Original Article

Comprehensive analysis of the immunological implication and prognostic value of MEAK7 in non-small cell lung cancer

Jing-Yu Miao[1], Zhen Lin[2]^

[1]Westlake Innovation Capital, Hangzhou, China; [2]Cancer Center, Department of Medical Oncology, Zhejiang Provincial People's Hospital (Affiliated People's Hospital), Hangzhou Medical College, Hangzhou, China

Contributions: (I) Conception and design: Z Lin; (II) Administrative support: Z Lin; (III) Provision of study materials or patients: Z Lin; (IV) Collection and assembly of data: JY Miao; (V) Data analysis and interpretation: JY Miao; (VI) Manuscript writing: Both authors; (VII) Final approval of manuscript: Both authors.

Correspondence to: Zhen Lin, MD. Cancer Center, Department of Medical Oncology, Zhejiang Provincial People's Hospital (Affiliated People's Hospital), Hangzhou Medical College, No. 158 Shangtang Road, Gongshu District, Hangzhou 313000, China. Email: linzhen.fau@gmail.com.

Background: The mechanistic target of rapamycin (mTOR)-associated protein Eak-7 homolog (MEAK7) is widely involved in the occurrence and development of various diseases, including tumors. However, the role of MEAK7 in non-small cell lung cancer (NSCLC) and its underlying mechanism in the tumor microenvironment remain unclear. The purpose of this paper is to explore the role of MEAK7 in the prognosis of NSCLC.

Methods: The expression levels of *MEAK7* were examined through the utilization of The Cancer Genome Atlas (TCGA) and the genotype-tissue expression project's pan-cancer dataset. Within this context, the relationships between *MEAK7* expression and various clinical features, as well as patient outcomes, were assessed using a comprehensive array of bioinformatics resources. Additionally, the link between *MEAK7* expression and the infiltration of immune cells was investigated employing CIBERSORT and ESTIMATE methodologies. Gene set enrichment analysis was performed to determine immune responses. Finally, the patient response to immunotherapy was predicted using the Tumor Immune Dysfunction and Exclusion (TIDE) algorithm and immune checkpoint score.

Results: *MEAK7* was highly expressed in many types of tumors including lung adenocarcinoma and lung squamous cell carcinoma. Elevated *MEAK7* expression was found to correlate with several key characteristics, including sex, age, the presence of metastasis, and pathological staging, and was identified as a significant predictor of poor prognosis in individuals with lung cancer. Subsequent analyses revealed a positive association between heightened *MEAK7* levels and the infiltration of immune cells, as well as the expression profiles of a variety of immune cell markers. *MEAK7* was closely linked to the pathways involved in immune regulation. Interestingly, patients with elevated *MEAK7* expression levels were sensitive to immunotherapy.

Conclusions: These findings provide compelling evidence that *MEAK7* may be involved in the progression of lung cancer and become a potential therapeutic target.

Keywords: Mechanistic target of rapamycin-associated protein Eak-7 homolog (MEAK7); non-small cell lung cancer (NSCLC); immune infiltrate; immunotherapy

Submitted Aug 18, 2024. Accepted for publication Dec 19, 2024. Published online Feb 26, 2025.
doi: 10.21037/tcr-24-1448
View this article at: https://dx.doi.org/10.21037/tcr-24-1448

^ ORCID: 0000-0001-5785-1496.

Introduction

Non-small cell lung cancer (NSCLC) is an extremely common and malignant tumor and the first leading cause of cancer-related death worldwide (1). Currently, great advances have been made in diagnostic and therapeutic techniques. Immunotherapy has emerged as a highly promising treatment option for various types, including NSCLC (2), breast cancer (3), liver cancer (4), colorectal cancer (5), melanoma (6) and so on. However, several large trials on immunotherapy reported within the last year have yielded disappointing results (7). The median overall survival (OS) and 5-year survival rates of patients with NSCLC remain very low (8). Hence, there is an urgent need to elucidate the mechanisms underlying the development and progression of NSCLC to identify prognostic markers and potential targets for therapeutic intervention. This research endeavor is important for advancing our understanding of NSCLC and for improving patient outcomes.

A previous study revealed that the mechanistic target of rapamycin (mTOR)-associated protein Eak-7 homolog (MEAK7) mainly activates mTOR signaling to regulate cell proliferation and migration (9). MEAK7 plays multifaceted roles in various diseases. Finelli *et al.* demonstrated that MEAK7 exerts neuroprotective effects against oxidative stress in neurological disorders (10). MEAK7 also regulates the formation of alternative mTOR complexes in cancer cells (11). MEAK7 is involved in regulating proliferation, migration, and invasion of pancreatic adenocarcinoma (12). However, little is known about its prognostic significance and the participation in the immune response in lung cancer. Furthermore, whether MEAK7 is associated with the response to immunotherapy remains unclear.

The primary objective of this study was to investigate the association between MEAK7 expression and its prognostic significance in NSCLC patients. Additionally, we examined the potential correlation between MEAK7 expression and levels of tumor-infiltrating immune cells in lung cancer. Furthermore, we aimed to explore the association between MEAK7 and potential response to immunotherapy in lung cancer, with the ultimate goal of providing guidance for personalized treatment strategies. In conclusion, our integrated analysis revealed immunological implications and prognostic value of MEAK7 expression in NSCLC. We present this article in accordance with the REMARK reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-1448/rc).

Highlight box

Key findings

- The mechanistic target of rapamycin-associated protein Eak-7 homolog (MEAK7) expression is significantly decreased in non-small cell lung cancer (NSCLC) compared to normal lung tissues ($P<0.05$).
- High *MEAK7* expression was associated with sex, age, metastasis, and pathological stage and significantly predicted an unfavorable prognosis in patients with lung cancer.
- *MEAK7* expression correlates with the functionality of anti-tumor immune cells, indicating its potential role in the tumor microenvironment.
- Patients with elevated *MEAK7* expression levels were sensitive to immunotherapy.

What is known and what is new?

- Previous research has highlighted MEAK7 as a potential biomarker for certain cancers.
- This study further establishes its significance in NSCLC prognosis. In addition, patients with elevated *MEAK7* expression levels were sensitive to immunotherapy.

What is the implication, and what should change now?

- These findings emphasize the potential of *MEAK7* as a prognostic biomarker for NSCLC, with implications for therapeutic strategies and prognostic assessments in clinical settings.

Methods

Data collection and analysis

This study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). To assess the expression patterns of MEAK7 and its associated clinical data across multiple cancer types, we obtained data from The Cancer Genome Atlas (TCGA) database, while normal tissues were sourced from both TCGA and Genotype-Tissue Expression (GTEx) databases. Normalized data were downloaded from the University of California Santa Cruz (UCSC) Xena database for further analysis (13) (https://xenabrowser.net/datapages/).

The University of ALabama at Birmingham CANcer data analysis Portal (UALCAN)

UALCAN (http://ualcan.path.uab.edu/) is a comprehensive, user-friendly, and interactive web resource that provides in-depth analyses of transcriptome data from TCGA and MET500 data (14). UALCAN was used to investigate

the expression of *MEAK7* and its correlation with diverse clinicopathological parameters in lung cancer, including sex, cancer stage, nodal metastasis status, age, race, and TP53 mutation status.

Kaplan-Meier plotter database analysis

We utilized the Kaplan-Meier plotter (http://kmplot.com), an online repository that amalgamates gene expression profiles and survival data from 1,925 clinical lung cancer patients, to elucidate the prognostic relevance of *MEAK7* in lung cancer (15). The cohort was stratified into two groups based on the median expression levels of *MEAK7*, delineating high and low expression groups, to evaluate the influence of *MEAK7* on OS, progression-free survival (PFS), and post-progression survival (PPS). For each group, hazard ratios (HRs) along with their respective 95% confidence intervals (CIs) and log-rank P values were meticulously computed to quantify the statistical significance of *MEAK7* expression on survival outcomes.

PrognoScan database analysis

We examined the association between *MEAK7* expression and survival in lung cancer using the PrognoScan database (http://dna00.bio.kyutech.ac.jp/PrognoScan/) (16). PrognoScan provides a valuable platform for exploring the relationship between *MEAK7* expression and patient prognosis, including OS and relapse-free survival (RFS), using a diverse range of publicly available cancer microarray datasets. For the purpose of dataset selection in this study, the inclusion criteria were meticulously defined: the "Cancer Type" was specified as lung cancer, with "Subtypes" limited to "adenocarcinoma" and "squamous cell carcinoma". HR along with their corresponding 95% CIs were determined for analysis. The significance threshold was set at a Cox P value threshold of less than 0.05.

Analysis of immune infiltration

The ESTIMATE is a powerful tool that enables the prediction of stromal and immune components within tumor tissues (17). By utilizing ESTIMATE, researchers can gain insights into the main compositions in lung cancer. To further enhance our understanding of immune components within tumors, we acquired immune cell infiltration scores from CIBERSORT database. These scores were obtained through a comprehensive study that utilized the CIBERSORT analytical tool, which has been previously published (18). By leveraging this dataset, we can uncover valuable insights into the diverse landscape of immune cell infiltration across lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC). TCGA lung cancer samples were divided into two groups according to median *MEAK7* expression (high versus low levels), and their immune cell infiltration levels were compared.

Correlation and enrichment analyses

Pearson's correlation analysis of the *MEAK7* messenger RNA (mRNA) and other mRNAs was performed for lung cancer using the LUAD and LUSC data. Gene set enrichment analysis (GSEA) was performed according to the Gene Ontology (GO) terms in the clusterProfiler package (19). Gene set variation analysis (GSVA) was used to provide an enrichment score for each sample, using the Biocarta items in the GSVA package (20). Following group comparing process was conducted by limma (21). The gene set for the immune checkpoints was derived from a previously published study (22,23).

Immunotherapeutic response prediction

We used the Tumor Immune Dysfunction and Exclusion (TIDE) algorithm to predict clinical responses to immune checkpoint inhibitors as previously described (24). Tumor mutational burden (TMB), measured as mutations per megabase, is an emerging and crucial indicator of sensitivity to immunotherapy. To determine the TMB scores for each patient with LUAD and LUSC, we employed a previously established computational method as described in the relevant literature (25). This standardized approach enabled us to gauge the extent of mutations within the tumor genome and evaluate their potential implications for immunotherapeutic responses. Meanwhile, Intratumor heterogeneity provides a simple, quantitative, and clinically practical biomarker for evaluating the relationship between intratumor genetic heterogeneity and outcomes in any type of cancer (26).

Statistical analyses

Data were expressed as the mean ± standard deviation. All data were analyzed using the R version 4.0.0 (R Foundation for Statistical Analysis Computing, Vienna, Austria).

Pairwise differences between groups were analyzed using Student's *t*-test. The significance was assessed at P<0.05.

Results

MEAK7 exhibits abnormally elevated expression levels in lung cancer

Initially, we conducted a comparative analysis of *MEAK7* expression in pan-cancer samples from TCGA, along with the corresponding normal samples obtained from TCGA and the GTEx database. *MEAK7* was highly expressed in most cancer types (17/33), including bladder urothelial carcinoma (BLCA), breast infiltrating carcinoma (BRCA), cervical squamous cell carcinoma and adenocarcinoma (CESC), cholangiocarcinoma (CHOL), colon adenocarcinoma (COAD), diffuse large B-cell lymphoma (DLBC), esophageal cancer (ESCA), pleomorphic glioma (GBM), head and neck squamous cell carcinoma (HNSC), renal papillary cell carcinoma (KIRP), brain low-grade glioma (LGG), LUAD, LUSC, pancreatic cancer (PAAD), rectal adenocarcinoma (READ), thymic cancer (THYM), and endometrial cancer (UCEC) (Wilcoxon rank sum test, P<0.05; *Figure 1A*). Secondly, we performed a comparative analysis of *MEAK7* expression within the TCGA-LUAD and TCGA-LUSC cohorts. The expression of *MEAK7* was significantly higher in tumor samples from LUAD (P<0.001; *Figure 1B*) and LUSC (P<0.001; *Figure 1C*). Furthermore, the difference remained significant in the comparison between tumor samples and matched para-cancerous samples (*Figure 1D,1E*). Subsequently, we conducted receiver operating characteristic (ROC) analysis to assess the discriminatory ability of *MEAK7* expression in distinguishing lung cancer tissues from normal tissues. The calculated areas under the curve (AUC) for *MEAK7* were 0.803 and 0.809, respectively, indicating its moderate potential as a diagnostic marker for lung cancer (*Figure 1F,1G*). The protein expression levels of MEAK7, determined by immunohistochemistry, were also upregulated in most lung cancer tissues, which can be summarized from the data on the Human Protein Atlas (HPA) website (Figure S1). These findings indicate prominent upregulation of MEAK7 expression in lung cancer, signifying its aberrant overexpression in this disease context.

MEAK7 expression is associated with clinical parameters of lung cancer patients

Using the UALCAN online tool, we conducted a comprehensive analysis of *MEAK7* expression across different patient groups, categorized by various clinical parameters. When considering sex as a determining factor, our findings revealed a significant upregulation of *MEAK7* expression in lung cancer samples from both male and female patients compared with their respective normal control counterparts. Interestingly, *MEAK7* expression of male is significantly higher than female. These results contribute to a better understanding of the role of *MEAK7* in lung cancer development and provide valuable insights into its potential as a diagnostic or therapeutic target for both sexes (*Figure 2A,2B*). Regarding tumor stage, a noteworthy upregulation of *MEAK7* expression was observed in both LUSC and LUAD patients across all stages (*Figure 2C,2D*). Furthermore, as the tumor stage progresses, the expression of *MEAK7* shows a mild elevation. According to the analysis of lymph node metastasis in cancer, *MEAK7* expression was higher in patients with LUAD and LUSC, specifically in those classified as N0, N1, or N2 stages (*Figure 2E,2F*). Compared with normal controls, the expression of *MEAK7* was found to be upregulated in both TP53-mutant and TP53 wild-type lung cancer patients (Figure S2A,S2B). Notably, the expression of *MEAK7* is significantly higher in the TP53 mutation group compared to the wild-type group in LUAD. With respect to age, *MEAK7* levels were markedly higher in lung cancer tissues across various patient age groups (21–40, 41–60, 61–80, and 81–100 years) in both LUAD and LUSC (Figure S2C,S2D). Additionally, *MEAK7* expression was substantially increased in lung cancer patients, irrespective of their smoking history. However, no significant difference was observed between smokers and non-smokers (Figure S2E,S2F). Collectively, these observations suggest a substantial correlation between *MEAK7* expression and tumor progression.

Elevated expression of MEAK7 is associated with unfavorable prognosis in individuals diagnosed with lung cancer

Given the association between the *MEAK7* expression and the progression, we also investigated the prognostic significance of the expression of *MEAK7*. Our analysis of the Kaplan-Meier plotter database revealed that lung cancer patients with heightened *MEAK7* expression had lower OS and PFS, whereas PPS did not show significant differences (*Figure 3A*). Additionally, an analysis conducted using the PrognoScan database demonstrated a significant association between elevated *MEAK7* expression and adverse outcomes

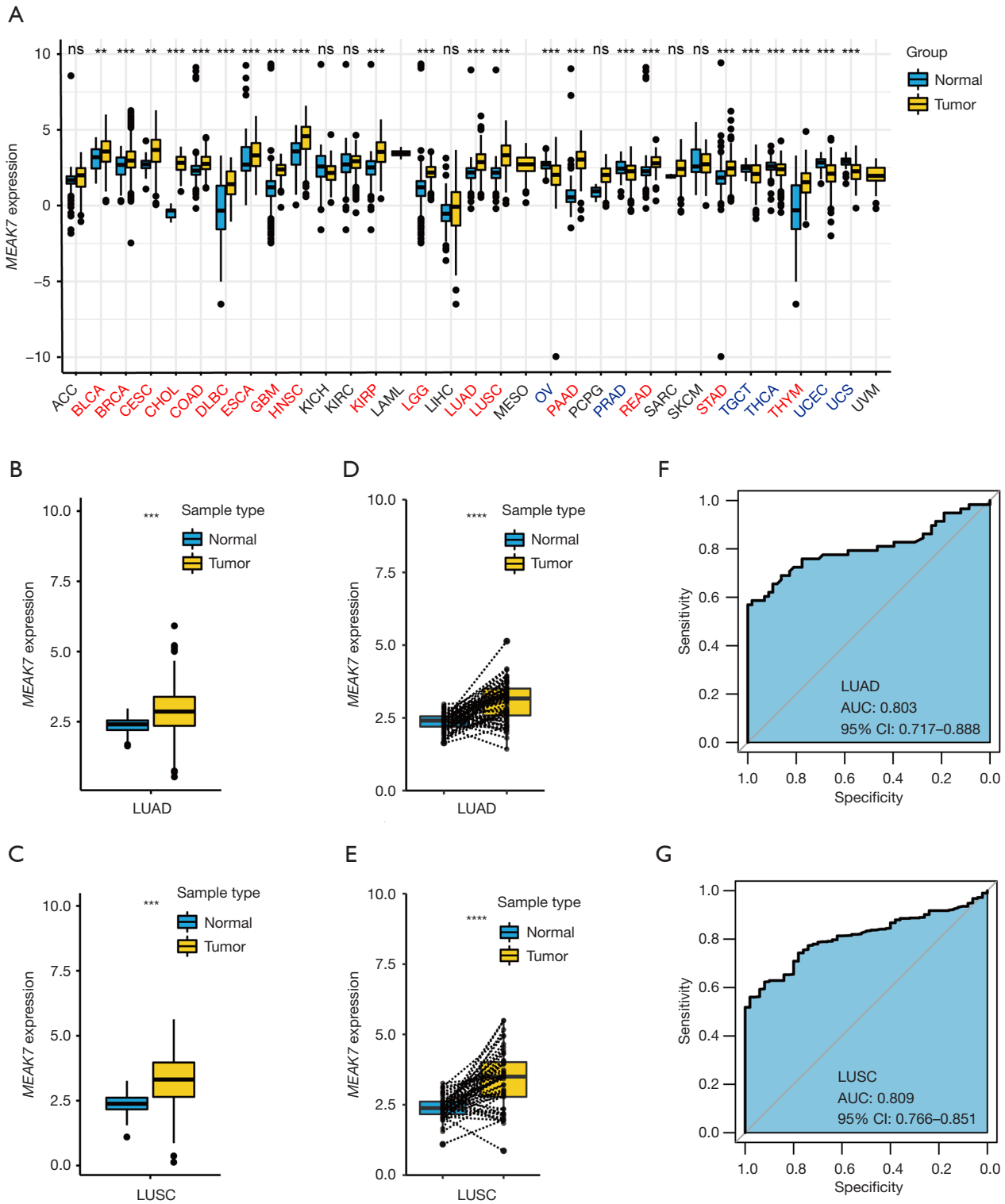


Figure 1 Different expression levels of *MEAK7* in different malignancies. (A) Increased or decreased *MEAK7* of various types of cancers compared with normal tissues in the TCGA and GTEx database. (B,C) Distinctive expression profiles of *MEAK7* in LUAD. (D,E) Distinctive expression profiles of *MEAK7* in LUSC. (F) A ROC curve was constructed to evaluate the sensitivity and specificity of *MEAK7* for identifying LUAD. (G) A ROC curve was constructed to evaluate the sensitivity and specificity of *MEAK7* for identifying LUSC.

ns, not significant; **, P<0.01; ***, P<0.001; ****, P<0.0001. *MEAK7*, MTOR associated protein, Eak-7 homolog; TCGA, The Cancer Genome Atlas; GTEx, Genotype-Tissue Expression; LUAD, lung adenocarcinoma; ROC, receiver-operating characteristic; LUSC, lung squamous cell carcinoma; AUC, area under the curve; CI, confidence interval; BLCA, bladder urothelial carcinoma; ACC, adrenocortical cancer; BRCA, breast infiltrating carcinoma; CESC, cervical squamous cell carcinoma and adenocarcinoma; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; DLBC, diffuse large B-cell lymphoma; ESCA, esophageal cancer; GBM, glioblastoma multiforme; HNSC, head and neck squamous cell carcinoma; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney papillary cell carcinoma; LAML, acute myeloid leukemia-like; LGG, brain low-grade glioma; LIHC, liver hepatocellular carcinoma; MESO, mesothelioma; OV, ovarian cancer; PAAD, pancreatic adenocarcinoma; PCPG, pheochromocytoma and paraganglioma; PRAD, prostate adenocarcinoma; READ, rectal adenocarcinoma; SARC, sarcoma; SKCM, skin cutaneous melanoma; STAD, stomach adenocarcinoma; THYM, thymic cancer; UCEC, uterine corpus endometrial carcinoma; UCS, uterine carcinosarcoma; UVM, uveal melanoma.

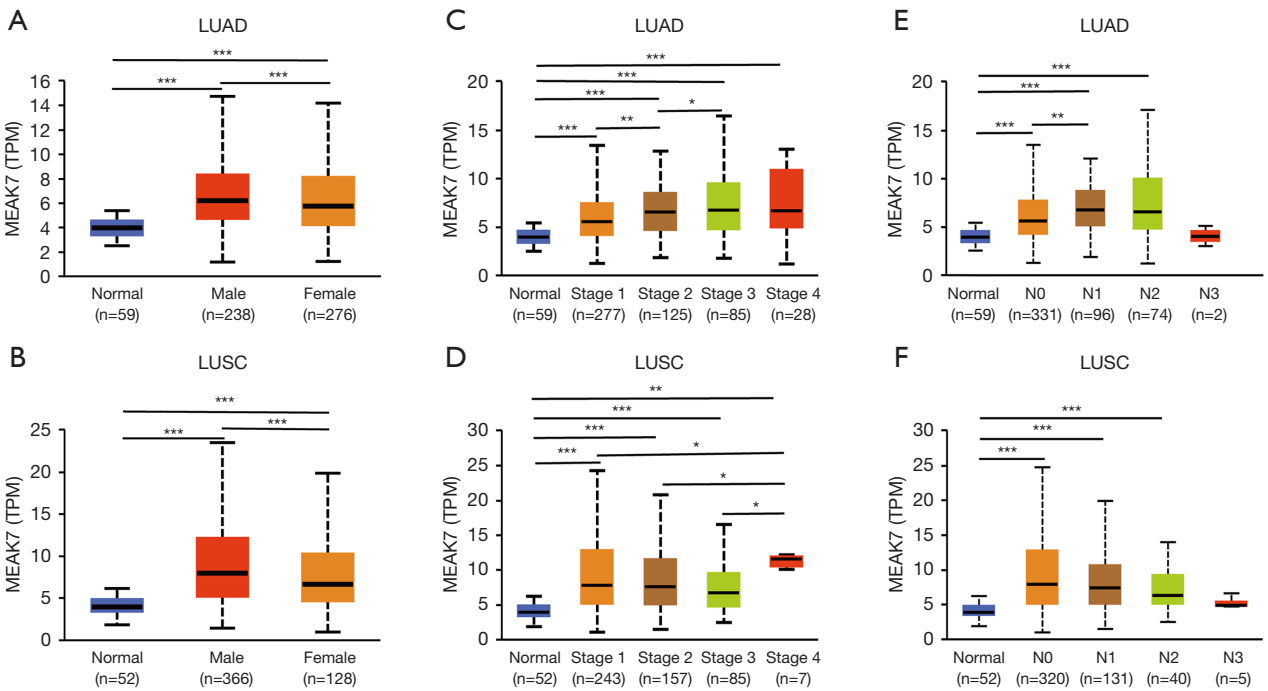


Figure 2 The expressions of *MEAK7* across patient groups categorized by clinical parameters were assessed by box plots utilizing the UALCAN database. A comparative analysis of the expression of *MEAK7* is shown for sex (A,B), tumor stage (C,D), and metastasis status (E,F) in LUAD and LUSC. Annotation for N class (N0: no regional lymph node metastasis; N1: metastases in 1 to 3 axillary lymph nodes; N2: metastases in 4 to 9 axillary lymph nodes; N3: metastases in 10 or more axillary lymph nodes). *, P<0.05, **, P<0.01; ***, P<0.001. *MEAK7*, MTOR associated protein, Eak-7 homolog; UALCAN, The University of ALabama at Birmingham CANcer data analysis Portal; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; TPM, transcripts per million.

in terms of OS and RFS across both the GSE31210 and GSE4573 cohorts (*Figure 3B*). To gain a deeper understanding of the prognostic significance of *MEAK7* expression in lung cancer, we investigated the relationship between *MEAK7* mRNA expression and various clinical characteristics using the Kaplan-Meier database. Intriguingly, our findings revealed a strong correlation between elevated *MEAK7* expression and unfavorable OS and PFS in both LUAD and LUSC, considering diverse covariates (*Figure 3C*). Elevated *MEAK7* expression was markedly correlated with diminished OS and PFS in lung cancer patients, irrespective of gender (*Figure 3C*). When considering various tumor stages, the association between high *MEAK7* expression and poor OS and PFS was observed exclusively in patients with stage I lung cancer (*Figure 3C*). Furthermore, a significant correlation was identified

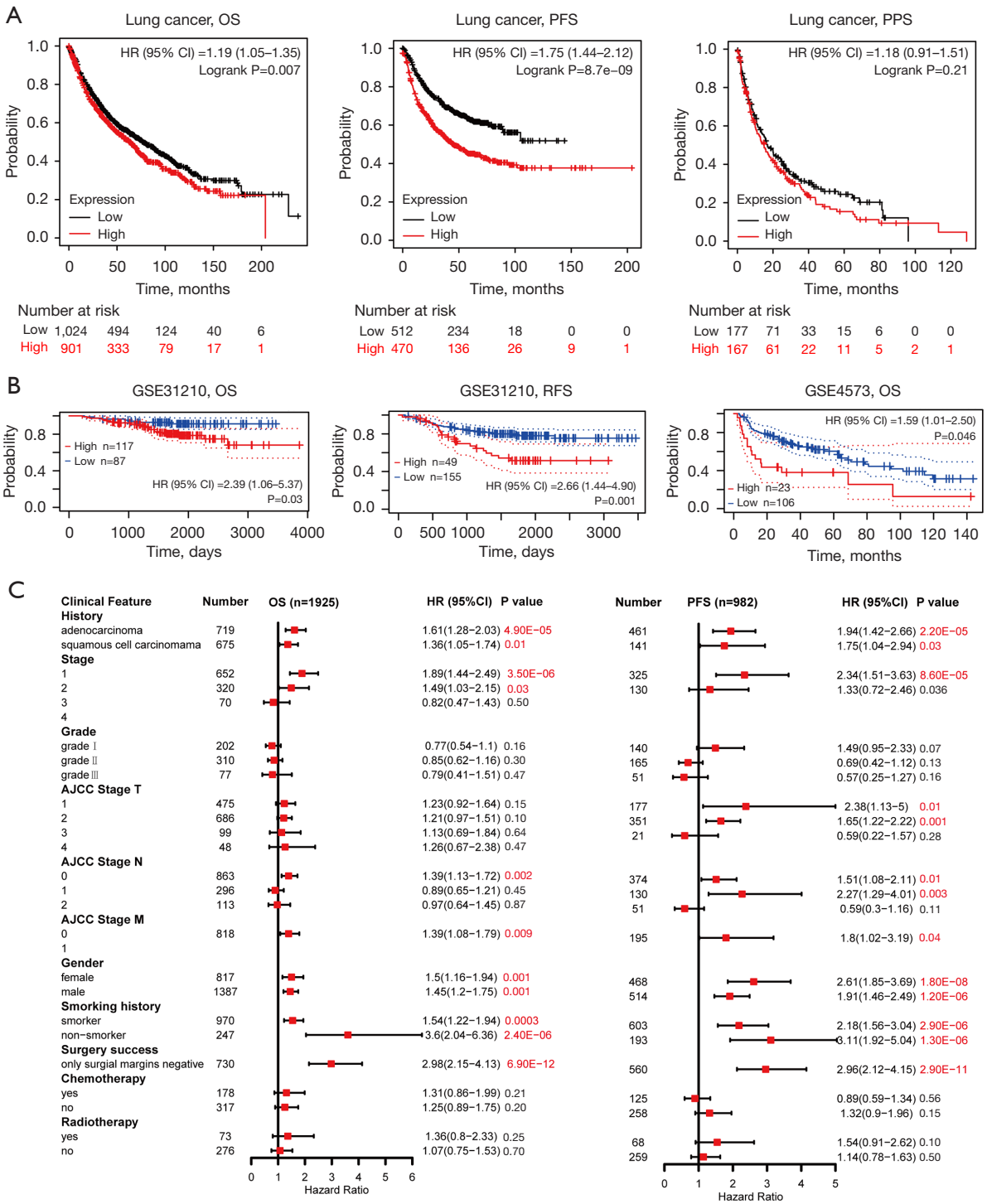


Figure 3 Survival analysis assessing the prognostic significance of *MEAK7*. (A) Survival curves depicting OS, PFS and PPS are illustrated using the Kaplan-Meier plotter. (B) Survival curves depicting the overall survival OS and RFS in the GSE31210 and GSE4573 datasets are presented utilizing the PrognoScan database. (C) The forest plot constructed to illustrate the correlation between *MEAK7* expression and clinical pathological parameters in patients with LUAD and LUSC. *MEAK7*, MTOR associated protein, Eak-7 homolog; OS, overall survival; PFS, progression-free survival; PPS, post-progression survival; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; HR, hazard ratio; CI, confidence interval.

between *MEAK7* expression and reduced PFS in lung cancer patients categorized as American Joint Committee on Cancer (AJCC) stages T1 and T2 (*Figure 3C*). Furthermore, we discovered a substantial link between *MEAK7* expression and adverse OS and PFS in both smokers and nonsmokers diagnosed with lung cancer (*Figure 3C*). Additionally, elevated *MEAK7* expression significantly correlated with inferior OS and PFS in patients with lung cancer who had negative surgical margins (*Figure 3C*). These findings provide compelling evidence that *MEAK7* expression has a substantial impact on the prognostic outcomes of patients diagnosed with lung cancer. Thus, *MEAK7* may serve as a valuable prognostic indicator for the clinical management of lung cancer.

MEAK7 is negatively correlated with anti-tumor immune components

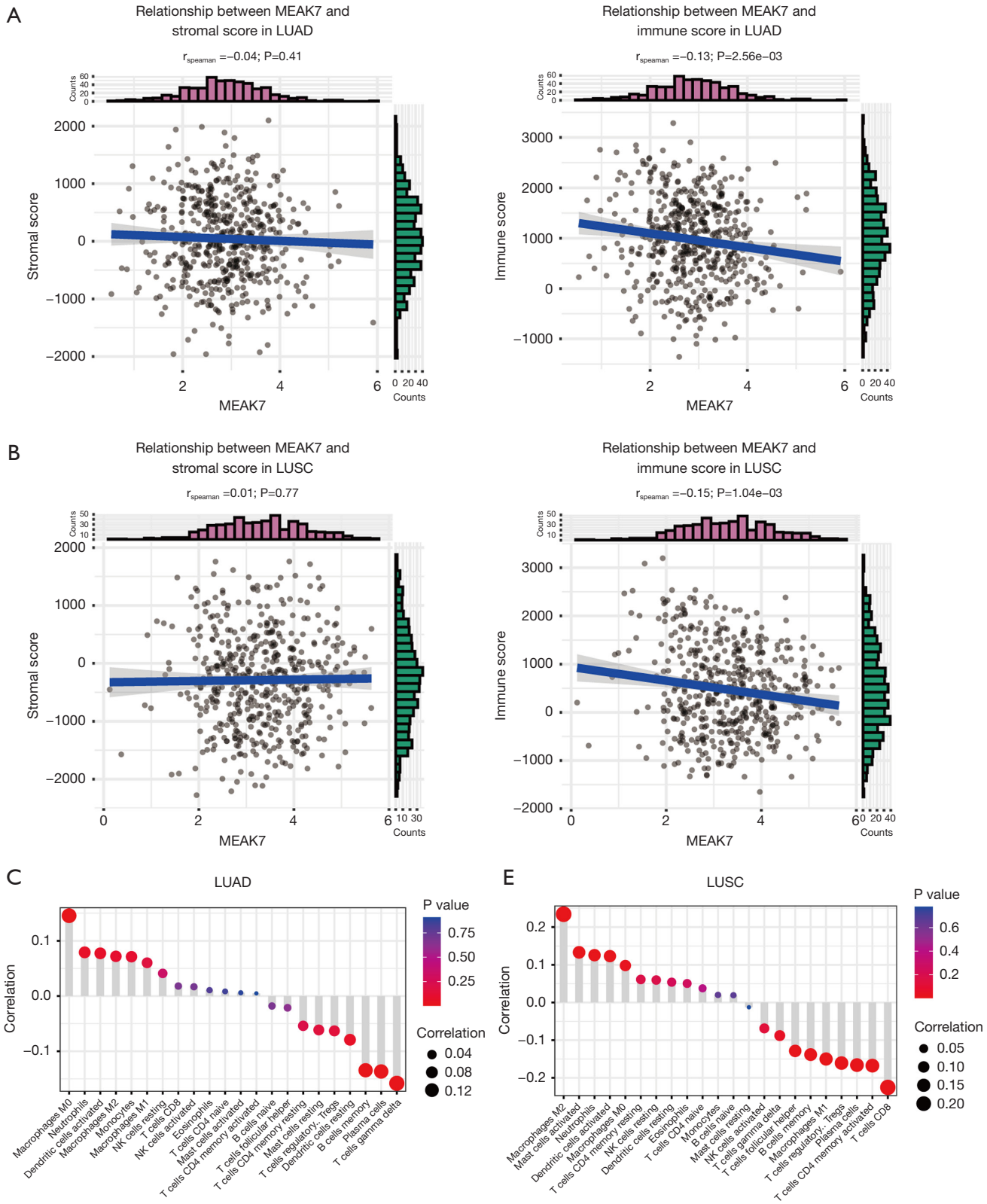
We employed the ESTIMATE algorithm to scrutinize the relationship between *MEAK7* expression and the infiltrating immune composition. Interestingly, our analysis revealed an inverse correlation between *MEAK7* expression and immune cell infiltration in both LUAD and LUSC, suggesting a possible role for *MEAK7* in the modulation of immune responses within these specific tumor types. However, no significant correlation was observed between *MEAK7* expression and stromal composition (*Figure 4A*,*4B*). To further investigate the impact of *MEAK7* on the tumor microenvironment (TME), we leveraged the computational tool CIBERSORT to evaluate the correlation between MEAK7 expression and the presence of various immune cell types. Our results highlighted significant positive correlations between *MEAK7* expression and the infiltration of M0 macrophages, neutrophils, dendritic cells, and M2 macrophages in LUAD patients. In contrast, *MEAK7* expression exhibited negative correlations with gamma delta T cells, plasma cells, and memory B cells (*Figure 4C*). Additionally, when clustering LUAD patients based on median expression, the *MEAK7*-high group exhibited a lower abundance of memory B cells, plasma B cells, and gamma-delta T cells, and a higher abundance of M0 and M2 macrophages (*Figure 4D*). Furthermore, we observed a significant positive correlation between *MEAK7* expression and M2 macrophage infiltration, whereas a negative association was found with $CD8^+$ T cells in LUSC (*Figure 4E*). When comparing different groups, the *MEAK7*-high group exhibited lower levels of memory B cells, plasma B cells, $CD8^+$ T cells, activated memory $CD4^+$ T cells, follicular helper T cells, and M1 macrophages, but higher levels of M2 macrophages (*Figure 4F*). These findings provide additional evidence supporting a significant association between *MEAK7* expression and immune infiltration, indicating that *MEAK7* plays a crucial role in the anticancer immune response within the TME of lung cancer.

MEAK7 diminishes the anti-tumor immune response in lung cancer

To gain deeper insights into the molecular mechanisms of *MEAK7* in lung cancer, we performed a GSEA analysis. Within the GO terms of MEAK7-regulated signaling pathways, our analysis revealed that immune-related activities were mainly suppressed. Specifically, the pathways that were identified as being downregulated include antigen processing and presentation, mast cell activation, immune response, macrophage activation, leukocyte-mediated cytotoxicity, export across plasma, positive regulation of immune effector processes, T cell-mediated immunity, activation of immune response, leukocyte migration, and the B cell receptor signaling pathway in LUAD (*Figure 5A*). Concurrently, we observed the activation of several hallmarks of malignant tumors, notably cell cycle checkpoints and DNA repair (*Figure 5A*). The same analysis was applied to the LUSC. Pathways such as the B cell receptor signaling pathway, B cell-mediated immunity, mast cell activation, activation of the immune response, regulation of leukocyte-mediated cytotoxicity, macrophage activation, alpha-beta T cell differentiation, export of plasma membrane, leukocyte migration, and T cell activation are suppressed. However, DNA repair, a positive cell cycle, and glycoprotein metabolic processes were activated (*Figure 5B*). To avoid bias due to method selection, we used the GSVA algorithm for terms from an independent Biacarta database. Similarly, a volcano plot revealed that the lymphocyte pathway is enriched in lung cancer (*Figure 5C*,*5D*). Correlation analysis revealed a significant negative relationship between *MEAK7* expression and lymphocyte pathway scores in both LUAD and LUSC (*Figure 5E*,*5F*). These findings provide strong evidence that *MEAK7* plays a crucial role in modulating immune response in lung cancer.

The expression of MEAK7 reflects the efficiency of immunotherapy in lung cancer

Immunotherapy, which focuses on targeting immune



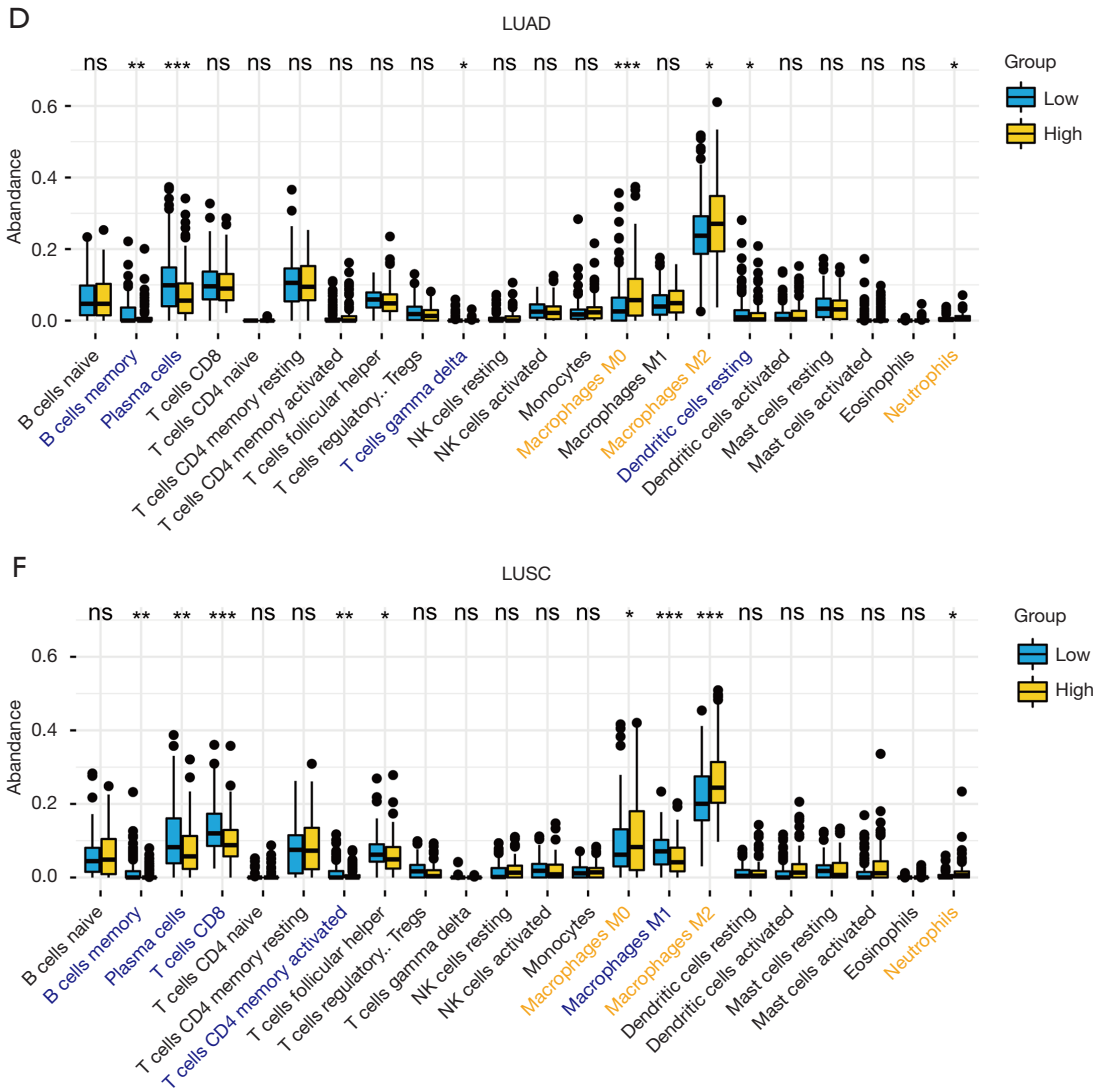


Figure 4 The correlation between *MEAK7* expression and tumor immune components. (A,B) The stromal component and immune component were calculated by ESTIMATE methods. (C) The correlation of immune cells and *MEAK7* expression in LUAD. (D) Comparison of the proportions of 22 types of immune infiltration by CIBERSORT methods in LUAD. (E) The correlation of immune cells and *MEAK7* expression in LUSC. (F) Comparison of the proportions of 22 types of immune infiltration by CIBERSORT methods in LUSC. ns, not significant; *, P<0.05, **, P<0.01; ***, P<0.001. *MEAK7*, MTOR associated protein, Eak-7 homolog; ESTIMATE, Estimation of STromal and Immune cells in MAlignant Tumor tissues using Expression data; CIBERSORT, cell-type identification by estimating relative subsets of known RNA transcripts.

checkpoints, has emerged as the primary treatment option for lung cancer. In this context, *MEAK7* is a promising candidate molecule for incorporation into therapeutic strategies. To explore the relationship between *MEAK7* expression and immunotherapy response, we analyzed the correlation between *MEAK7* expression and a series of widely used biomarkers (27-29). First, we incorporated the TIDE algorithm into our analysis, which encompasses T-cell dysfunction and exclusion score (24). As expected, the patients with high *MEAK7* presence significantly lower TIDE scores, lower dysfunction scores, and higher exclusion scores (*Figure 6A*,*6B*). Furthermore, GSEA indicated significant enrichment of the immune checkpoint pathway (*Figure 6C*,*6D*). These results indicated that

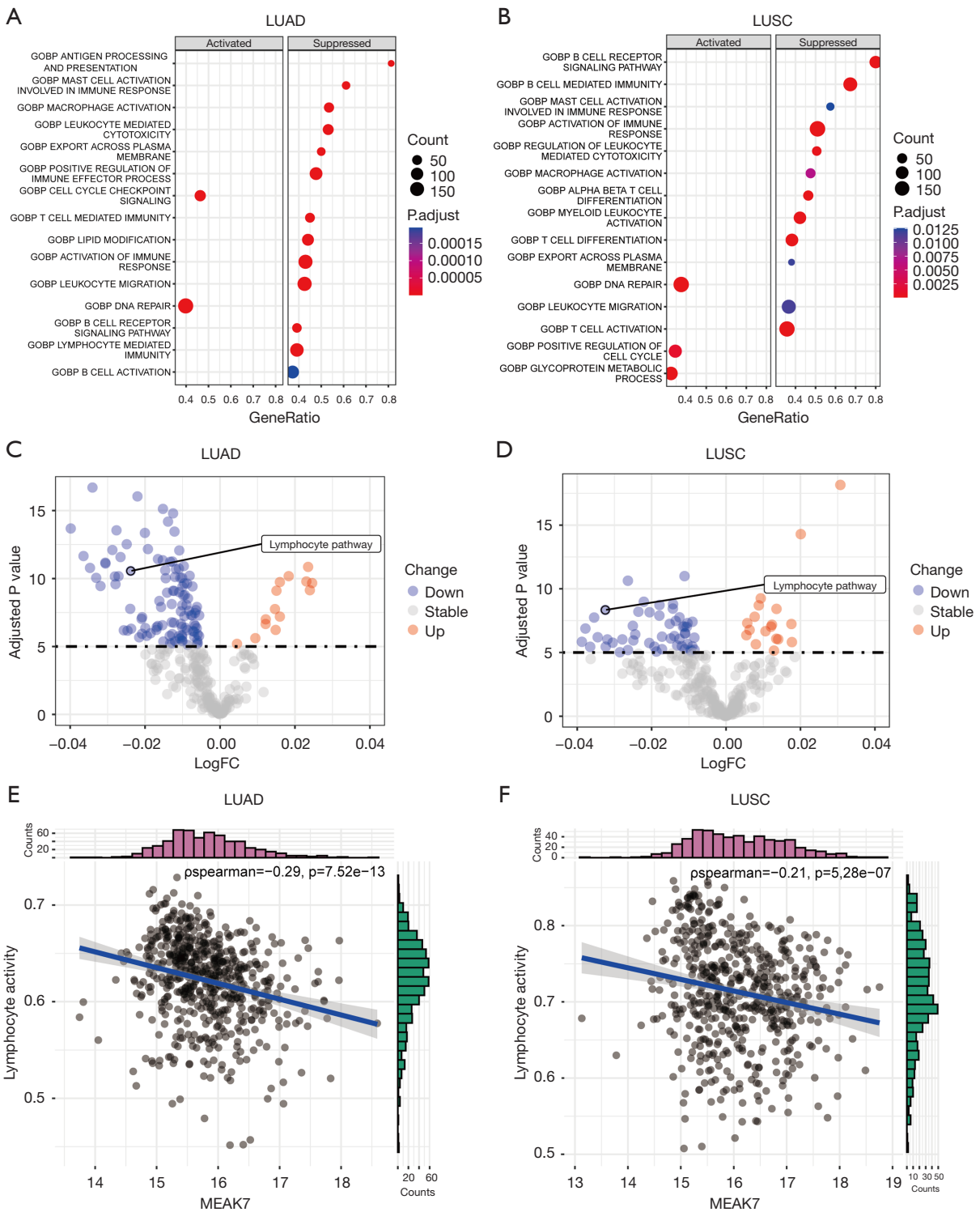


Figure 5 The *MEAK7* expressions are negatively correlated with immune response in lung cancer. (A,B) GO enrichment analysis to uncover the functional implications of the genes that are correlated with *MEAK7*. (C,D) The volcano plot shows a significant down-regulation of lymphocytes in Biocarta database. (E,F) Scatter plots were conducted to investigate the correlation between the activity of the lymphocyte pathway and *MEAK7* expression. *MEAK7*, MTOR associated protein, Eak-7 homolog; GO, Gene Ontology.

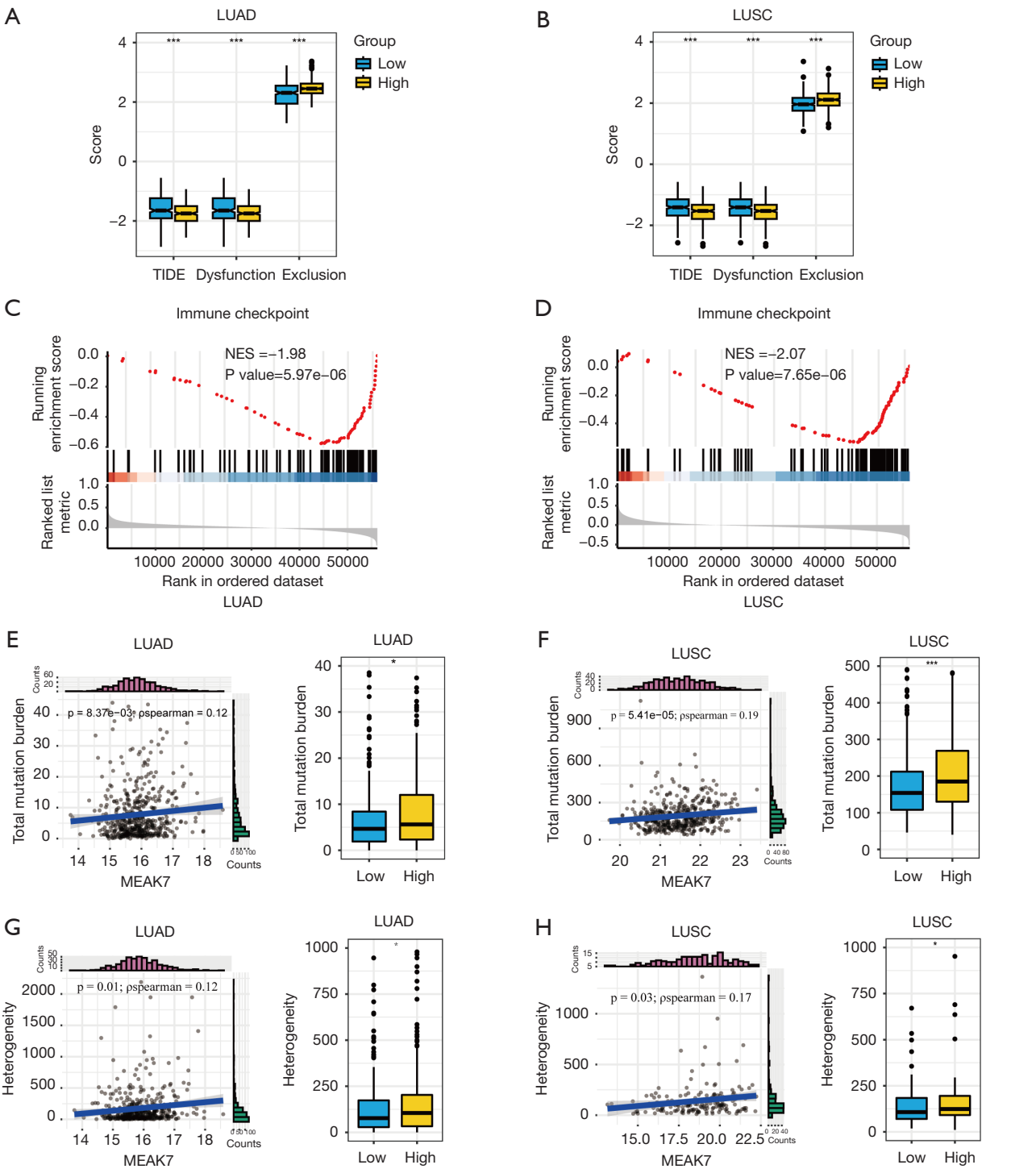


Figure 6 The expression of *MEAK7* reflected the efficiency of immunotherapy in lung cancer. (A,B) The correction of TIDE score and the *MEAK7* expression in LUAD and LUSC separately. (C,D) The GSEA analysis of immune checkpoint gene set. (E,F) The correlation between mutation burden and the *MEAK7* expression in LUAD and LUSC separately. (G,H) The correlation between heterogeneity and the *MEAK7* expression in LUAD and LUSC separately. *, P<0.05; ***, P<0.001. *MEAK7*, MTOR associated protein, Eak-7 homolog; TIDE, Tumor Immune Dysfunction and Exclusion. LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; GSEA, gene set enrichment analysis; NES, normalized enrichment score.

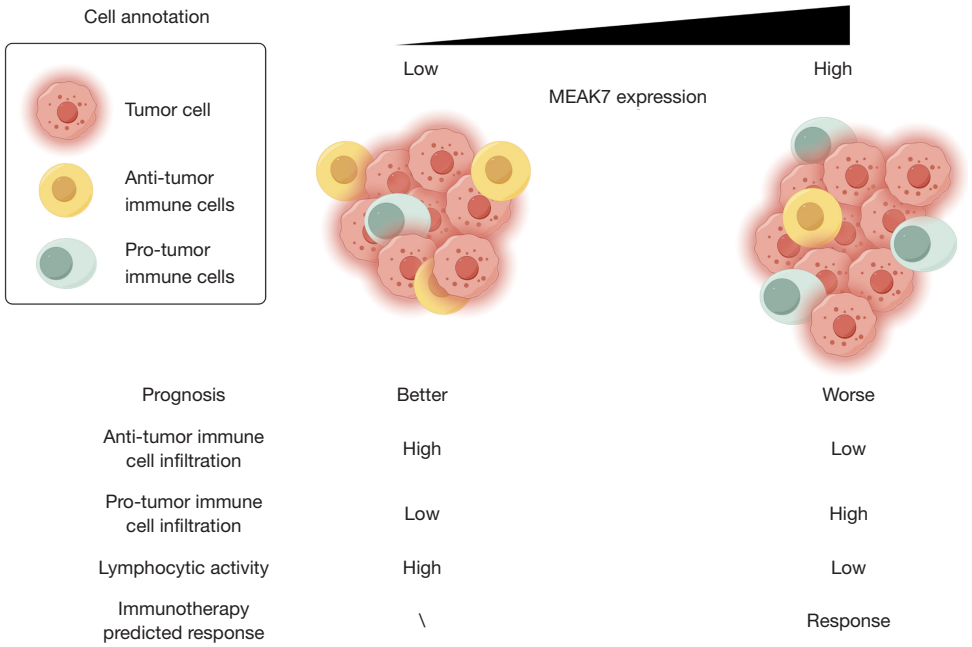


Figure 7 Graphical abstract. *MEAK7* plays a role in predicting the tumor immunophenotype and clinical outcomes in NSCLC patients (By Figdraw). *MEAK7*, MTOR associated protein, Eak-7 homolog; NSCLC, non-small cell lung cancer.

patients with high *MEAK7* expression may be suitable candidates for immunotherapy, particularly immune checkpoint blockade. There is consensus that patients with a high TMB or a considerable level of heterogeneity are more likely to attain a higher objective response rate when treated with immunotherapy (30). Hence, we performed calculations to assess the TMB and heterogeneity in LUAD and LUSC patients separately. Our findings revealed a substantial mutation load in patients with high *MEAK7* expression (*Figure 6E,6F*). Additionally, patients within the MEAK7-high cohort displayed higher heterogeneity (*Figure 6G,6H*). This finding highlights the potential of targeting the MEAK7 pathway to enhance the effectiveness of immunotherapeutic approaches, as it appears to play a crucial role in modulating TME and immune response.

Discussion

In this study, we identified *MEAK7* as a strong prognostic factor of NSCLC. Moreover, the increased expression of *MEAK7* suggested a decreased infiltration of antitumor lymphocytes and a good response to immunotherapy (*Figure 7*).

Initially, our bioinformatics analysis leveraging the TCGA and GTEx public databases revealed that *MEAK7* expression levels in lung cancer are markedly elevated compared to those in normal lung tissue. Building on this observation, we proceeded to investigate the clinical prognostic significance of *MEAK7* expression in lung cancer patients. Our findings indicated that increased *MEAK7* expression is significantly correlated with various patient characteristics, including sex, age, clinical stage, histological grade, and the presence of metastasis. Moreover, our Kaplan-Meier survival analyses demonstrated a pronounced difference in survival rates among lung cancer patients stratified by *MEAK7* expression levels, with those exhibiting high *MEAK7* expression experiencing significantly poorer survival outcomes compared to those with low expression. Collectively, these results provide compelling evidence suggesting that *MEAK7* could be a promising independent prognostic biomarker for lung cancer.

However, there is insufficient research of MEAK7 in the field of tumor. MEAK7 is reported to promote the proliferation and migration of pancreatic adenocarcinoma cells (12). In the two widely recognized NSCLC cell lines, H1975 and H1299, stem-like $CD44^+/CD90^+$ cells, exhibited increased protein levels of mEAK-7, S6K2, n-cadherin (marker for the epithelial-mesenchymal transition state in cancer stem cells), and phosphorylated S6 and 4E-BP1. These findings suggest an activation of the mTOR

signaling pathway, which is associated with an enhanced invasive capacity when compared to the non-stem-like $CD44^{-}/CD90^{-}$ cells (11). Regarding the limited knowledge of MEAK7 in NSCLC, our discovery holds promise for advancing the field of targeted precision oncology.

TILs have been recognized as significant prognostic markers that have a crucial impact on tumor development and treatment responses within the TME (31). Previous studies have found that many genes can influence the prognosis of patients with tumors by affecting the infiltration of immune cells and anti-tumor immune responses (32-36). Therefore, we selected the ESTIMATE and CIBERSORT methods to evaluate the heterogeneity in the diversity of tumor-infiltrating immune cells. The *MEAK7*-high group contained more immunosuppressive cells and fewer cytolytic immune cells. To further validate the involvement of immune competency, we predicted that *MEAK7* might be involved in pathways related to immune regulation. This was proven through GSEA using the GO and Biocarta term databases. We found that most immunosuppressive pathways were enriched in the *MEAK7*-high group in lung cancer patients. These findings suggested a strong correlation between *MEAK7* expression and immune regulation. Tumors with elevated *MEAK7* expression levels may be immunosuppressive condition in patients. Considering the strong correlation between *MEAK7* and immune cells mentioned above, TIDE was used to predict the potential response to immunotherapy. Immune checkpoint genes validated the immune response in LUAD and LUSC. Previous studies have shown that heterogeneity and mutation burden are important hallmarks of cancers (25,27). Interestingly, *MEAK7* exhibited a noteworthy correlation with levels of heterogeneity and mutation load in lung cancer. In conclusion, our findings suggest that *MEAK7* is a potential biomarker and a therapeutic target for drug treatment in clinical practice.

The present study has contributed to a deeper comprehension of the relationship between *MEAK7* and lung cancer. Nonetheless, it is not without its limitations. Predominantly, the analyses in this study were confined to mRNA levels of *MEAK7*, and a more robust assessment that includes protein expression levels would bolster the credibility of our findings. Additionally, the study did not address the diagnostic and prognostic relevance of *MEAK7* in small cell lung cancer and large-cell lung cancer, which represents a significant gap in our current understanding. Future research should aim to bridge these gaps to provide a more holistic view of MEAK7's role in the spectrum of lung cancer.

Conclusions

We conducted a thorough evaluation of *MEAK7* to determine its potential role in promoting cancer and its significance as a prognostic marker for patients. Elevated levels of *MEAK7* correlate with tumor immunosuppression, suggesting the potential efficacy of immune checkpoint inhibitors. Consequently, these findings hold great value for enhancing our current comprehension of MEAK7's role and its potential translational application in determining lung cancer prognosis and guiding therapeutic selection.

Acknowledgments

The authors express their gratitude to TCGA database for data availability.

Footnote

Reporting Checklist: The authors have completed the REMARK reporting checklist. Available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-1448/rc

Peer Review File: Available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-1448/prf

Funding: None.

Conflicts of Interest: Both authors have completed the ICMJE uniform disclosure form (available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-1448/coif). J.M. reports that she was employed by Westlake Innovation Capital and declares that there is no conflict of interest regarding the publication of this paper. The other author has no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

References

1. Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018;68:394-424.
2. Meyer ML, Fitzgerald BG, Paz-Ares L, et al. New promises and challenges in the treatment of advanced non-small-cell lung cancer. Lancet 2024;404:803-22.
3. Keenan TE, Tolaney SM. Role of Immunotherapy in Triple-Negative Breast Cancer. J Natl Compr Canc Netw 2020;18:479-89.
4. Li M, Bhoori S, Mehta N, et al. Immunotherapy for hepatocellular carcinoma: The next evolution in expanding access to liver transplantation. J Hepatol 2024;81:743-55.
5. He R, Lao Y, Yu W, et al. Progress in the Application of Immune Checkpoint Inhibitor-Based Immunotherapy for Targeting Different Types of Colorectal Cancer. Front Oncol 2021;11:764618.
6. Long GV, Menzies AM, Scolyer RA. Neoadjuvant Checkpoint Immunotherapy and Melanoma: The Time Is Now. J Clin Oncol 2023;41:3236-48.
7. Thomas A, Giaccone G. Why has active immunotherapy not worked in lung cancer? Ann Oncol 2015;26:2213-20.
8. Ferlay J, Colombet M, Soerjomataram I, et al. Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. Int J Cancer 2019;144:1941-53.
9. Nguyen JT, Ray C, Fox AL, et al. Mammalian EAK-7 activates alternative mTOR signaling to regulate cell proliferation and migration. Sci Adv 2018;4:eaao5838.
10. Finelli MJ, Sanchez-Pulido L, Liu KX, et al. The Evolutionarily Conserved Tre2/Bub2/Cdc16 (TBC), Lysin Motif (LysM), Domain Catalytic (TLDc) Domain Is Neuroprotective against Oxidative Stress. J Biol Chem 2016;291:2751-63.
11. Nguyen JT, Haidar FS, Fox AL, et al. mEAK-7 Forms an Alternative mTOR Complex with DNA-PKcs in Human Cancer. iScience 2019;17:190-207.
12. Yuan P, Tang C, Chen B, et al. miR-32-5p suppresses the proliferation and migration of pancreatic adenocarcinoma cells by targeting TLDC1. Mol Med Rep 2021;24:752.
13. Goldman MJ, Craft B, Hastie M, et al. Visualizing and interpreting cancer genomics data via the Xena platform. Nat Biotechnol 2020;38:675-8.
14. Chandrashekar DS, Bashel B, Balasubramanya SAH, et al. UALCAN: A Portal for Facilitating Tumor Subgroup Gene Expression and Survival Analyses. Neoplasia 2017;19:649-58.
15. Lánczky A, Győrffy B. Web-Based Survival Analysis Tool Tailored for Medical Research (KMplot): Development and Implementation. J Med Internet Res 2021;23:e27633.
16. Mizuno H, Kitada K, Nakai K, et al. PrognoScan: a new database for meta-analysis of the prognostic value of genes. BMC Med Genomics 2009;2:18.
17. Yoshihara K, Shahmoradgoli M, Martínez E, et al. Inferring tumour purity and stromal and immune cell admixture from expression data. Nat Commun 2013;4:2612.
18. Newman AM, Liu CL, Green MR, et al. Robust enumeration of cell subsets from tissue expression profiles. Nat Methods 2015;12:453-7.
19. Yu G, Wang LG, Han Y, et al. clusterProfiler: an R package for comparing biological themes among gene clusters. OMICS 2012;16:284-7.
20. Hänzelmann S, Castelo R, Guinney J. GSVA: gene set variation analysis for microarray and RNA-seq data. BMC Bioinformatics 2013;14:7.
21. Ritchie ME, Phipson B, Wu D, et al. limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res 2015;43:e47.
22. Lin Z, Meng X, Wen J, et al. Intratumor Heterogeneity Correlates With Reduced Immune Activity and Worse Survival in Melanoma Patients. Front Oncol 2020;10:596493.
23. Thorsson V, Gibbs DL, Brown SD, et al. The Immune Landscape of Cancer. Immunity 2018;48:812-830.e14.
24. Jiang P, Gu S, Pan D, et al. Signatures of T cell dysfunction and exclusion predict cancer immunotherapy response. Nat Med 2018;24:1550-8.
25. Chalmers ZR, Connelly CF, Fabrizio D, et al. Analysis of 100,000 human cancer genomes reveals the landscape of tumor mutational burden. Genome Med 2017;9:34.
26. Mroz EA, Rocco JW. MATH, a novel measure of intratumor genetic heterogeneity, is high in poor-outcome classes of head and neck squamous cell carcinoma. Oral Oncol 2013;49:211-5.

27. Dagogo-Jack I, Shaw AT. Tumour heterogeneity and resistance to cancer therapies. Nat Rev Clin Oncol 2018;15:81-94.
28. Lyu GY, Yeh YH, Yeh YC, et al. Mutation load estimation model as a predictor of the response to cancer immunotherapy. NPJ Genom Med 2018;3:12.
29. He X, Xu C. Immune checkpoint signaling and cancer immunotherapy. Cell Res 2020;30:660-9.
30. Hellmann MD, Ciuleanu TE, Pluzanski A, et al. Nivolumab plus Ipilimumab in Lung Cancer with a High Tumor Mutational Burden. N Engl J Med 2018;378:2093-104.
31. Gentles AJ, Newman AM, Liu CL, et al. The prognostic landscape of genes and infiltrating immune cells across human cancers. Nat Med 2015;21:938-45.
32. Zou J, Chen Y, Ji Z, et al. Identification of C4BPA as biomarker associated with immune infiltration and prognosis in breast cancer. Transl Cancer Res 2024;13:25-45.
33. Wang K, Chen X, Liu Y, et al. SOX11 as a prognostic biomarker linked to m6A modification and immune infiltration in renal clear cell carcinoma. Transl Cancer Res 2024;13:3536-55.
34. Lin Z, Xie YZ, Zhao MC, et al. Xanthine dehydrogenase as a prognostic biomarker related to tumor immunology in hepatocellular carcinoma. Cancer Cell Int 2021;21:475.
35. Gao L, Wang W, Ma H, et al. Bioinformatics analysis reveals SOD1 is a prognostic factor in lung adenocarcinoma. Transl Cancer Res 2024;13:5522-34.
36. Wang H, Wang Y, Tan P, et al. Prognostic value and anti-tumor immunity role of TMED9 in pan-cancer: a bioinformatics study. Transl Cancer Res 2024;13:5429-45.

Cite this article as: Miao JY, Lin Z. Comprehensive analysis of the immunological implication and prognostic value of MEAK7 in non-small cell lung cancer. Transl Cancer Res 2025;14(2): 1085-1100. doi: 10.21037/tcr-24-1448