA) method from the GSVA package was used to analyze the immune infiltration in order to show the relationship between *MRPS24* and the levels of immune cell infiltration. We downloaded the immune data set from the web [20,21]. Additionally, the TIMER web and TIMER2.0 web was used to investigate the relationship between the *MRPS24* expression levels and the immune cell infiltration in LUAD [11,12,22]. Finally, the TISIDB database were performed to verified the relationship of the immune cell infiltration levels and *MRPS24* expression.

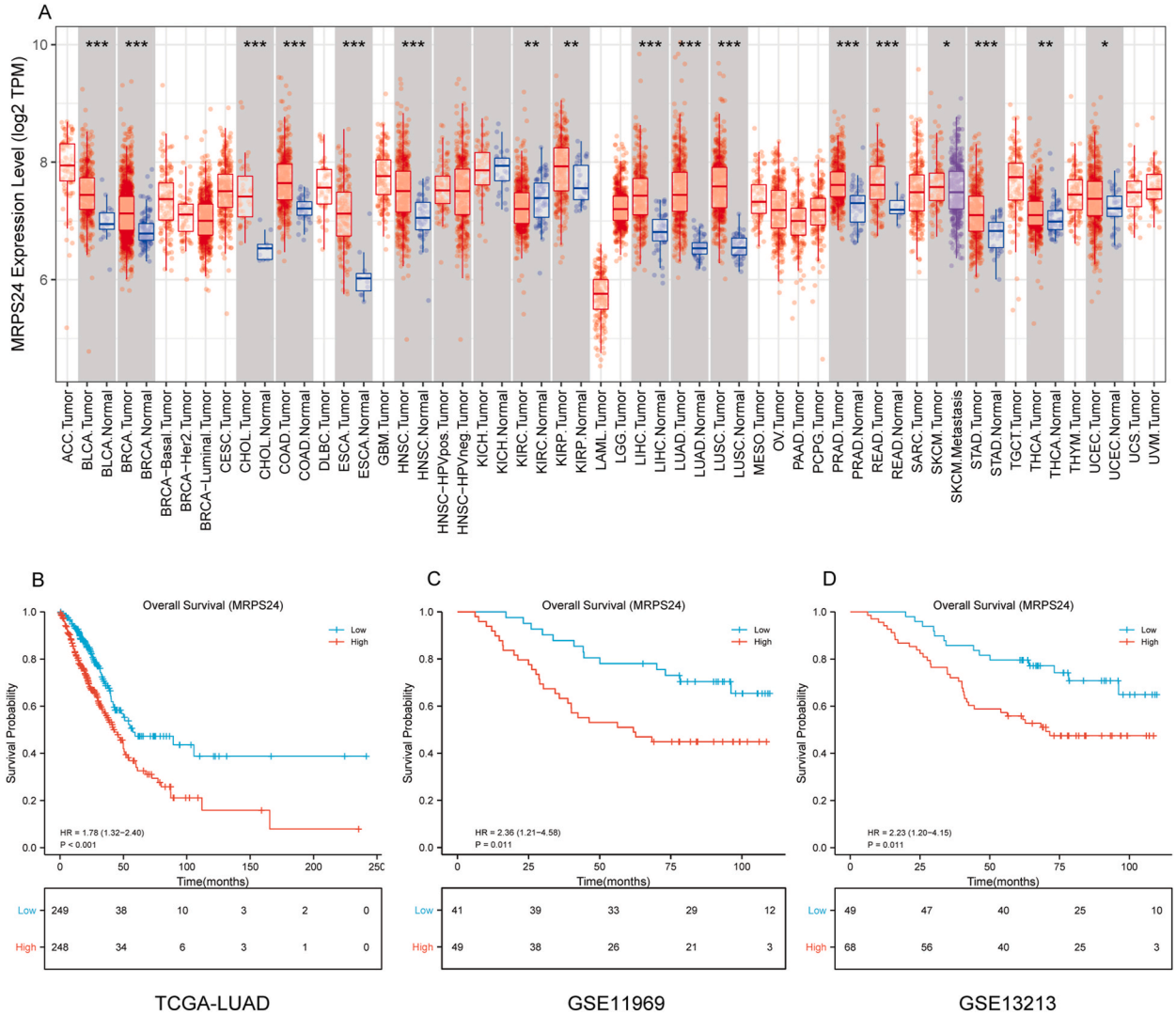

Fig. 2. The TCGA database's data on *MRPS24* expression levels in various tumor types and the prognostic significance of *MRPS24* expression in LUAD. (A) Levels of the *MRPS24* expression in various types of tumors; (B) Curve of survival for OS derived from TCGA data (n = 497); (C) Curves of survival for OS derived from GSE11696 data (n = 90); (D) Curves of survival for OS derived from GSE13213 data (n = 117).

2.9. Statistical analysis

All data were analyzed using SPSS version 22.0 and R version 4.0.2. We have obtained a copyright license of SPSS statistical software. Wilcoxon test was carried out to investigate the correlation between *MRPS24* expression and clinical characteristics, including T stage, N stage, M stage, clinicopathological stage, paired samples, and non-paired samples. The survival analyses were analyzed by the Kaplan-Meier curve and log-rank test. The survival R package and the survminer R package generated the survival curve. The death risk was evaluated using univariate and multivariate Cox regression analyses. The risk factors with $p < 0.05$ were included in the multivariate analysis to identify independent prognostic factors of LUAD. To assess the relationship between the various *MRPS24* expression groups and immune cell infiltration, the Spearman correlation was used. In all statistical analyses, $p < 0.05$ was considered to be statistically significant.

3. Results

3.1. Clinical characteristics

Patients' characteristics were shown in Table 1. The data, sourced from TCGA, comprise gene expression and clinical data from 497 patients. We collected patients' data, encompassing gender, age, number pack years smoked, race, tumor details (site, EGFR, ALK, KRAS status), and staging information (T, N, M, TNM), as well as vital status and *MRPS24* expression. The study included 228 male patients (45.9%) and 269 female patients (54.1%). There were 327 (65.8%) patients aged≤70 years and 160 (32.2%) aged >70 years. The clinical stage was I for 267 patients (53.7%), II for 118 patients (23.7%), III for 80 patients (16.1%), and IV for 25 patients (5.0%). Besides, there were 249 (50.1%) and 248 (49.9%) patients with low and high expression of *MRPS24*, respectively. Finally, a total of 180 patients (36.2%) died, while 317 patients (63.8%) were alive.

3.2. MRPS24 is overexpressed in lung adenocarcinoma

The results revealed that the mRNA expression levels of *MRPS24* were higher than those in normal samples ($p < 0.001$) (Fig. 1A). In addition, *MRPS24* expression in LUAD was significantly higher than that of the adjacent normal tissues of paired specimens ($p < 0.001$) (Fig. 1B). Importantly, the higher expression of *MRPS24* had a positive relation with topography distribution (Fig. 1C, $p < 0.01$), lymph node metastasis (Fig. 1D, $p < 0.001$), pathologic stage (Fig. 1E, $p < 0.05$). On the contrary, there was no difference between *MRPS24* expression and distant metastasis (Fig. 1F, $p > 0.05$). The ROC indicated that the *MRPS24* expression in LUAD was 0.824 (0.786–0.862) (Fig. 1G). We further explore the protein expression of *MRPS24* in LUAD. As shown in Fig. 1H and I, the expression of *MRPS24* was not detected in normal lung tissues, while high protein expression of *MRPS24* was observed in LUAD tissues. In normal lung tissues, the quantity was not detected in HPA073947. In LUAD tissues, the quantity was scored as 75%–25% in HPA073947. In normal lung tissues, the staining intensity was scored as negative for HPA073947. While the staining intensity in the LUAD tissues of HPA073947 was scored as strong. Immunohistochemistry was used to determine the geographic distribution of *MRPS24* in LUAD. The location of *MRPS24* was cytoplasmic/membranous. Finally, we explored *MRPS24* expression using the pan-cancer RNA-seq data from TCGA. The results revealed that the expression levels of *MRPS24* in almost all cancers were higher than those in normal tissues

Table 2
The univariate and multivariate survival analyses in the TCGA database.

Clinicopathologic variable	Total(N)	HR (95% CI)	p-value
a.			
Gender (Male vs. Female)	497	0.954 (0.711–1.279)	0.752
Age (>70 vs.≤70)	487	1.464 (1.081–1.982)	0.014
number pack years smoked (>40 vs.≤40)	341	1.026 (0.714–1.475)	0.888
Race (Other vs. White)	497	1.265 (0.797–2.008)	0.061
Tumor site (Upper lobe vs. Other)	497	1.156 (0.862–1.552)	0.333
EGFR status (Mut vs. Wt)	266	1.265 (0.797–2.008)	0.319
ALK status (Mut vs. Wt)	236	1.713 (0.938–3.128)	0.080
KRAS status (Mut vs. Wt)	302	1.257 (0.778–2.032)	0.351
T stage (T2/T3/T4 vs. T1)	494	1.678 (1.187–2.373)	0.003
N stage (N2/N3 vs. N0/N1)	486	2.274 (1.589–3.255)	<0.001
M stage (M1 vs. M0)	355	2.129 (1.243–3.648)	0.006
Pathologic stage (Stage II/Stage III/Stage IV vs. Stage I)	490	2.629 (1.924–3.591)	<0.001
MRPS24 (High vs. Low)	497	1.781 (1.319–2.403)	<0.001
b.			
Age (>70 vs.≤70)		1.437 (1.053–1.962)	0.022
T stage (T2/T3/T4 vs. T1)		1.322 (0.919–1.900)	0.132
N stage (N2/N3 vs. N0/N1)		1.163 (0.776–1.743)	0.463
Pathologic stage (Stage II/Stage III/Stage IV vs. Stage I)		2.253 (1.575–3.223)	<0.001
MRPS24 (High vs. Low)		1.431 (1.043–1.964)	0.027

TCGA, The Cancer Genome Atlas; HR, hazard ratio; CI, confidence interval; EGFR, epithelial growth factor receptor; Mut, mutation type; Wt, wild type; ALK, anaplastic lymphoma kinase; KRAS, kirsten rat sarcoma viral oncogene; *MRPS24*, Mitochondrial Ribosomal Protein S24.

(Fig. 2A).

3.3. *MRPS24* overexpression predicts a poor overall survival and serves as an independent prognostic factor in LUAD

We chose overall survival (OS) to explore the prognosis of *MRPS24* in LUAD. The results showed that patients with high expression of *MRPS24* had significantly shorter OS durations compared to those with low expression of *MRPS24* in LUAD patients ($p < 0.001$) (Fig. 2B). To further validate the relationship between *MRPS24* expression and prognosis, we explored the GSE11969 and GSE13213 datasets. The results also revealed that high *MRPS24* expression had an unfavorable OS than low *MRPS24* expression in LUAD patients ($p < 0.05$) (Fig. 2C and D). We used univariate and multivariate Cox regression analysis to estimate the relationship between *MRPS24* expression and prognosis in LUAD from TCGA database. Univariate analysis showed that *MRPS24* expression was important prognostic

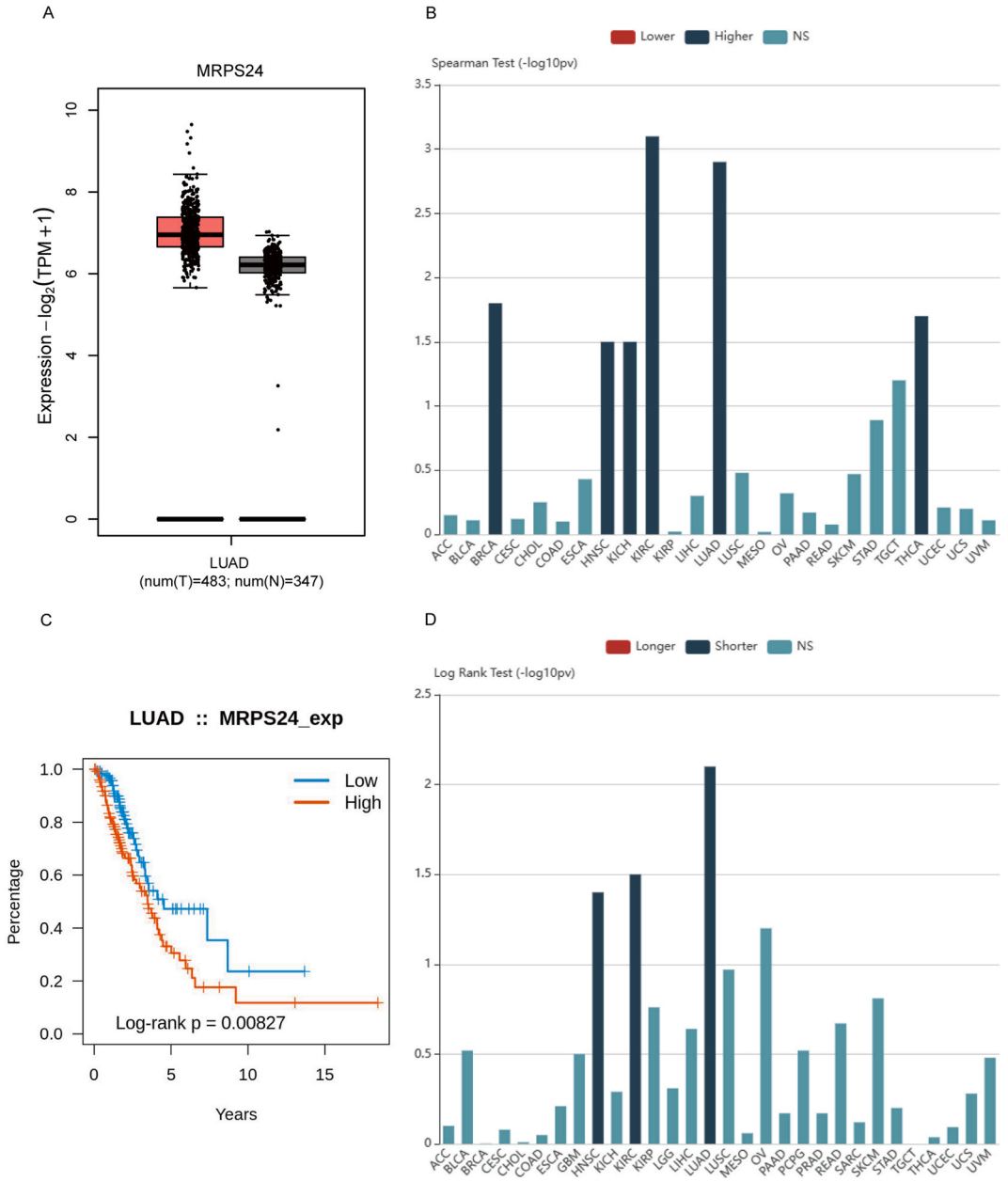

Fig. 3. The validation of the *MRPS24* expression level, tumor stage, and prognosis of *MRPS24*. (A) *MRPS24* expression levels in LUAD tissue and normal tissue (GEPIA database); (B) The correlation between the expression of *MRPS24* and the stage of tumor in various human cancers (TISIDB database); (C) The correlations between *MRPS24* expression and overall survival in LUAD (TISIDB database); (D) The correlations between the expression of *MRPS24* and overall survival in various human cancers (TISIDB database).

factor of OS in LUAD. The M stage was excluded from the multivariate analysis due to missing data in excess of 20%. In addition, *MRPS24* expression were identified as an independent prognostic factor in LUAD by multivariate analysis (Table 2). These results suggested that *MRPS24* expression was a marker for an unfavorable prognosis for LUAD.

3.4. Validation of the expression level and prognosis of MRPS24

We verified that the *MRPS24* gene was highly expressed in LUAD tumor tissues compared to normal tissues ($p < 0.05$) (Fig. 3A). Fig. 3B illustrated that a higher level of *MRPS24* expression is linked to a more advanced stage of the tumor. Finally, the high *MRPS24* expression was significantly linked to a lower overall survival rate in LUAD (Fig. 3C and D).

3.5. Establishment and validation of the prognostic models for LUAD

We constructed a nomogram model based on independent prognostic factors in the multifactorial analysis (Fig. 4A). The C-index was 0.68 of *MRPS24* with 1000 bootstrap resamples for the nomogram. Besides, the calibration curve assessed the performance of the nomogram (Fig. 4B–D). The calibration curve revealed favorable consistency between the observed probability and predicted probability.

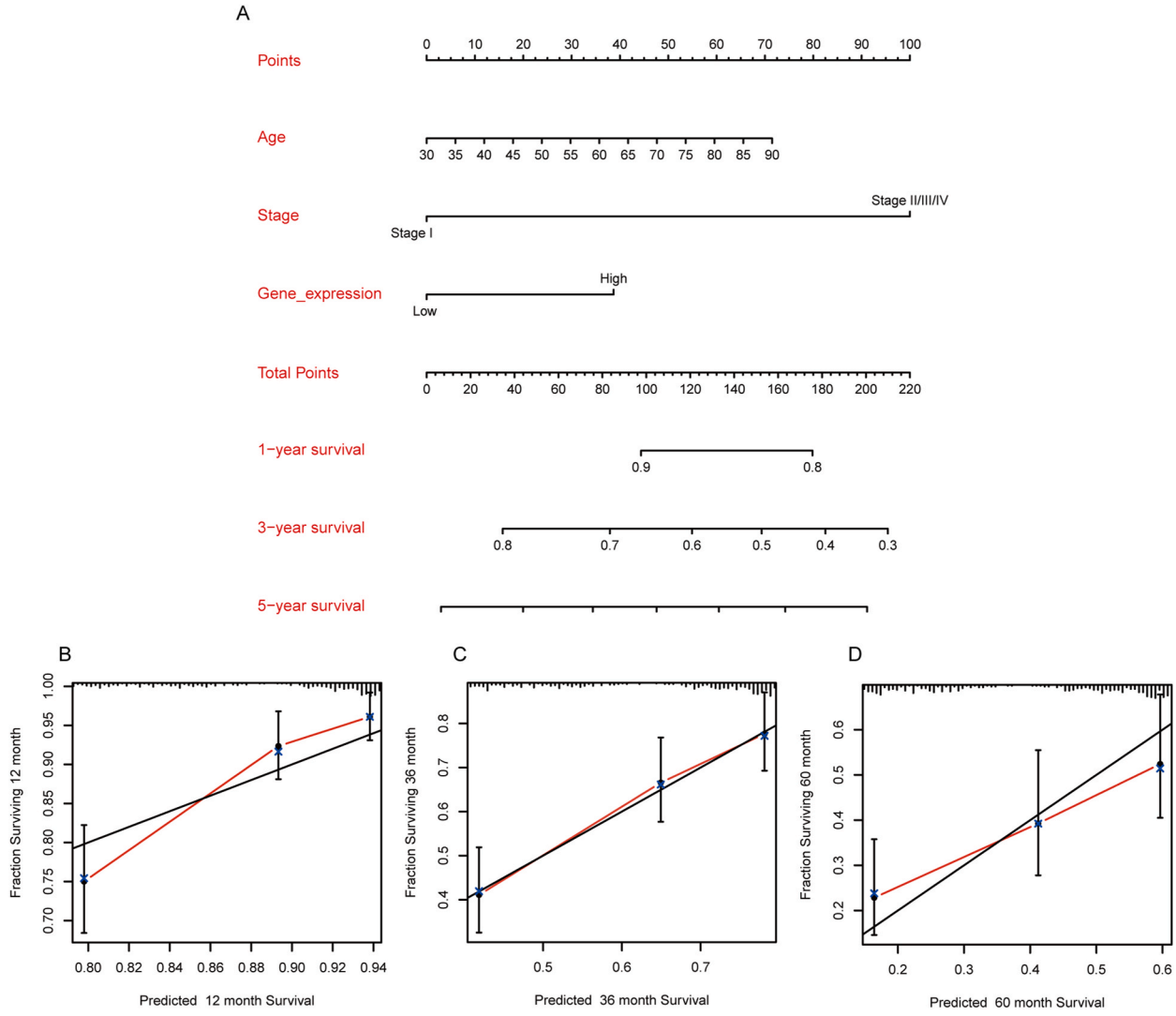

Fig. 4. Nomogram and calibration curve for predicting the probability of 1-, 3- and 5-year OS for LUAD patients. (A) A nomogram based on TCGA data that incorporates *MRPS24* expression and other prognostic factors in LUAD; (B–D) The curve of calibration for the nomogram.

3.6. Functional enrichment analysis of low- and high- MRPS24 expression groups

To further investigate the potential effect of *MRPS24* in tumorigenesis, we carried out GSEA GO and GSEA KEGG analyses in order to determine the essential functions and pathways that are associated with *MRPS24*. Several functional groups were involved with the GSEA GO enrichment items. The GSEA GO analyses revealed that 16 biological processes and 4 cellular components were enriched. The functions of *MRPS24* were mainly involved in the epidermal cell differentiation, intermediate filament, epigenetic regulation of gene expression, gene silencing and nuclear chromatin (Fig. 5A and B). Additionally, the GSEA KEGG analysis indicated that the significantly enriched pathways were mainly involved in proliferation, cell cycle, amplified in lung cancer, DNA methylation, DNA replication, and immune (Fig. 5C and D).

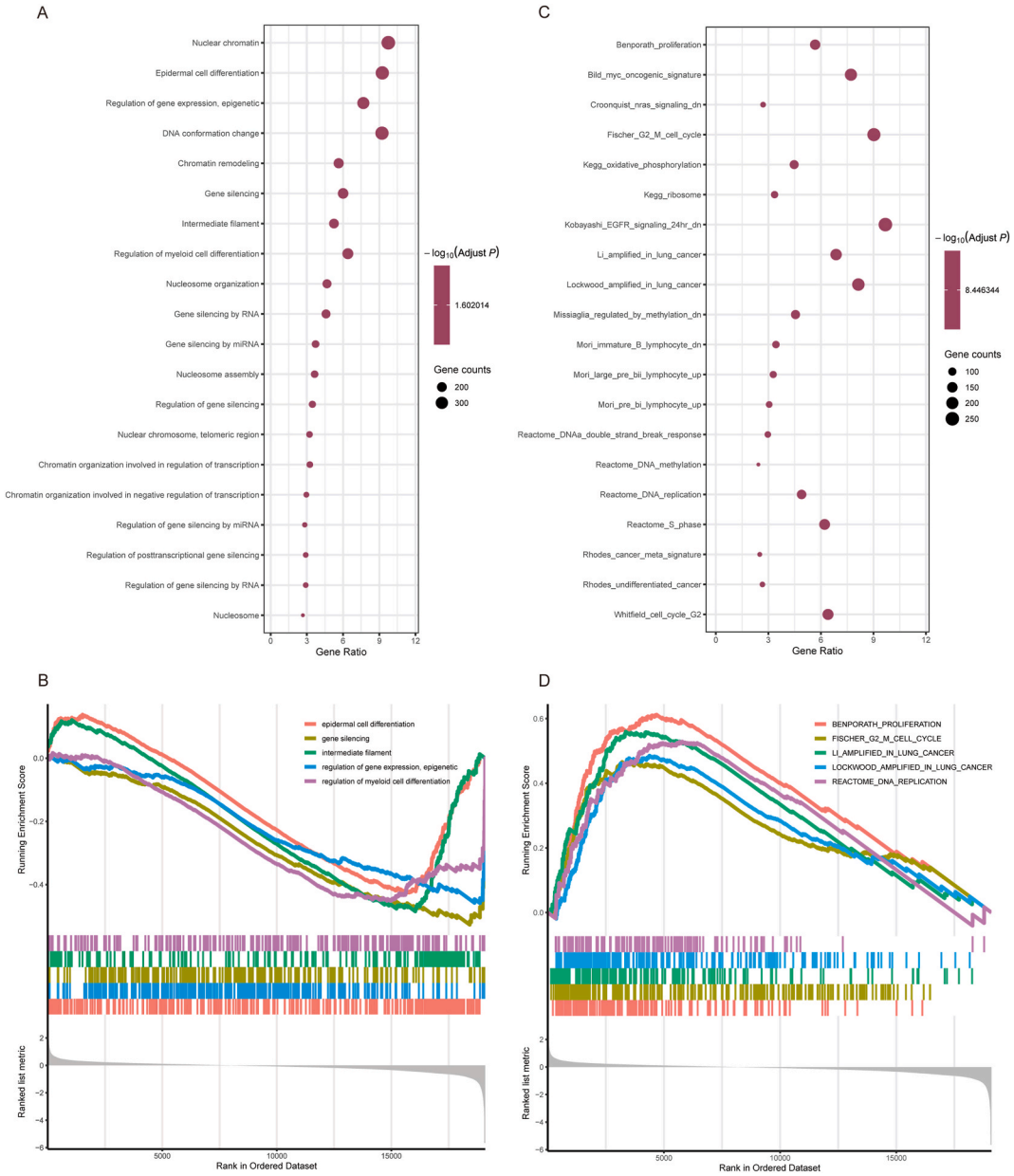

Fig. 5. Functional enrichment analysis of *MRPS24* in LUAD. (A–B) Analysis of differentially expressed genes by GSEA GO enrichment in samples with high and low *MRPS24* expression; (C–D) Analysis of differentially expressed genes by GSEA KEGG enrichment in samples with high and low *MRPS24* expression.

3.7. Analysis of MRPS24 genetic alteration, copy number variations, methylation, and prognosis

We investigated the associations between genetic alteration and prognosis of *MRPS24* in LUAD patients. The results showed a high alteration rate of *MRPS24* was observed in LUAD patients (Fig. 6A). The genetic alteration was found in 111 of the 501 LUAD patients, with an alteration rate of 22%. In addition, the results demonstrated that a genetic alteration in *MRPS24* was linked to a shorter OS in LUAD patients (Fig. 6B). The mRNA expression and CNVs data for *MRPS24* in LUAD were then analyzed using cBioPortal. The level of *MRPS24* expression in LUAD was found to be higher in patients with *MRPS24* amplification of CNVs (Fig. 6C). In light of the findings of the GSEA GO and GSEA KEGG enrichment analyses, which suggested that *MRPS24* may play a role in the process of methylation, we conducted additional research on *MRPS24* methylation as well as *MRPS24* expression. According to the findings, the expression level of

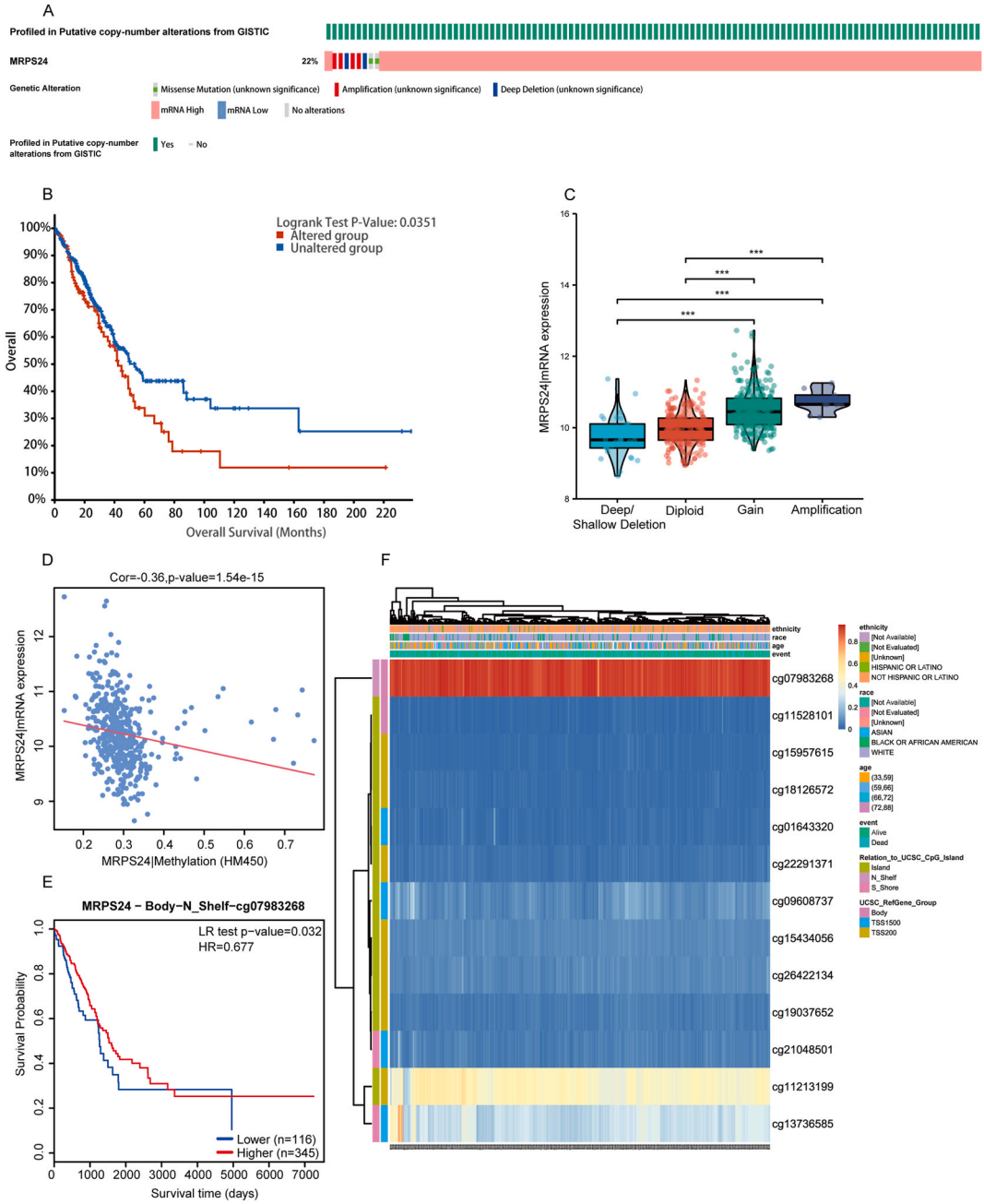

Fig. 6. *MRPS24* gene alteration, copy number variations, and methylation in LUAD. (A–B) *MRPS24* genetic alteration and its relationship to LUAD patient survival; (C) *MRPS24* expression levels in different CNVs group; (D) The relationship between *MRPS24*'s expression level and methylation; (E) Kaplan-Meier curve for the methylation of *MRPS24*; (F) Visualization of the relationship between methylation level and *MRPS24* expression.

MRPS24 had a negative correlation with the level of methylation in LUAD ($r = -0.36$, $p < 0.001$) (Fig. 6D). Moreover, the MethSurv analysis revealed that patients with low *MRPS24* methylation had a worse prognosis than those with high methylation ($p < 0.05$). We found that a CpG island marker, cg07983268, was correlated with an unfavorable outcome (Fig. 6E). Given that *MRPS24* has a low level of methylation (Fig. 6F), the expression of *MRPS24* may be correlated with the hypomethylation level.

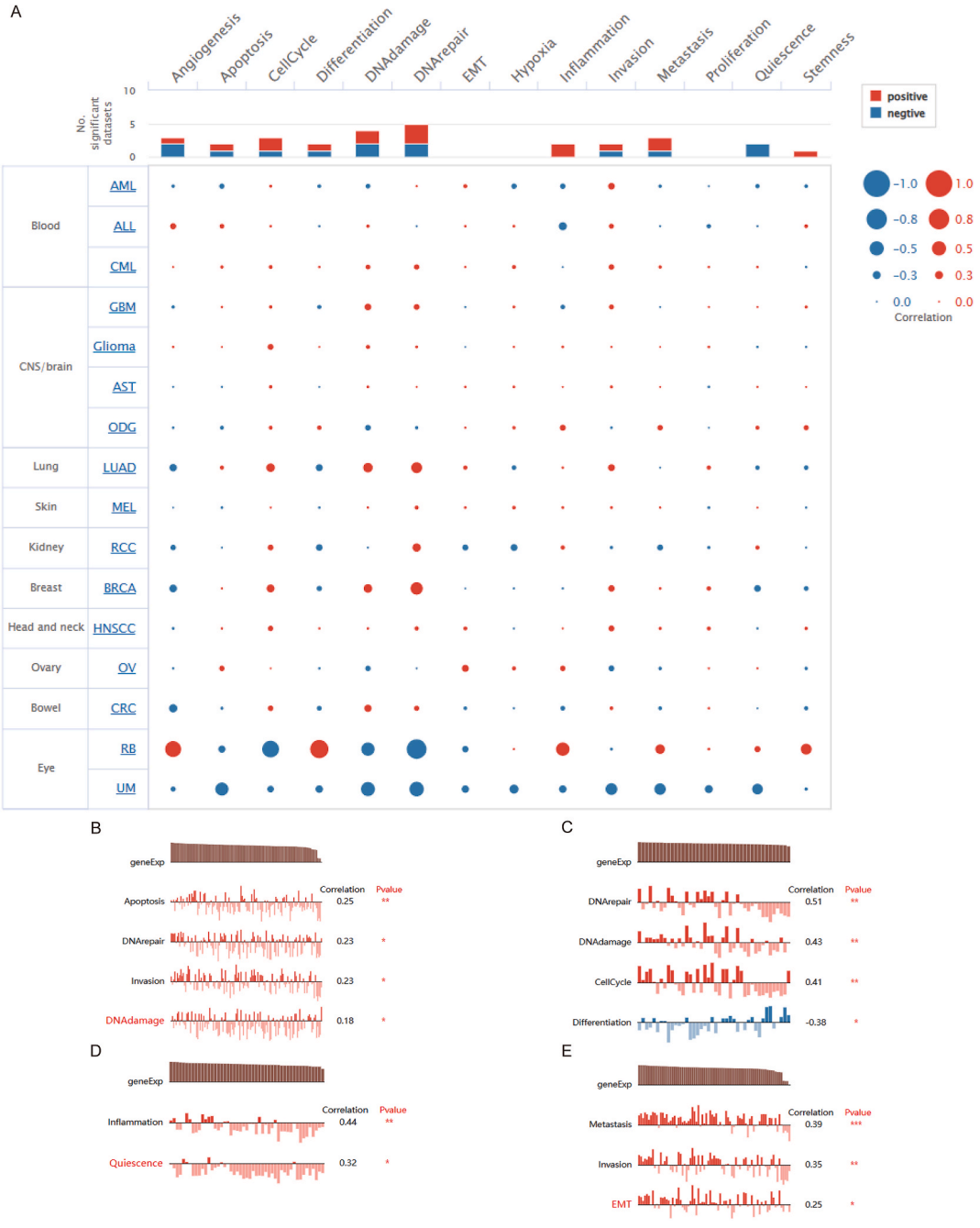

Fig. 7. CancerSEA database analysis of *MRPS24* function state in various human cancers. (A) Functional relevance of *MRPS24* in various cancer types; (B) Details of the functional relevance of *MRPS24* in LUAD (EXP0066); (C) Details of the functional relevance of *MRPS24* in LUAD (EXP0067); (D) Details of the functional relevance of *MRPS24* in lung cancer (PDX, LC-MBT-15); (E) Details of the functional relevance of *MRPS24* in lung cancer (PDX, LC-PT-45).

3.8. Single-cell functional analysis of MRPS24

To further investigate the potential role of *MRPS24* in tumors, we used CancerSEA to explore *MRPS24*'s function at the single-cell level (Fig. 7A–E). The results indicated that *MRPS24* was positively linked with inflammation, metastasis, invasion, apoptosis, DNA damage, DNA repair, and cell cycle of LUAD. Besides, *MRPS24* had a negative relationship with differentiation.

3.9. The correlation between MRPS24 expression and the infiltration of immune cells

Since both functional enrichment analysis and single-cell functional analysis suggested that *MRPS24* is associated with immunity and inflammation, we further applied ssGSEA and TIMER database to explore the relationship between *MRPS24* expression and immune cell infiltration level in LUAD. We discovered that *MRPS24* expression negatively correlated with activated B cell, central memory CD4 T cell, effector memory CD4 T cell, effector memory CD8 T cell, immature B cell, regulatory T cell, T follicular helper cell, Type 1 T helper cell, Type 2 T helper cell, Type 17 T helper cell, activated dendritic cell, eosinophil, immature dendritic cell, macrophage, mast cell, Natural killer cell, and plasmacytoid dendritic cell (all $p < 0.05$) (Table 3). Moreover, the results by TIMER revealed that the expression level of *MRPS24* was negatively linked with the infiltration of B cell ($r = -0.22$, $p < 0.001$), $CD8^+$ T cell ($r = -0.151$, $p < 0.001$), $CD4^+$ T cell (r = -0.225, $p < 0.001$), macrophage ($r = -0.264$, $p < 0.001$), neutrophil ($r = -0.188$, $p < 0.001$), and dendritic cell ($r = -0.173$, $p < 0.001$) (Fig. 8A–F). The results analyzed by CIBERSORT-ABS, EPIC, MCP-COUNTER, QUANTISEQ, and x-Cell of TIMER2.0 database also demonstrated that the *MRPS24* expression was inversely linked to the immune cell infiltration in LUAD (Fig. 9A-T and Fig. 10A-P). Finally, we also applied TISIDB database to validate the relationship of the immune cell infiltration levels and *MRPS24* expression. The results revealed that the *MRPS24* expression was inversely linked to the infiltration of the immune cell in LUAD, which were the same as our previous results (Fig. 11A-T). In summary, the result indicated that the *MRPS24* expression was associated with cold tumors. The association between *MRPS24* expression and cold tumors was shown in Fig. 12. The figure was drawn by Generic Diagramming Platform (https://gdp.renlab.cn/#/).

4. Discussion

Cancer development is a multi-step process that involves numerous signaling pathways and genes. The signaling network that regulates pathogenesis is still not fully understood. Targeting the protein kinases activity as part of a therapeutic approach to fight LUAD is made possible by the presence of driver mutations in genes. Nonetheless, the prognosis for LUAD patients who lack these driver mutations is generally dismal. As a result, immunotherapy has been suggested as a current treatment option for LUAD without driver mutations. Immunotherapy advancements have resulted in significant changes in the epidemiology, treatment, and prevention of lung cancer over the past few decades. Contrary to conventional therapies, immunotherapy patients benefit from durable antitumor immune responses that are dependent on immunomodulation between the tumor microenvironment and cancer cells [23]. Tumor-infiltrating immune cells are prominent factors that maintain the balance of the TME, thereby significantly influencing cancer development and prognosis. Before treatment, the immune microenvironment of the tumor can be classified into three immune phenotypes, named immune excluded, immune inflamed, or immune desert, depending on the degree to which it responds to immunotherapy. Immunotherapy is ineffective for the majority of tumor patients with immune desert and immune excluded types, highlighting the significance of the immune microenvironment in tumor development and treatment [24,25]. Hence, not all patients will benefit from immunotherapy. To better identify patients at risk for a poor immune response, it is necessary to gain insight into the infiltration and distribution characteristics of immune cells in the immune microenvironment of LUAD patients. Consequently, it is

Table 3
The relationship between *MRPS24* expression levels and the infiltration level of immune cells in the tumor microenvironment.

Immune cell	Correlation coefficient (r)	p-value
Activated B cell	−0.272	<0.001
Central memory CD4 T cell	−0.143	0.001
Effector memory CD4 T cell	−0.185	<0.001
Effector memory CD8 T cell	−0.144	0.001
Immature B cell	−0.297	<0.001
Regulatory T cell	−0.101	0.021
T follicular helper cell	−0.114	0.009
Type 1 T helper cell	−0.170	<0.001
Type 17 T helper cell	−0.113	0.010
Type 2 T helper cell	−0.122	0.005
Activated dendritic cell	−0.087	0.048
Eosinophil	−0.291	<0.001
Immature dendritic cell	−0.149	0.001
Macrophage	−0.107	0.014
Mast cell	−0.212	<0.001
Natural killer cell	−0.200	<0.001
Plasmacytoid dendritic cell	−0.253	<0.001

MRPS24, Mitochondrial Ribosomal Protein S24.

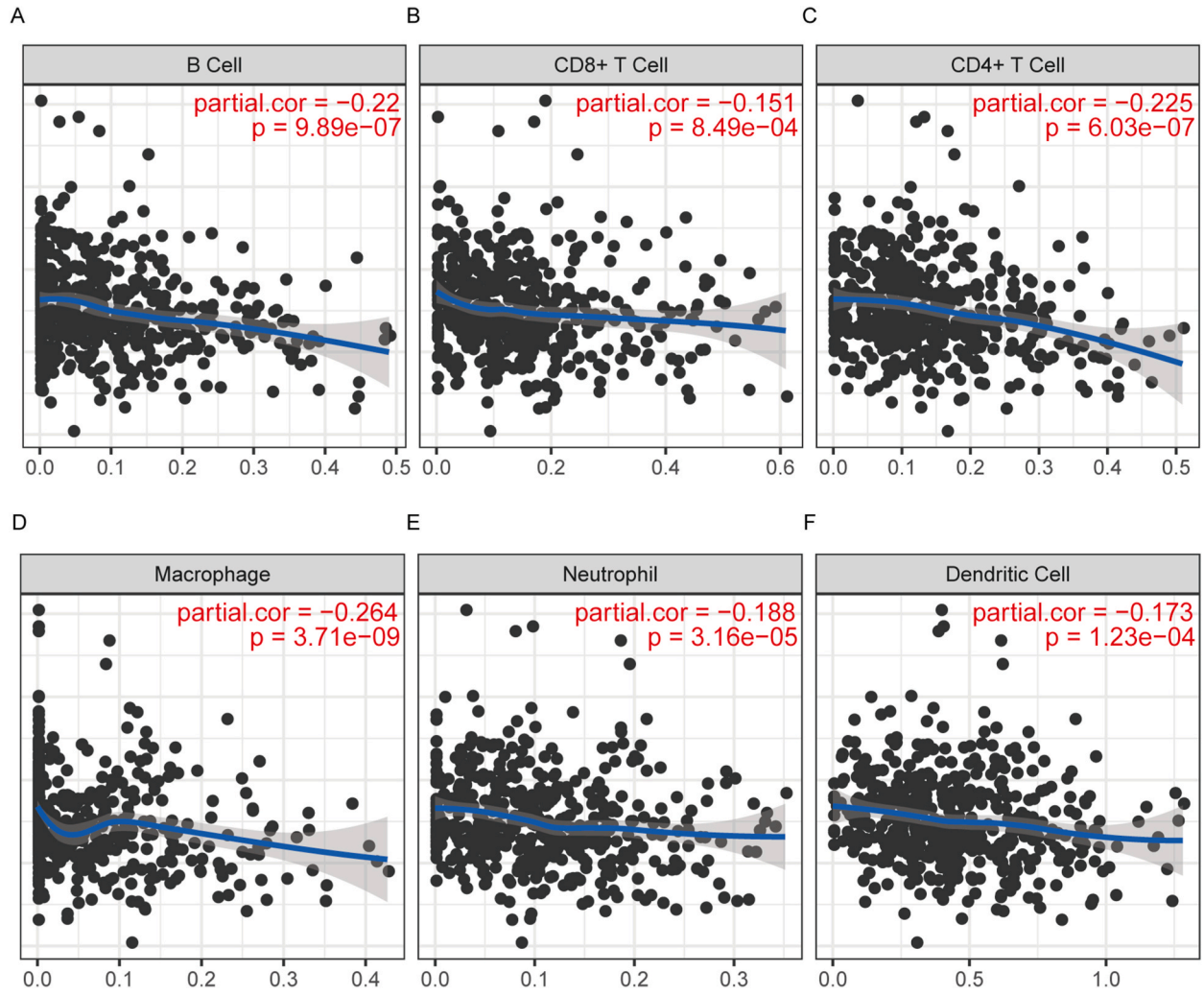

Fig. 8. Relationship between *MRPS24* and the infiltration of immune cells in LUAD. (A–F) The expression of *MRPS24* had a significant inverse correlation with the infiltration of B cell, $CD8^+$ T cell, $CD4^+$ T cell, macrophage, neutrophil, and dendritic cell by Timer database.

urgent to identify useful biomarkers to predict the efficacy of immunotherapy.

Currently, searching for LUAD markers through bioinformatics is a common practice. Efficient markers can be screened by combining high-throughput omics data with patient's clinical data. As a result, they can be utilized as credible indicators. In this study, we focus on *MRPS24* to explore its role in LUAD after rigorous data analysis. Through the analysis of multi-database data from LUAD samples, we found that the mRNA and protein expression level of *MRPS24* in the tumor tissues is higher than that in nontumor tissues. *MRPS24* was also highly expressed in pan-cancer. The increased expression level of *MRPS24* was linked with advanced stage and poor outcome in LUAD. In addition, *MRPS24* was an independent prognostic factor in LUAD based on the univariate and multivariate analyses. Clinical data and the expression of *MRPS24* were then included in a prognostic nomogram that could be used to more accurately identify patients who are at high risk. A worse clinical outcome was associated with a higher nomogram score. Accordingly, we speculated that *MRPS24* might serve as a promising prognostic biomarker in the treatment of LUAD patients.

At present, there is no available report on the functional enrichment analysis of *MRPS24* in LUAD. The GSEA GO and GSEA KEGG analyses were performed to deeply explore the potential functions of *MRPS24*. The functions of *MRPS24* were mainly involved in the epidermal cell differentiation, intermediate filament, epigenetic regulation of gene expression, gene silencing and nuclear chromatin. Additionally, the GSEA KEGG pathway analysis indicated that the *MRPS24* was mainly related to proliferation, cell cycle, amplified in lung cancer, DNA methylation, DNA replication, and immune. These findings suggested that *MRPS24* overexpression may be linked to the occurrence and progression of LUAD. We further used some databases from cBioportal, MethSurv, and CancerSEA to explore the molecular characteristics of *MRPS24* in LUAD, including gene expression, prognosis, gene alterations, DNA methylation, and functional analyses to clarify its potential regulatory pathways and function in the development of LUAD. We discovered that patients with *MRPS24* amplification of CNVs had higher levels of *MRPS24* expression in LUAD. It suggested that CNVs may be the cause of *MRPS24*'s elevated expression. Cancer is a clonal process at the genetic level, and when mutations accumulate in somatic cells, they lead to

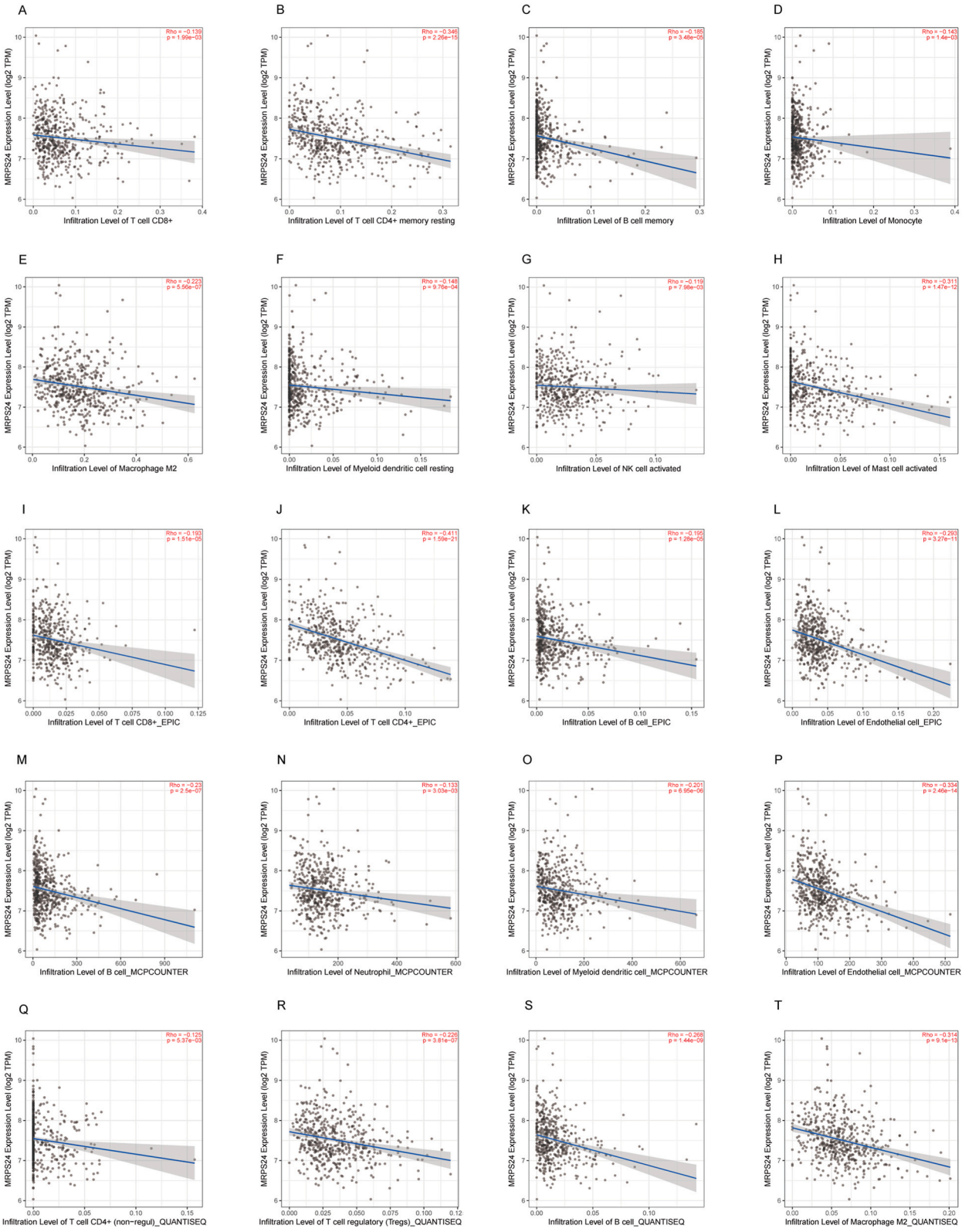

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Fig. 9. The validation of the correlation between the expression of *MRPS24* and the immune cell infiltration in patients with LUAD. (A–H) The correlation between *MRPS24* expression and immune cell infiltration levels in patients with LUAD (CIBERSORT-ABS tool); (I–L) The correlation between *MRPS24* expression and immune cell infiltration levels in patients with LUAD (EPIC tool); (M–P) The correlation between *MRPS24* expression and immune cell infiltration levels in patients with LUAD (MCP-COUNTER tool); (Q–T) The correlation between *MRPS24* expression and immune cell infiltration levels in patients with LUAD (QUANTISEQ tool).

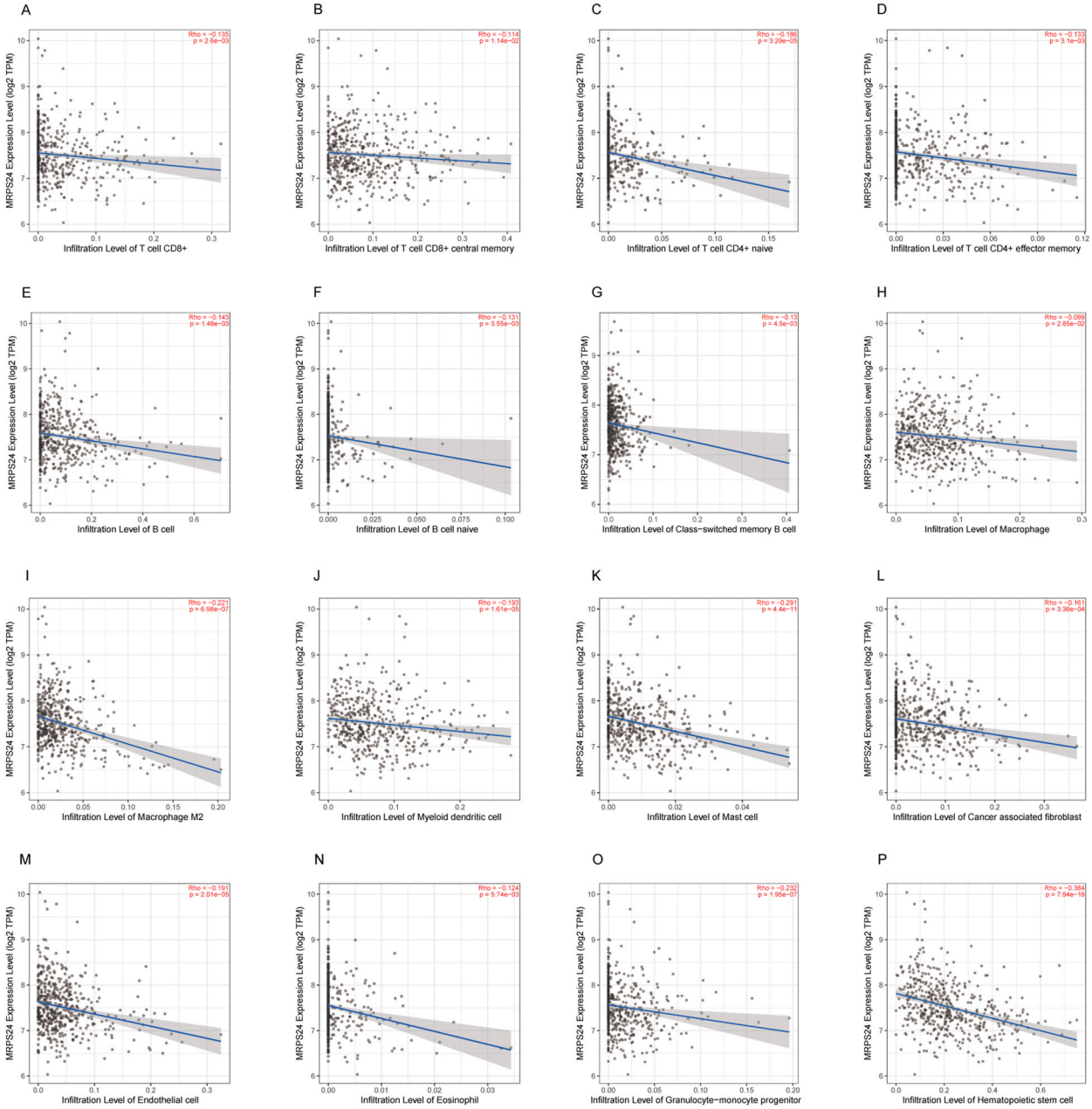

Fig. 10. The validation of the correlation between the expression of *MRPS24* and the immune cell infiltration in patients with LUAD. (A–P) The correlation between *MRPS24* expression and immune cell infiltration levels in patients with LUAD (xCell tool).

abnormal growth of normal cells. Furthermore, *MRPS24* promoter methylation is lower in LUAD than in normal tissue, and *MRPS24* expression is negatively correlated with methylation. It is interesting that *MRPS24* methylation was found to be associated with the prognosis of LUAD. Hypomethylated patients have a poorer overall survival, which is consistent with the fact that the expression of this gene has prognostic value. In the present study, we identified 1 CpG sites of *MRPS24* which was correlated with prognosis. Through

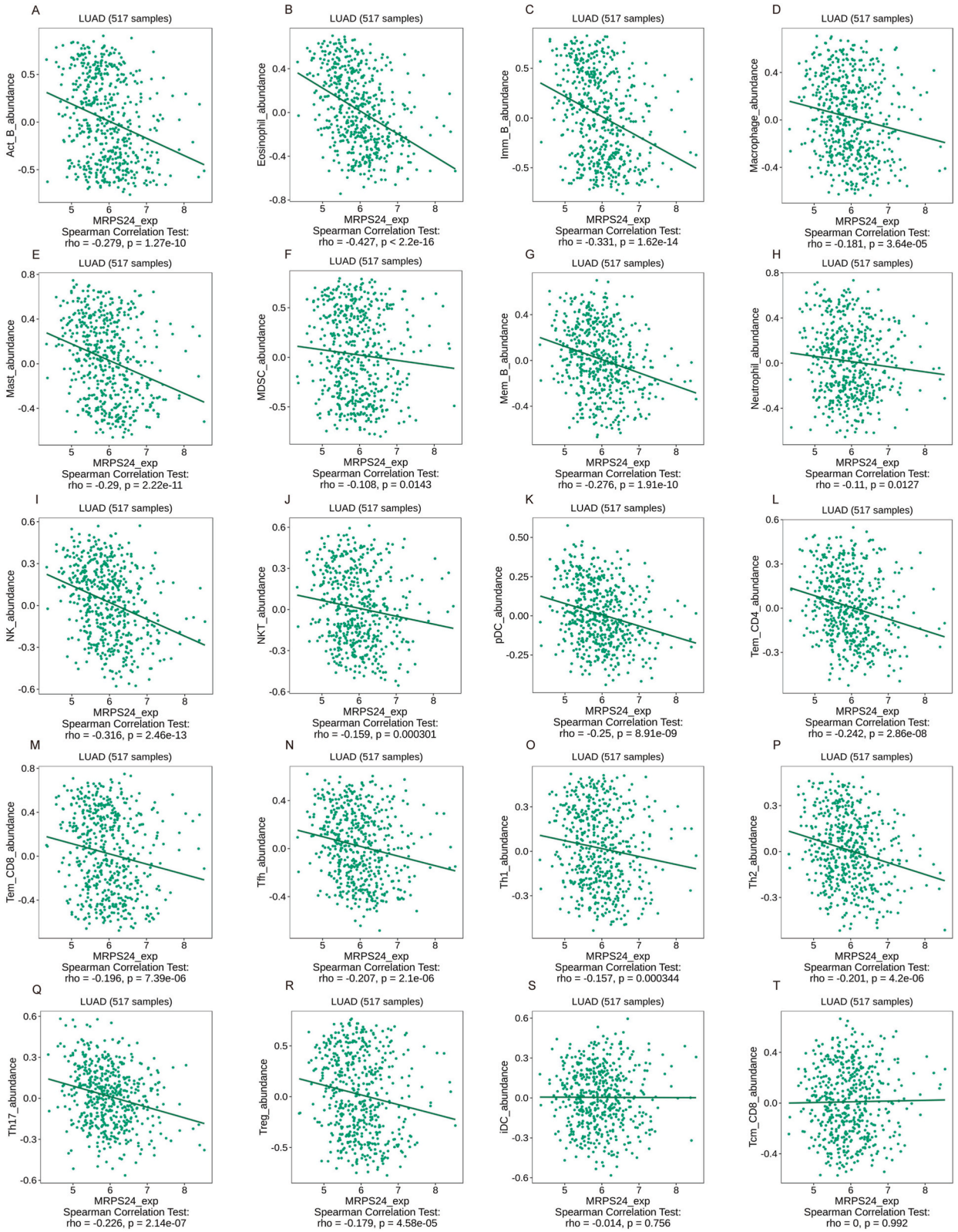

Fig. 11. The validation of the correlation between the expression of *MRPS24* and the immune cell infiltration in patients with LUAD. (A–T) The correlation between *MRPS24* expression and immune cell infiltration levels in patients with LUAD (TISIDB database).

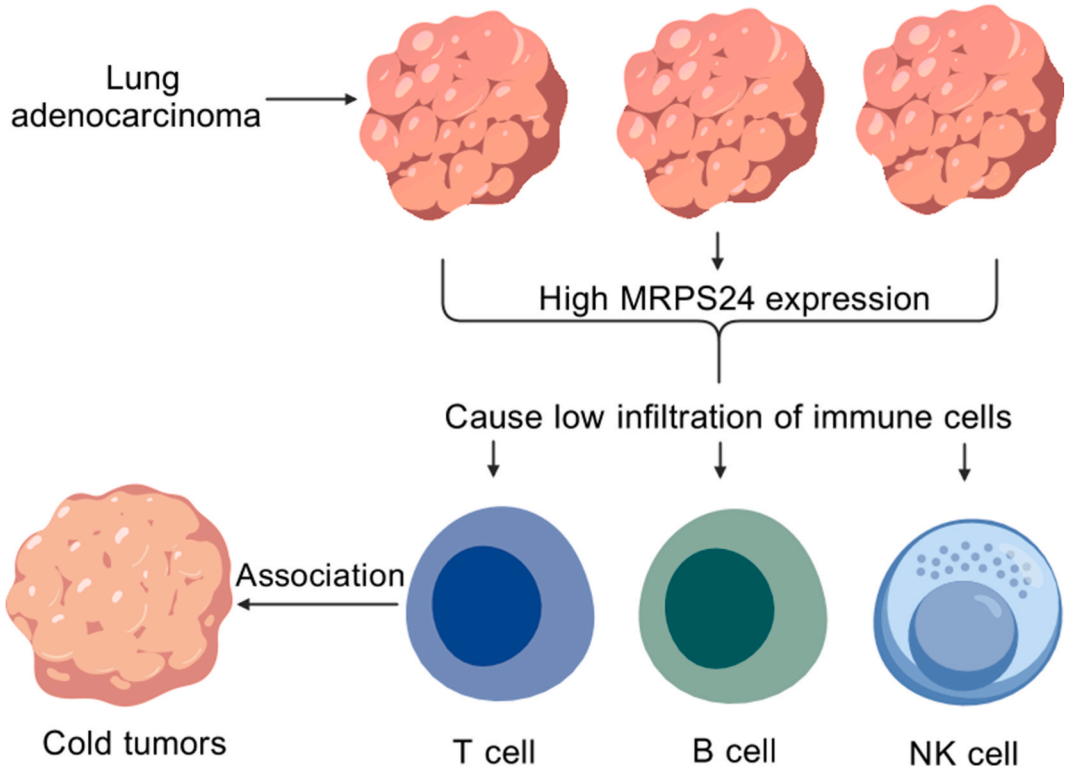

Fig. 12. The association between *MRPS24* expression and cold tumors.

methylation, *MRPS24* may have influenced the prognosis of cancer patients. Finally, single-cell function analysis revealed that *MRPS24* was positively related to inflammation, metastasis, invasion, apoptosis, DNA damage, DNA repair, and cell cycle in LUAD. Study has demonstrated that abnormal DNA methylation can speed up the development of cancer by controlling cell growth and causing apoptosis or senescence [26]. Hence, the upregulation of *MRPS24* in LUAD could be partially attributed to *MRPS24* hypomethylation and CNVs.

LUAD is a type of aggressive cancer that is characterized by a high degree of genetic and heterogeneity [27]. DNA methylation is a type of epigenetic modification that occurs frequently. Cancer cells may have abnormal DNA methylation [28]. In order to fully understand how genes are controlled, an examination of DNA methylation is essential [29]. The process of DNA methylation is prominent to regulating gene expression, maintaining genomic integrity, and promoting tumor development. DNA methylation has been shown to be a useful adjunct biomarker for clinical cancer diagnosis and prognosis [30–32]. Hypermethylated genes can be used as a therapeutic target in cancer treatment because DNA methylation is reversible. Our results illustrated that *MRPS24* might contribute to the tumorigenesis by affecting methylation.

It is generally accepted that people with impaired immunological systems may be more susceptible to cancer, while people with a normally functioning immune system may protect against and even prevent the development of malignant tumors [33,34]. The tumor microenvironment is a critical element in the development of cancer, and immune evasion is a crucial phase in the development and therapeutic resistance of tumor [35]. As an essential component of the immune microenvironment, infiltrating immune cells serve as necessary biomarkers for determining the effectiveness of immunotherapy [36]. Predictive biomarkers are required for individualized therapy due to the intricate interaction between the host immune system and the tumor immune microenvironment. It is important to note that patients with high *MRPS24* expression had low levels of immune cell infiltration. The results suggested that *MRPS24* interacted with immune cells and tumor cells in LUAD. The overexpression of *MRPS24* in LUAD may contribute to an immunosuppressive microenvironment. These findings suggest that overexpression of *MRPS24* may be associated with cold tumors. These tumor cells are usually cunning, with a low number of mutations, thus evading the immune system and making it difficult for immune cells to penetrate inside the tumor, which in turn leads to a low likelihood of immune cells recognizing and killing the malignant tumor. Hence, we speculated that the high *MRPS24* expression in LUAD will have inferior benefits after immunotherapy. It might be possible that a novel target for the immunotherapy of LUAD can be found by manipulating the expression of *MRPS24* at the gene level. Additionally, this will enhance accurate immunotherapy response forecasting, which is crucial for guiding the dissemination of clinical practice and the implementation of therapy decision-making.

Although the effect of *MRPS24* in LUAD was described, some limitations are supposed to be acknowledged in our study. Firstly, since this study was conducted retrospectively using data from the TCGA and GEO database, some specific clinical information about LUAD may have been lacking. Secondly, all results were based on data that was made available to the public and should be verified by

additional basic experiments on clinical samples. Thirdly, due to the uneven distribution of the number of LUAD samples within each group, this research is unable to reveal a comprehensive relationship between the clinical characteristics and immune infiltration characteristics. Besides, we need to verify the roles of *MRPS24* in LUAD on tumor immunity and antitumor immunotherapy through more fundamental experiments. Finally, further studies of *MRPS24* are required to gain a better understanding of the potential correlation between tumor microenvironment and the immunotherapy response of LUAD.

5. Conclusions

In conclusion, our study systematically explored that *MRPS24* expression was significantly correlated with prognosis, tumorigenesis, genetic alteration, copy number variations, methylation, and immune cell infiltration in LUAD. *MRPS24* might be a potential immune-related predictor of immunotherapy response in LUAD. These results provide clues for future research into carcinogenesis and development, biomarker selection for immune therapy efficacy prediction, and therapeutic target identification in LUAD.

Data availability statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Ethics approval and consent to participate

Not applicable.

CRediT authorship contribution statement

Yanni Gao: Writing – original draft, Software, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Yilin Yu:** Writing – original draft, Software, Methodology, Formal analysis, Data curation. **Haixia Wu:** Writing – original draft, Formal analysis, Data curation. **Zhenzhou Xiao:** Writing – review & editing, Validation, Supervision, Project administration, Investigation, Conceptualization. **Jiancheng Li:** Writing – review & editing, Visualization, Validation, Supervision, Project administration, Conceptualization.

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.

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List of abbreviations

MRPS24	Mitochondrial Ribosomal Protein S24
LUAD	lung adenocarcinoma
TME	tumor microenvironment
TCGA	The Cancer Genome Atlas
GSEA	gene set enrichment analysis
CNVs	copy number variations
GEO	Gene Expression Omnibus
ROC	receiver operating characteristic
GO	gene ontology
KEGG	Kyoto Encyclopedia of Genes and Genomes
NES	normalized enrichment score
FDR	false discovery rate

ssGSEA	single-sample Gene Set Enrichment Analysis
OS	overall survival
EGFR	epithelial growth factor receptor
Mut	mutation type
Wt	wild type
ALK	anaplastic lymphoma kinase
KRAS	kirsten rat sarcoma viral oncogene
HR	hazard ratio
CI	confidence interval
TPR	true positive rate
FPR	false positive rate

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