OncoTargets and Therapy

Dovepress
open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

Apolipoprotein E is a Potential Biomarker for Predicting Cancer Prognosis and is Correlated with Immune Infiltration

Jinji Chen[1,*], Herong Zhu[2,*], Shaohua Chen[3], Hua Mi[1]

[1]Department of Urology, the First Affiliated Hospital of Guangxi Medical University, Nanning, Guangxi Zhuang Autonomous Region, People's Republic of China; [2]Department of Urology, Guangdong Provincial Hospital of Integrated Traditional Chinese and Western Medicine, Foshan, People's Republic of China; [3]Department of Urology, Guangxi Medical University Cancer Hospital, Nanning, Guangxi Zhuang Autonomous Region, People's Republic of China

*These authors contributed equally to this work

Correspondence: Hua Mi, Department of Urology, the First Affiliated Hospital of Guangxi Medical University, Nanning, Guangxi Zhuang Autonomous Region, People's Republic of China, Email mihua2019@163.com; Shaohua Chen, Department of Urology, Guangxi Medical University Cancer Hospital, Nanning, Guangxi Zhuang Autonomous Region, People's Republic of China, Email silverchan1994@163.com

Background: Apolipoprotein E (APOE) is a polymorphic protein that plays a role in lipoprotein transformation and metabolism. It is involved in numerous physiological processes within the body and is closely associated with tumor growth and metastasis. However, the role of APOE in pan-cancer has yet to be evaluated. Therefore, studying the association between APOE and various cancer types is crucial for providing a basis for individualized treatment strategies and clinical prognosis assessment.

Methods: We investigated the diagnostic and prognostic significance of APOE across 33 tumor types, as well as its correlation with tumor mutation burden (TMB) and microsatellite instability (MSI). Additionally, we employed the ESTIMATE and CIBERSORT algorithms to analyze the potential impact of APOE on the immune system. Furthermore, gene set enrichment analysis (GSEA) was conducted to explore its underlying physiological function.

Results: Based on observations from a pan-cancer dataset, APOE expression was significantly different between cancer and normal tissues, and was simultaneously associated with survival outcomes in terms of cancer type, clinical annotation, TMB, MSI, and TICs abundance. In addition, the results also showed that expression of APOE may respond to a variety of cancer chemotherapy.

Conclusion: The findings from this study strongly indicate a close association between APOE and tumor development. Moreover, APOE shows promise as a potential biomarker for predicting prognosis and response to immunotherapy in patients with pan-cancer.

Keywords: apolipoprotein E, APOE, pan-cancer, prognosis, immune infiltration

Introduction

Cancer is the leading cause of premature death and reduced life expectancy in humans, imposing a significant burden on patients, their families, and society as a whole.[1] Immunotherapy with immune checkpoint blockade (ICB) has been a major breakthrough for cancer patients, but it is important to note that not all patients benefit from it.[2] Despite significant advancements in cancer research and management, there is still a need to explore better treatment options and strategies.[3] Furthermore, there is currently a lack of reliable biomarkers that can accurately predict the response to ICB therapy.[4] Therefore, there is an urgent need to study novel biomarkers that can be used to predict patient prognosis and monitor treatment responses, in order to advance personalized medicine and improve overall patient survival rates.

APOE is a component of triglyceride-rich lipoproteins and is associated not only with various neurodegenerative diseases, including Alzheimer's and Parkinson's diseases, but also with evidence indicating its involvement in tumor progression and metastasis.[5–8] For example, previous studies have demonstrated that APOE enhances the proliferation and growth of papillary thyroid cancer cells by promoting cellular glycolysis.[9] Additionally, lung adenocarcinoma

Received: 30 October 2023
Accepted: 12 March 2024
Published: 19 March 2024

patients with malignant pleural effusion presenting with APOE over-expression had a shorter overall survival time.[10] The expression of APOE is essential for the growth and survival of ovarian cancer cells and high serum APOE level can be used to assist in the diagnosis of ovarian cancer.[11] In gastric cancer, tumor-associated macrophages (TAMs) promote tumor cell metastasis by releasing exosomes that secrete APOE, thereby activating the PI3K-Akt signaling pathway.[8] In prostate cancer, tumor cells promote senescence in TREM2+ immunosuppressive neutrophils through the secretion of APOE.[12] However, APOE has been shown to exert anti-tumor effects and fulfill an essential role in reversing the immune suppressive phenotypes in the tumor microenvironment (TME).[13] There is evidence that APOE could decrease the abundance of myeloid-derived suppressor cells (MDSCs), an immunosuppressive cell population, and enhance T cell activation.[14] These findings highlight the dual role of APOE in tumors, indicating its important functions and influence in the tumor microenvironment, but whether it can serve as a reliable prognostic biomarker and predictor of immunotherapy response remains unknown.

In this study, we utilized The Cancer Genome Atlas (TCGA) and cBioPortal databases to investigate the prognostic value of APOE in pan-cancer. We explored the association between APOE and tumor mutational burden (TMB) as well as microsatellite instability (MSI). Additionally, we employed the ESTIMATE and CIBERSORT algorithms to calculate immune scores, stromal scores, and relative levels of tumor-infiltrating immune cell (TIC) subtypes. Gene set enrichment analysis (GSEA) was performed to reveal the potential biological mechanisms of APOE in pan-cancer. The findings suggest that APOE can serve as a diagnostic and prognostic biomarker for pan-cancer patients, contributing to a deeper understanding of tumor immunological characteristics and the development of individualized treatment strategies.

Materials and Methods

Patient Information Processing and Differential Expression Analysis

We used the University of California Santa Cruz (UCSC Xena https://xena.ucsc.edu/) browser to download RNA sequencing and related clinical data from the TCGA database, containing 12,591 samples from 33 cancers. The expression matrix of APOE in all samples was extracted using the Practical Extraction and Report Language (PERL) script. Differences in APOE expression were analyzed and visualized between cancer and healthy tissues using R packages "ggpubr". All expression data were normalized via log2 conversion. Additionally, APOE expression in different cancer types was compared using the cBioPortal database (https://www.cbioportal.org).

Patients

Prostate adenocarcinoma (PRAD) tissues and normal tissues were surgically resected from patients at The First Affiliated Hospital of Guangxi Medical University between December 2022 and March 2023. Prostate adenocarcinoma tissues and normal tissues (n = 4) were collected from the same patients and promptly preserved in liquid nitrogen for further experiments. The patients participating in this study provided informed consent. The Ethics Committee of Guangxi Medical University approved this study (Approval Number: 2022-E390-01), and it was conducted in accordance with the principles outlined in the Declaration of Helsinki.

Association of APOE with Survival Information and Clinical Annotations

Overall survival (OS), disease-specific survival (DSS), disease-free interval (DFI), and progression-free interval (PFI) were evaluated. Univariate Cox regression and Kaplan-Meier analyses were conducted to explore the influence of APOE on patient survival in pan-cancer using the R packages "survminer" and "survival". Clinical annotations correlation analyses were performed using the "limma" package and visualized with the "ggpubr" R package.

Correlation Between APOE Expression and Tumor Mutation Burden, Tumor Microsatellite Instability, and Therapeutic Responses

APOE expression was correlated with MSI and TMB based on Spearman correlation analysis and visualized with radar plots using the R package "fmsb". Additionally, the associations of APOE expression levels with chemotherapy responses in patients were analyzed using the ROC plotter server.

Correlation of APOE Expression with the Tumor Immune Microenvironment and Tumor-Infiltrating Immune Cell Profiles of Pan-Cancer Patients

The ESTIMATE algorithm was used to calculate the immune and stromal scores using the R packages "estimate" and "limma" in order to determine the proportion of infiltrating stromal/immune cells in pan-cancer samples. The immune cell infiltrations were assessed using the CIBERSORT algorithm. Correlation analysis of APOE expression and TICs levels was conducted using Spearman correlation analysis and visualized with the R packages "ggplot2", "ggpubr", and "ggExtra".

Relationship Between APOE Expression with Immune-Related Genes and Gene Set Enrichment Analysis with APOE in Pan-Cancer

Co-expression analysis was conducted using the "limma" R package and visualized with "'RColorBrewer" and "reshape2". GSEA was employed to uncover potential biological mechanisms of APOE in pan-cancer, and functional analysis was performed using the R-packages "limma", "clusterProfiler", and "enrichplot".

RNA Extraction and qRT-PCR

According to the manufacturer's protocol, total RNA was isolated using Axygen reagent (CORNING, Nanning, China). The primers sequences used for qRT-PCR were obtained from Sangon Biotech (Shanghai, China) as follows (5'-3'): APOE forward - GTTGCTGGTCACATTCCTGG, reverse - GCAGGTAATCCCAAAAGCGAC. β-actin forward - GTCATTCCAAATATGAGATGCGT, reverse - GCTATCACCTCCCCTGTGTG. Relative expression levels were calculated using the 2-ΔΔCt method. Statistical analyses were performed with GraphPad Prism.

Immunohistochemical Staining

Immunohistochemical (IHC) staining was performed after deparaffinization in xylene and rehydration in graded ethanol. The antigen was retrieved using sodium citrate buffer (pH 6.0) at 95 °C for 20 min. A 3% H2O2 solution was used to quench endogenous peroxidase activity and a 1% bovine serum albumin buffer was used to block non-specific binding. Subsequently, sections were incubated with the primary antibodies over night at 4 °C and then exposed to secondary antibodies for 40 minutes at room temperature after going through several washes. 3.3-diaminobenzidine tetrahydrochloride (DAB) was then applied to the sections. ImageJ software was used to analyze the intensity of staining. The slices were finally observed under a microscope, followed by optical density (OD) measurement by ImageJ software. The relative expression of APOE was presented as average optical density.

Statistical Analysis

The statistical analyses and diagram drawing were performed using R software (v4.1.2), ImageJ software (v1.8), and GraphPad Prism (v9.4). The expression patterns of APOE protein in tumor and healthy control tissues were compared using the *t*-test. Kaplan-Meier curves and univariate Cox regression analyses were conducted to determine the association between APOE expression and survival. A P-value< 0.05 was considered statistically significant.

Results

Differential Expression of APOE in Pan-Cancer

Based on the TCGA data, a differential analysis of APOE expression was conducted between cancer and healthy samples from 33 cancers (Figure 1A). The results revealed that APOE gene expression was highly elevated in invasive breast carcinoma (BRCA), esophageal carcinoma (ESCA), glioblastoma multiforme (GBM), head and neck squamous cell carcinoma (HNSC), liver hepatocellular carcinoma (LIHC), prostate adenocarcinoma (PRAD), stomach adenocarcinoma (STAD), thyroid carcinoma (THCA), and uterine corpus endometrial carcinoma (UCEC). In contrast, APOE expression levels were significantly downregulated in cholangiocarcinoma (CHOL), pheochromocytoma and paraganglioma (PCPG), and pancreatic adenocarcinoma (PAAD) tumor tissues compared to healthy tissues. Based on the cBioPortal database, genetic variations in the APOE gene were further studied. The findings demonstrated that APOE was altered in 149 of the 10,953

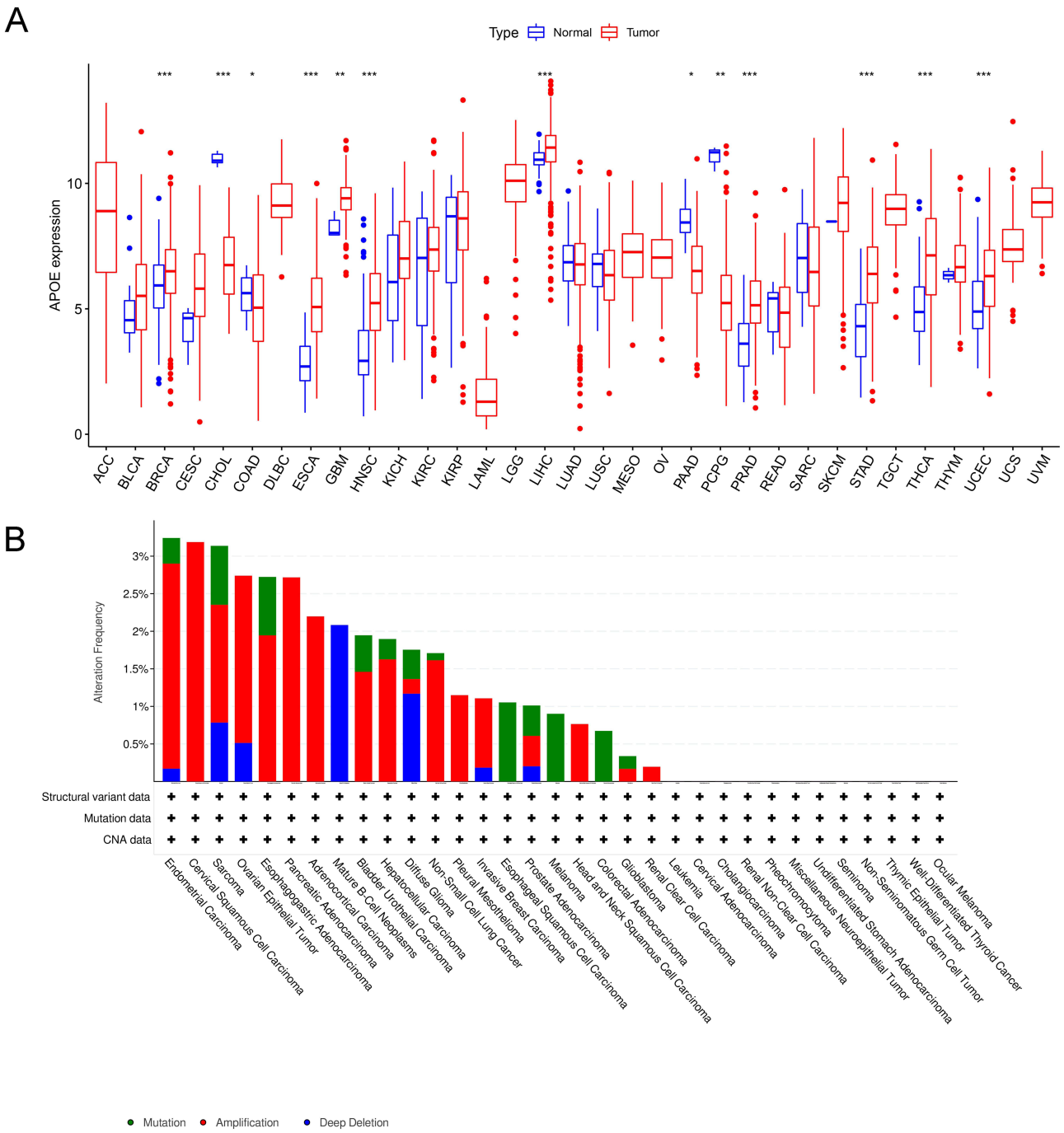

Figure 1 Differential expression and alteration of APOE. (**A**) Comparison of APOE levels between tumor and normal tissues. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$. (**B**) Alteration frequency of APOE mutations in multiple cancer types.

patients. Various alterations were present, including amplifications, mutations, and deep deletions, with amplifications being the most frequent alteration. Among the pan-cancer types, the alteration frequency of sarcoma was particularly significant (Figure 1B). Taken together, these results suggested the abnormal expression and alteration of APOE in pan-cancer.

Impact of APOE on Pan-Cancer Prognosis

Based on comprehensive correlation analyses of OS, DSS, DFI, and PFI, we aimed to investigate the expression level of APOE and its association with tumor prognosis in various tumor types. Kaplan-Meier survival analysis revealed

a positive correlation between elevated APOE expression and the prognosis of eight different types of cancer, including cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC) (OS: $P = 0.043$; DSS: $P = 0.028$; PFI: $P = 0.043$), lymphoid neoplasm diffuse large B-cell lymphoma (DLBC) (OS: $P = 0.013$; DSS: $P = 0.025$), brain lower grade glioma (LGG) (OS: $P = 0.026$), thyroid carcinoma (THCA) (DSS: $P = 0.048$; PFI: $P = 0.008$), uveal melanoma (UVM) (PFI: $P = 0.031$), and bladder urothelial carcinoma (BLCA) (DFI: $P = 0.041$). However, six other types of cancer exhibited a positive correlation between downregulated APOE expression and positive outcomes, including thymoma (THYM) (OS: $P = 0.019$; DSS: $P = 0.043$; PFI; $P = 0.042$), adrenocortical carcinoma (ACC) (DSS: $P = 0.029$; PFI: $P = 0.019$), PRAD (DSS: $P = 0.045$, PFI: $P = 0.015$), and STAD (DFI: $P = 0.027$). We observed a crossing of curves in LGG, THCA, and STAD, which prompted us to conduct validation of the proportional hazard assumption and perform interaction analysis on Cox regression models. The results showed that the P-value of interaction analysis was greater than 0.05, and the curves in the quadratic log survival curve of Cox regression were approximately parallel, indicating that Cox regression fulfilled the assumption of equal proportional hazards. Obviously, the differential expression of APOE exhibits distinct effects on the survival outcomes of cancer patients (Figure 2A-D).

Additionally, univariate Cox proportional hazards model analysis demonstrated that certain cancers exhibited improved survival rates when APOE expression was upregulated. These cancers included THYM (OS: HR = 2.173, $P = 0.004$; DSS: HR = 3.490, $P = 0.018$) (Figure 3A and B), STAD (DFI: HR = 1.249, $P = 0.045$; PFI: HR = 1.127, $P = 0.042$) (Figure 3C and D), and PRAD (DFI: HR = 1.375, $P = 0.043$; PFI: HR = 1.362, $P < 0.001$) (Figure 3C and D). In contrast, APOE expression had a negative impact on the prognosis of KIRC (OS: HR = 1.153, $P = 0.014$; DSS: HR = 1.216, $P = 0.007$; PFI: HR = 1.146, $P = 0.021$) (Figure 3A, B and D), THCA (OS: HR = 0.690, $P = 0.006$; DSS: HR = 0.485, $P = 0.004$; PFI: HR = 0.856, $P = 0.022$) (Figure 3A, B and D), UVM (OS: HR = 0.658, $P = 0.025$; DSS: HR = 0.660, $P = 0.03$; PFI: HR =0.647, $P = 0.012$) (Figure 3A, B and D), and LGG (OS: HR = 0.834, $P = 0.004$; PFI: HR = 0.845, $P = 0.009$) (Figure 3A and D).

Relationship Between APOE Expression with Clinical Phenotypes in Various Cancers

The relevance of tumor stage, age, and gender with APOE was analyzed to determine the correlation between APOE expression and the clinical phenotypes of various cancer types. Figure 4A depicts that APOE expression was higher in the advanced stage of BRCA ($P = 0.0055$), ESCA ($P = 0.0056$), and KIRC ($P = 0.0012$). In contrast, stages I and II of LIHC ($P = 0.024$), THCA ($P = 0.019$), and testicular germ cell tumors (TGCT) ($P = 0.022$) exhibited high APOE expression levels (Figure 4A). Intriguingly, patients aged ≥ 65 years demonstrated higher APOE expression levels in BLCA ($P = 0.0031$), PRAD ($P = 0.045$), THCA ($P = 0.0035$), and UCEC ($P = 0.04$) (Figure 4B), while patients aged < 65 years with STAD ($P = 0.037$) and LGG ($P = 0.0029$) (Figure 4B) had higher APOE expression levels. Moreover, male KIRC patients ($P = 0.0096$) and SARC patients ($P = 0.014$), as well as female patients with lung squamous cell carcinoma (LUSC) ($P = 0.00026$), exhibited high APOE expression levels (Figure 4C). These results highlight significant differences and provide important insights for guiding the clinical treatment of patients.

Relationship Between APOE Expression with Tumor Mutation Burden and Tumor Microsatellite Instability

We analyzed the correlations between APOE expression levels, TMB, and MSI to investigate the potential of APOE in predicting immunotherapy responses. Specifically, we found that APOE expression was positively correlated with TMB in six types of tumors, namely BRCA, COAD, PRAD, KIRP, KIRC, and BLCA. In contrast, APOE expression showed a negative correlation with TMB in five other types of tumors, namely THCA, STAD, SKCM, PAAD, and LGG (Figure 5A). Furthermore, the results indicated a positive correlation between APOE expression and MSI in SKCM, PRAD, DLBCL, COAD, and BRCA. Nevertheless, APOE expression showed a negative correlation with MSI in ACC, TGCT, STAD, PCPG, OV, LUSC, and acute myeloid leukemia (LAML) (Figure 5B). Subsequently, we investigated the impact of APOE expression on the response to chemotherapy in several commonly occurring cancers. Significantly, patients with ovarian and breast cancers exhibiting high APOE expression showed a reduced responsiveness to chemotherapy, while patients with glioblastoma and colorectal cancer displaying low APOE expression after

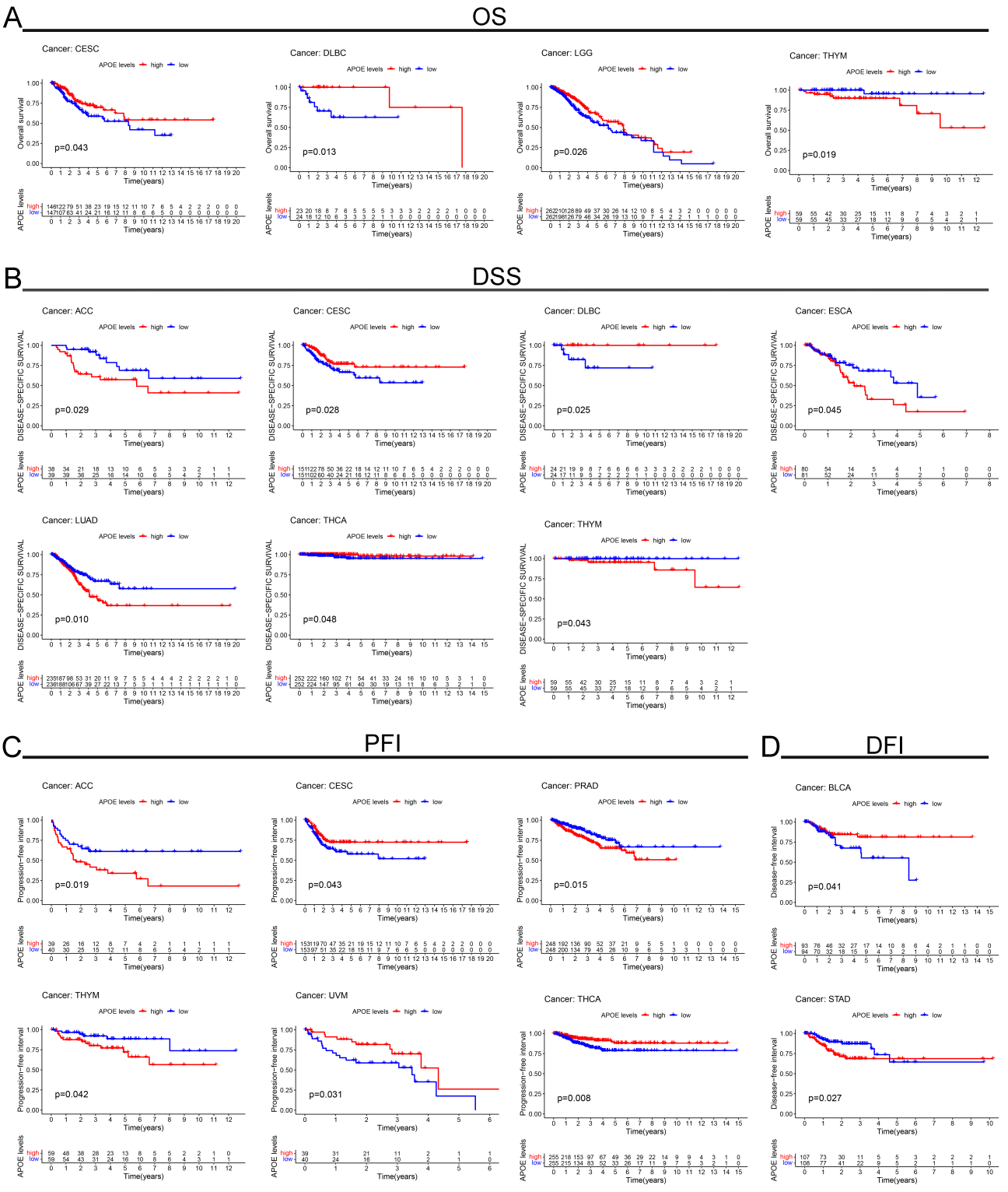

Figure 2 An analysis of Kaplan–Meier survival data showing the correlation between APOE expression levels and survival times. (**A**) OS for APOE expression in CESC, DLBC, LGG, and THYM. (**B**) DSS for APOE expression in ACC, CESC, DLBC, ESCA, lung adenocarcinoma (LUAD), THCA, and THYM. (**C**) PFI for APOE expression in ACC, CESC, PRAD, THYM, UVM, and THCA. (**D**) DFI for APOE expression in BLCA and STAD.

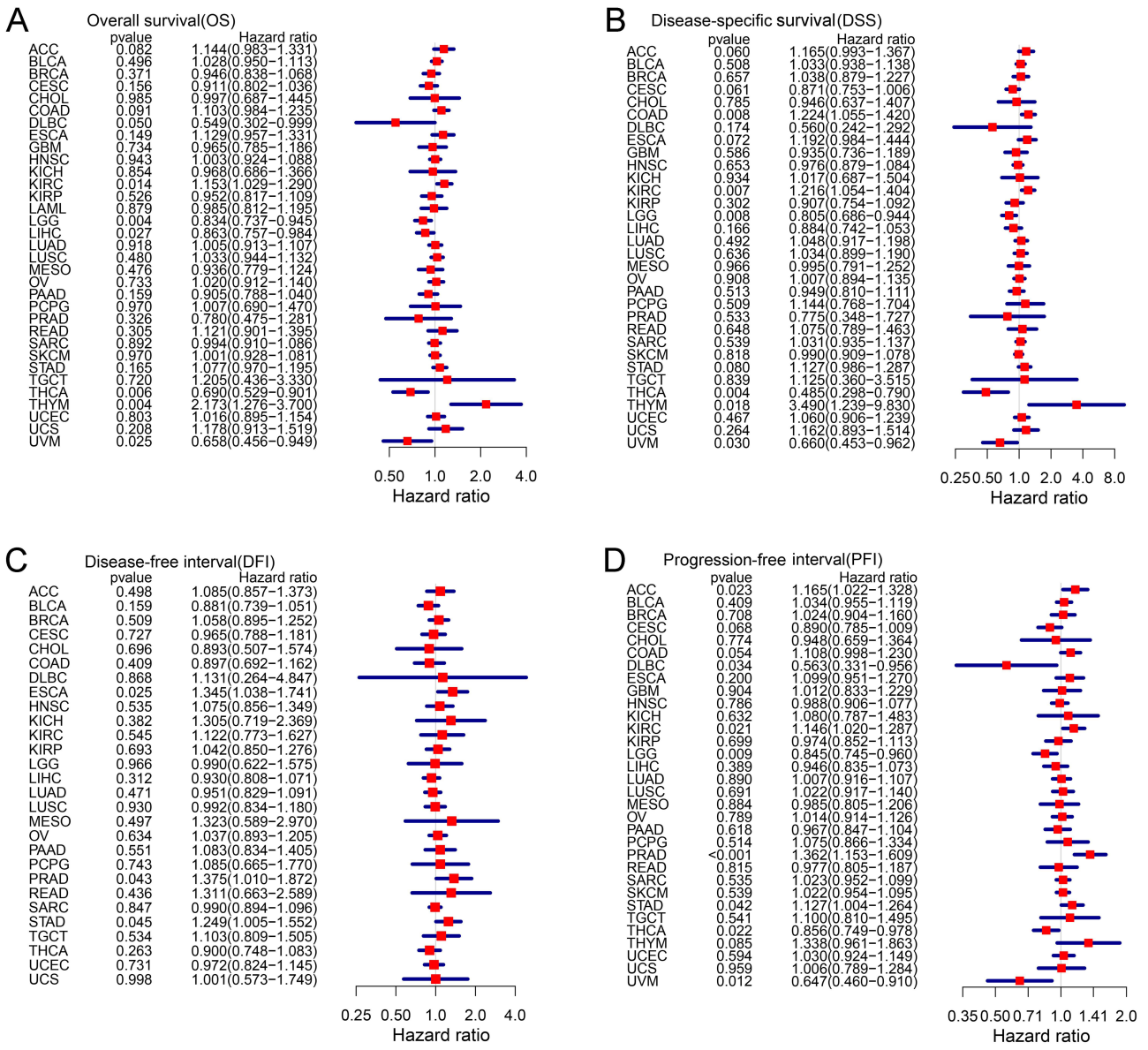

Figure 3 Analysis of univariate Cox data showing the relationship between APOE expression level and survival time. (**A**) OS for APOE expression. (**B**) DSS for APOE expression. (**C**) DFI for APOE expression. (**D**) PFI for APOE expression.

chemotherapy demonstrated improved survival outcomes (Figure 5C-F). Overall, these findings suggest that APOE may serve as a potential biomarker for predicting anti-tumor therapeutic efficacy.

Relationship Between APOE Expression with the Tumor Microenvironment and Immune Cell Infiltration

To clarify the correlation between APOE expression and TME, which can regulate cancer progression and influence therapeutic outcomes,[15] we further explore the components of TME in various types of cancer. The six types of tumors (CESC, THCA, LGG, ESCA, LUAD, and THYM) were categorized into a high-risk group (Figure 6A) and a low-risk group (Figure 6B) based on Kaplan-Meier survival curves for patients with cancer.

We calculated immune and matrix scores for these cancer types and found a positive correlation between APOE expression and these scores. Next, we examined the correlation between APOE expression and TICs in CESC, THCA, LGG, ESCA, LUAD, and THYM. Our data demonstrated a positive association between APOE expression and M1

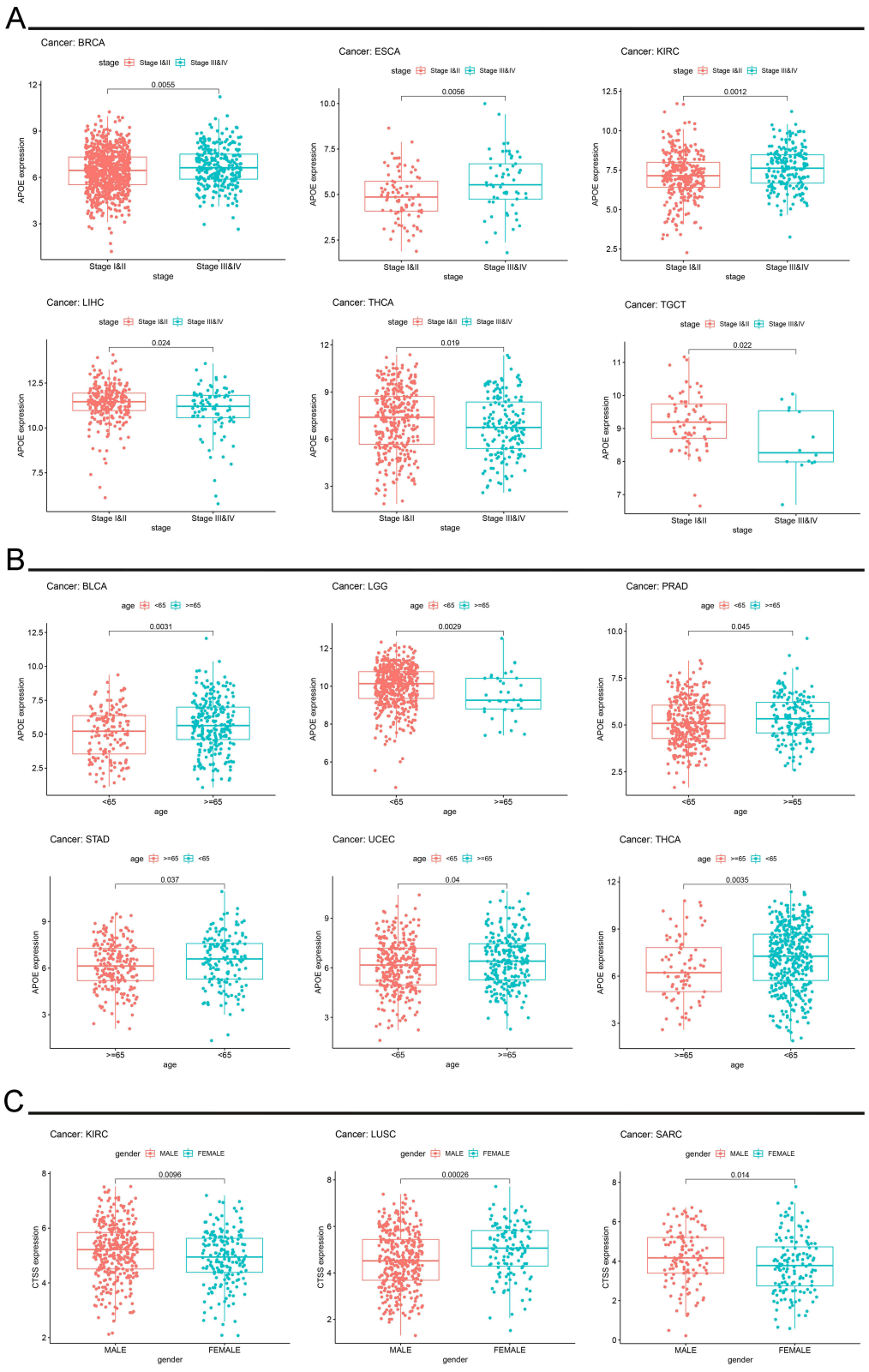

Figure 4 An analysis of APOE expression levels and clinical annotations. (**A**) Relationship between APOE expression and tumor stages in BRCA, ESCA, KIRC, LIHC, THCA, and TGCT. (**B**) Relationship between APOE expression and age in BLCA, LGG, PRAD, STAD, UCEC, and THCA. (**C**) Relationship between APOE expression level and gender in KIRC, LUSC, and SARC.

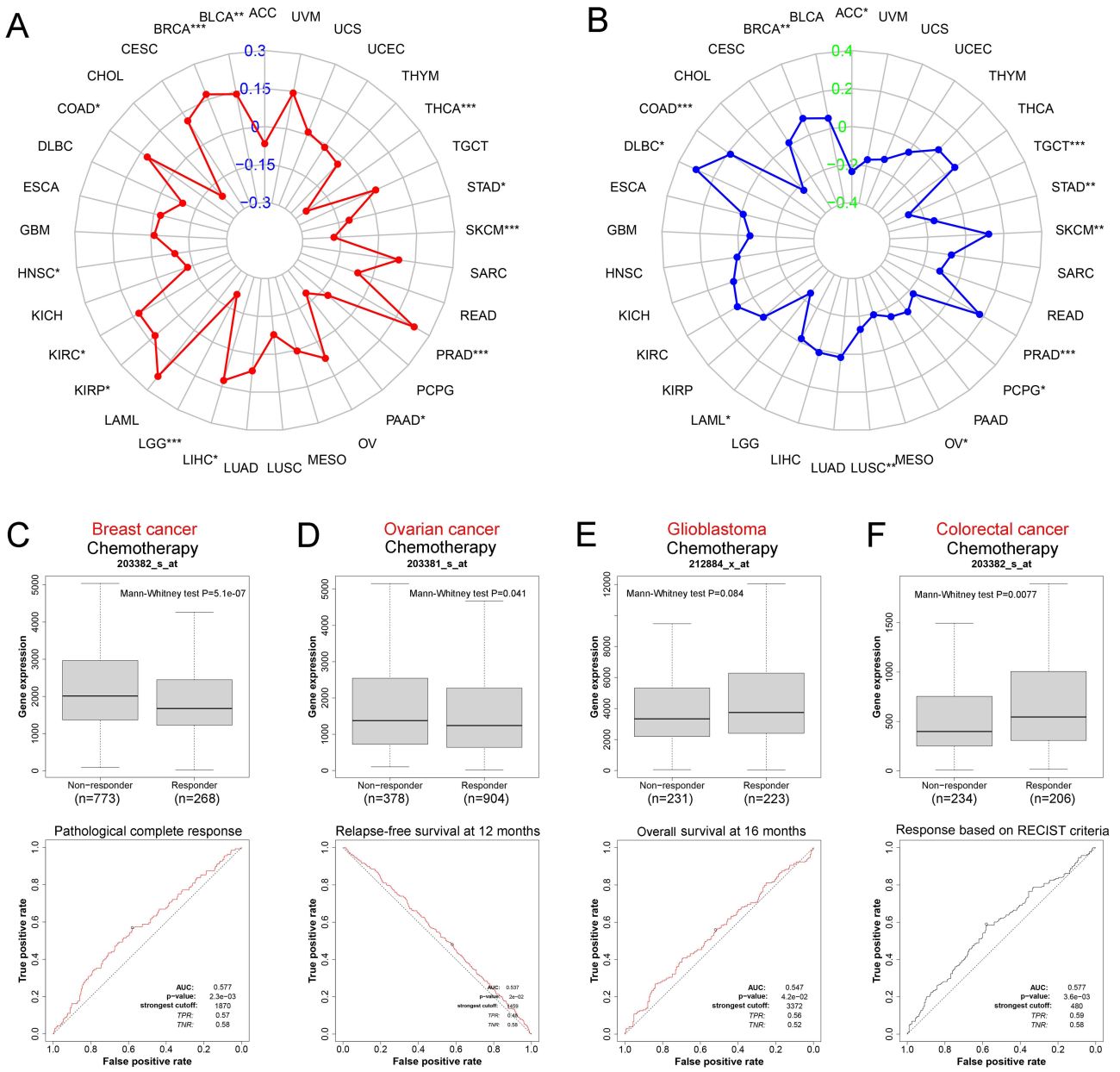

Figure 5 The association between APOE expression and TMB, MSI, and therapeutic responses in different types of cancer. (**A**) Relationship between APOE expression and TMB. (**B**) Relationship between APOE expression and MSI. (**C-F**) APOE expression is associated with chemotherapy responses in the breast, brain, colorectal, and ovarian cancer cohorts according to the receiver operating characteristic curve (ROC). *P < 0.05, **P < 0.01, and ***P < 0.001.

macrophages, M2 macrophages, and infiltrating CD8 T cells in CESC within the low-risk group (Figures 7A-C). Additionally, a negative association was observed between APOE expression and infiltrating activated mast cells, resting CD4 memory T cells, NK cells resting, and dendritic cells activated (Figures 7D-G). Similarly, in THCA, APOE expression showed a positive association with infiltrating memory B cells, infiltrating gamma delta T cells, and M2 macrophages (Figures 7H-K). However, a negative association was observed with infiltrating naive B cells, infiltrating monocytes, and infiltrating activated dendritic cells (Figures 7L-N). In LGG, APOE expression showed a positive association with the levels of infiltrating monocytes (Figures 7O). However, a negative correlation was observed with M0 macrophages, M1 macrophages, and infiltrating neutrophils (Figures 7P-R). In the high-risk group, APOE expression showed a positive association with the levels of infiltrating plasma cells, M2 macrophages, and activated CD4 memory T cells in THYM (Figures 8A-C). Moreover, APOE expression was positively associated with

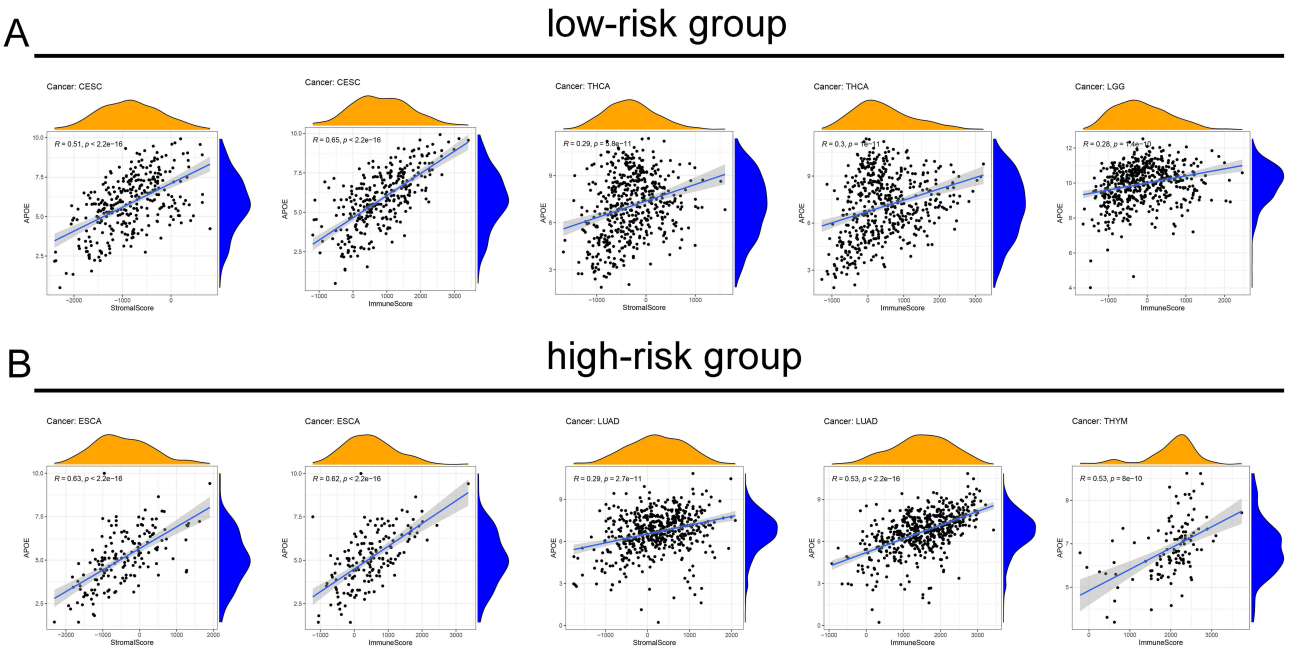

Figure 6 An analysis of the relationship between APOE expression and TME. (**A**) Relationship between APOE expression with stromal score and the immune score of the low-risk group. (**B**) Relationship between APOE expression with stromal score and the immune score of the high-risk group.

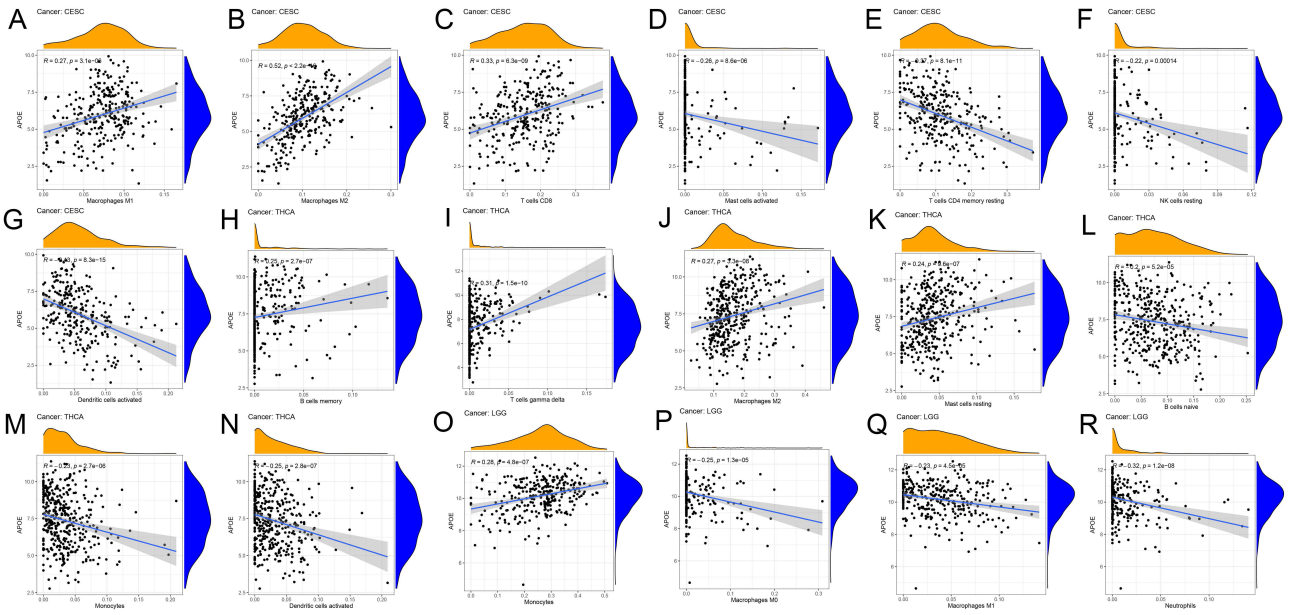

Figure 7 Association of infiltrating levels of immune cells with APOE expression in the low-risk group. (**A-G**) Relationship between each TIC and APOE expression in CESC. (**H-N**) Relationship between each TIC and APOE expression in THCA. (**O-R**) Relationship between each TIC and APOE expression in LGG.

the levels of M2 macrophages in ESCA (Figure 8D). Similarly, APOE expression was positively correlated with different subsets of tumor infiltrating T cells, including activated CD4 memory T cells, and infiltrating CD8 T cells. It showed a positive correlation with multiple subgroups of infiltrating macrophages, including M0 macrophages, M1 macrophages, and M2 macrophages but correlated negatively with resting CD4 memory T cells, infiltrating activated dendritic cells, and infiltrating naive B cells in LUAD (Figures 8E-L). Interestingly, we observed a correlation between APOE expression and the level of TAMs infiltration in various tumors. In summary, these results indicate that APOE expression can influence the development of cancer cells by reshaping the compositions in the TME. Next, we

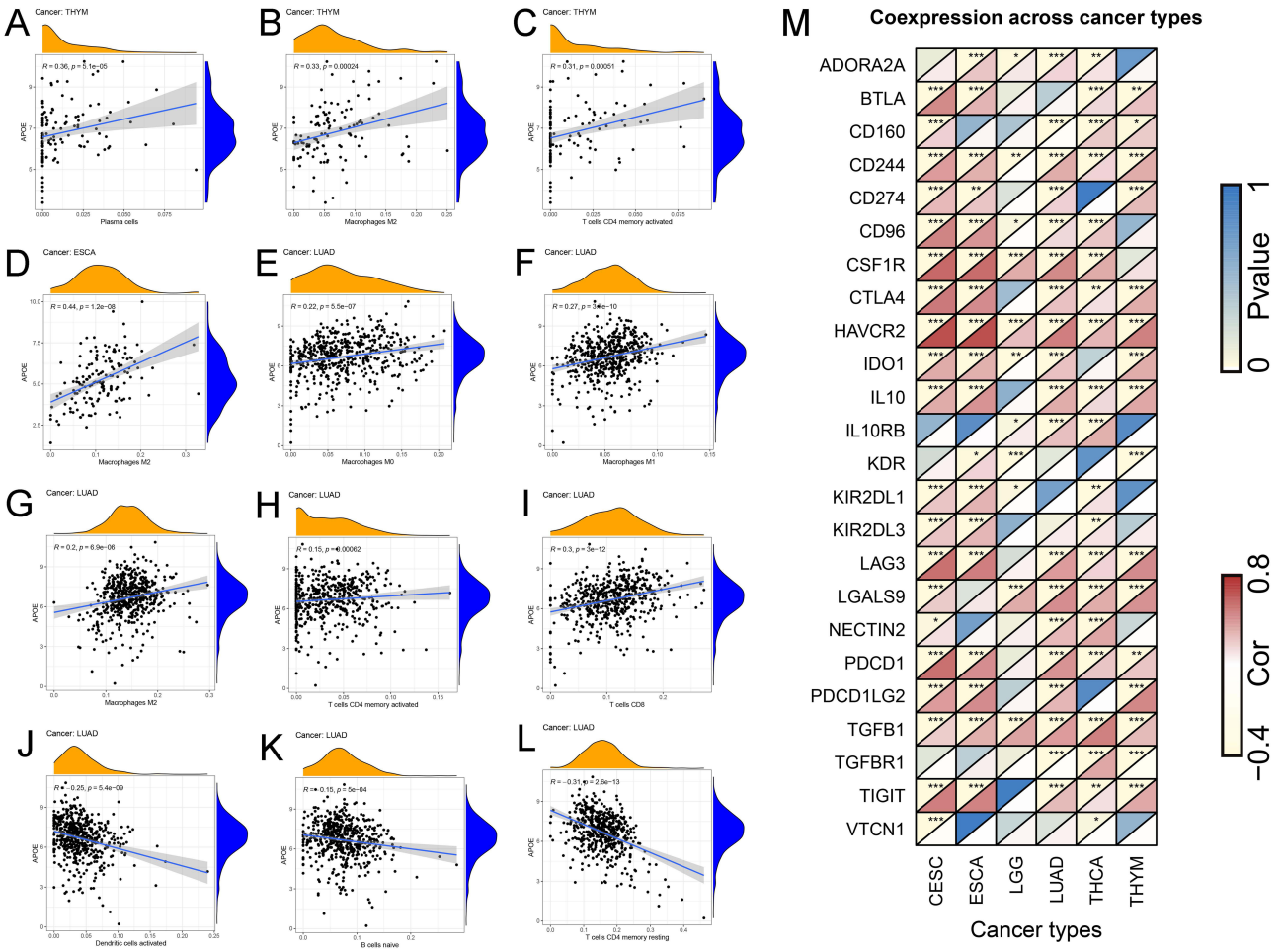

Figure 8 Association of infiltrating levels of TICs with APOE expression in the high-risk group and the relationship between immune-related genes and APOE expression. (**A-C**) Relationship between each TIC and APOE expression in THYM. (**D**) Relationship between each TIC and APOE expression in ESCA. (**E-L**) Relationship between each TIC and APOE expression in LUAD. (**M**) Relationship between immune-related genes and APOE expression. **P* < 0.05, ***P* < 0.01, and ****P* < 0.001.

performed co-expression analysis to confirm the associations between APOE and immune-related genes (Figure 8M). We found a positive correlation between APOE and several immune-related genes in all selected cancers, such as CSF1R, HAVCR2, PDCD1, and TGFB1. These results suggest that APOE is directly involved in tumor immunity or may play a potential role in tumor immunity.

Enrichment Analysis of Cancers in Different Groups

To investigate the potential biological function of APOE in pan-cancer, we conducted GSEA analysis for CESC, THCA, LGG, ESCA, LUAD, and THYM. The results of the GO functional annotation revealed that APOE was negatively correlated with the biological processes of membrane potential regulation, sensory perception of smell, and detection of chemical stimulus in LGG, LUAD, and THYM. In contrast, APOE was positively correlated with several immune-related functions in CESC, ESCA, THCA, and THYM. These activities included lymphocyte-mediated immunity, T cell receptor complex, keratinocyte differentiation, and B cell-mediated immunity (Figure 9A). KEGG pathway analysis revealed a positive correlation between APOE and several crucial pathways, such as the chemokine signaling pathway, ribosome, and olfactory transduction.[16–18] Finally, APOE showed a negative correlation with neuroactive ligand-receptor interaction and calcium signaling pathway in LGG, as well as valine, leucine, and isoleucine degradation, and glycosphingolipid biosynthesis globo-series in THCA (Figure 9B). In summary, the above results demonstrated the biological processes and immune-related pathways of APOE in several cancers.

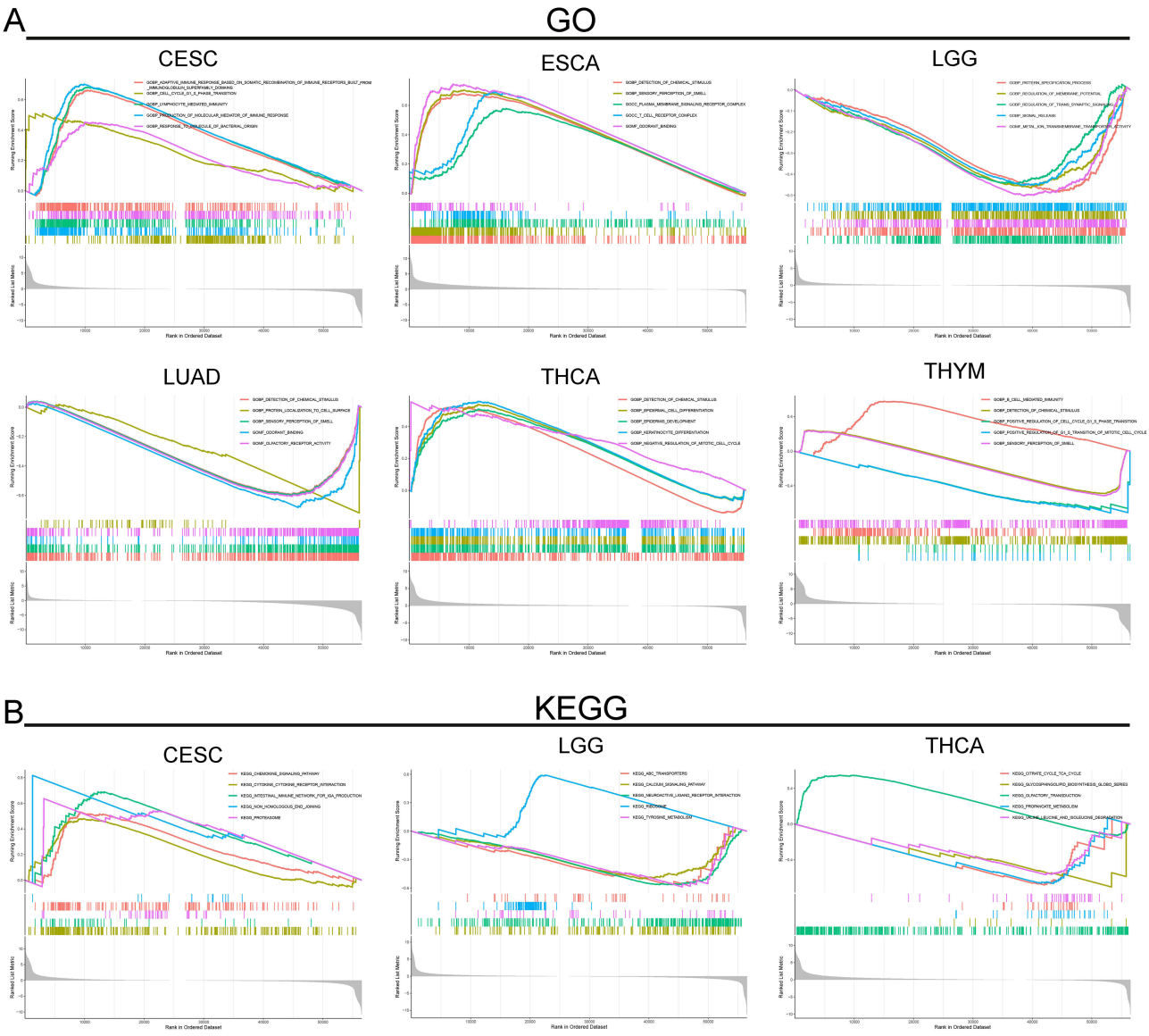

Figure 9 GSEA analysis of APOE gene in different groups. (**A**) GO functional terms of APOE. (**B**) KEGG pathway analysis of APOE.

Differential Expressions of APOE Between PRAD and Normal Samples

Using the TCGA database, we identified a significant up-regulation of APOE expression in PRAD. ($P < 0.001$; Figure 1A). At the histological level, we performed IHC staining to validate the differential expression of APOE in PRAD samples (Figure 10A, B). Furthermore, qRT-PCR results revealed an increase in APOE mRNA levels in PRAD tumor tissues (Figure 10C).

Discussion

In recent years, cancer has claimed tens of thousands of lives worldwide each year. It ranks second only to heart disease among all causes of death, resulting in family disruption and overwhelming financial burdens.[1] Over the past twenty-five years, the clinical application of targeted and immune-based therapies has significantly enhanced cancer treatment.[19] Despite researchers' efforts to improve the treatment outcomes of various cancers, the overall prognosis and survival rates remain discouraging.[3] Therefore, the discovery of biomarkers predicting cancer prognosis represents a valuable scientific pursuit providing crucial insights for the development of treatment strategies for cancer patients.

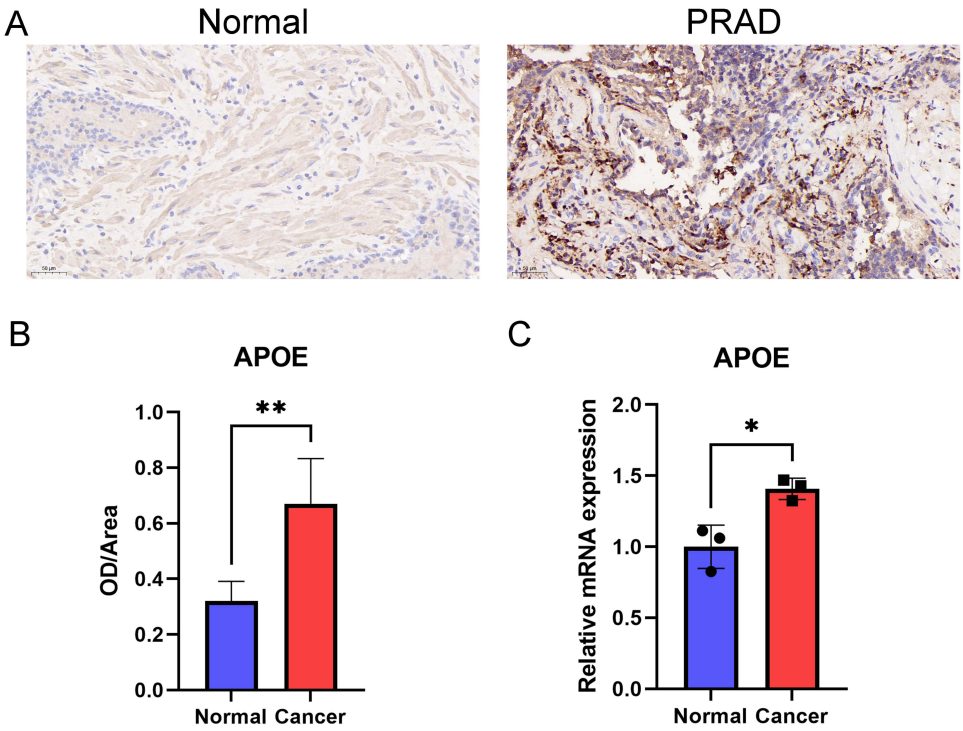

Figure 10 IHC staining validation and qRT-PCR. (**A**) IHC staining was applied to validate the differential expressions of APOE using PRAD samples. Scale bar = 50 μm. (**B**) ImageJ software was used to evaluate the relative expression of APOE, which was expressed as optical density (OD). (**C**) Comparison of APOE gene mRNA expression between normal and tumor tissues in PRAD. *P < 0.05, **P < 0.01. Data are expressed as mean ± SEM.

Pan-cancer analysis can uncover genetic aberrations between different types of tumors, offering insights into cancer treatment protocol design.[20] Recently, a substantial body of evidence has elucidated the relationship between cancer types and epigenome variation, methylation signatures, and cancer immunotherapy response. So far, certain members of the apolipoprotein family have demonstrated excellent diagnostic performance in specific types of cancer. For example, APOEBC-mediated mutations serve as favorable prognostic factors and predictors for immunotherapy in bladder cancer patients.[21] Studies have demonstrated that APOE, a member of the vertebrate exchangeable apolipoprotein family, also exerts a significant influence on cancer development.[22,23] In acute myeloid leukemia (AML), the binding of APOE is associated with T-cell suppression and tumor infiltration, which occurs via downstream signaling mediated by LILRB4 (Leukocyte immunoglobulin-like receptor B4) in AML cells.[24] In colorectal cancer, patients with elevated APOE mRNA levels demonstrated shorter overall survival and progression-free survival. Additionally, in vitro studies indicated that APOE facilitated the migration and invasion of colorectal cancer cells through its binding to LRP1 (low-density lipoprotein receptor-associated protein 1).[25] In this study, abnormal expression of APOE was observed in 13 cancer types, with high expression observed in prostate cancer cells, consistent with our experimental results and previous findings.[26] In contrast, APOE expression levels were lower in CHOL, COAD, PAAD, and PCPG in healthy tissues. However, due to database limitations, our analysis results exhibit some inconsistencies with previous studies. For example, in a large validation cohort, APOE exhibited significant enrichment in postoperative recurrent KIRC, whereas our results showed no difference in expression.[27] Moreover, survival analysis suggested that APOE expression was associated with the prognosis of some cancer patients. Notably, APOE was significantly related to tumor stages, age, and gender in various cancer types. These results are critical for guiding different patient populations in choosing the appropriate treatment. Also, APOE expression was correlated with infiltrating levels of TICs and indicated that APOE was directly involved in tumor immunity or could play a potential role in tumor immunity. Importantly, APOE also exerts an anti-tumor effect, and its expression significantly influences the response of several cancers to chemotherapy. Taken together, the above-mentioned findings suggested that APOE could be used as a biomarker for cancer prognosis.

Immunotherapy has gained significant attention in cancer research, particularly with the advent of immune checkpoint inhibitors, as an innovative therapeutic approach under clinical development. In recent years, researchers have been looking for ways to improve prognostic accuracy for patients. Several markers, including MSI, TMB, PD-1, and PD-L1 have been widely recognized for their roles in predicting the prognosis and their response to immunotherapy.[28–31] Previous studies have shown that colorectal cancer patients with microsatellite instability-high (MSI-H) may have better outcomes and increased sensitivity to immune checkpoint inhibitors.[32] Similarly, tumors with higher TMB have demonstrated longer overall survival and higher response rates to immune checkpoint blockade.[33] Higher mutation burden enables immune checkpoint inhibitors (ICIs) to block immune checkpoint proteins, such as PD-1 and PD-L1, resulting in enhanced therapeutic effects.[34] However, despite the long availability of immunotherapy for cancer in clinics, there is a need to enhance its therapeutic effect. A study has demonstrated that the utilization of APOE inhibitors in conjunction with anti-PD-1 therapy can effectively suppress tumor progression and facilitate tumor regression.[35] The present study provided compelling evidence supporting APOE serve as a predictor of immunotherapy effectiveness. We found a significant correlation between APOE expression and TMB as well as MSI. These findings suggest that APOE expression levels influence cancer TMB and MSI, ultimately impacting patient response to immune checkpoint inhibitor therapy. Consequently, APOE may serve as a valuable indicator for predicting the response rates of specific cancer types to immunotherapy and guiding clinical decision-making.

The TME consists of diverse cell types and extracellular components that play a pivotal role in tumor initiation, development, and metastasis.[36] Changes in TME components can impact the survival of tumor patients. Stromal components, as a key component of TME, can not only influence tumorigenesis and tumor progression but is also play a crucial role in the tumor resistance. For example, the stromal component can impede the effective delivery of therapeutic drugs to the target tissue due to fibrosis and high interstitial pressure.[37] Tumor-associated endothelial cells have been found to induce CD8+ T cell tolerance and are associated with adverse prognosis in cancer patients.[38,39] Immune cells within TME play a dual role in tumor progression.[40,41] In normal conditions, they function as surveillance agents, monitoring cancer cells and suppressing tumor growth.[42,43] However, certain immune cells facilitate immune evasion and assist tumor cells in evading immune surveillance through diverse mechanisms, thus avoiding immune-mediated destruction.[44] Immune cells play a crucial role in regulating tumor angiogenesis by interacting with vascular endothelial cells, an area of research that is currently highly active.[45] In addition, studies have reported that myeloid cells derived from the bone marrow, including macrophages, neutrophils, and MDSCs, have a positive impact on tumor blood vessel formation and maintenance.[46] Studies have shown that APOE induces the expression of immunosuppressive factors CXCL1 and CXCL5 in pancreatic tumor cells through LDL receptor and NF-κB signaling pathways, thereby inhibiting T cell infiltration in PDAC.[47] Our study demonstrated that APOE was significantly associated with immune and stromal scores in CESC, THCA, ESCA, and LUAD. Overall, the results herein suggested that APOE could be involved in TME remodeling, thereby affecting tumor progression.[13] Finally, the GSEA results suggested that APOE was associated with a pan-oncogenic immunomodulatory pathway, thereby shedding light on the underlying mechanisms of APOE in TME. Furthermore, these results suggest that APOE may impact tumor development by modulating the immune status of the TME immune status and influencing the survival outcomes of cancer patients.

In prostate cancer, cancer cells promote immunosuppressive neutrophil senescence in prostate cancer by activating the APOE/TREM2/ERK signaling pathway.[12] In addition, studies have shown that APOE is involved in reverse cholesterol transport, and cholesterol can be metabolized to androgens in prostate cells, thereby stimulating androgen receptors to promote tumor development.[48] Our immunohistochemistry and real-time PCR results showed that APOE was highly expressed in prostate cancer tissues, which was consistent with previous reports.

While the present study reached some key conclusions, it also had certain limitations. Firstly, the tumor cohort in this study was exclusively obtained from the TCGA database, and further validation supported by multiple databases is required. Furthermore, it is crucial to consider the inherent limitations of the database, including potential sample selection bias and limited clinical information. Secondly, one of the limitations of this study is the absence of information on the three subtypes of APOE (E2, E3, and E4) in the database we utilized. Although APOE was highly expressed in several tumors and associated with poor prognosis, the specific underlying mechanism was not explored. Therefore,

further investigation is needed to elucidate the specific role of APOE in each tumor. Future studies should focus on validating the expression and function of APOE both in vivo and in vitro.

Conclusion

In summary, the findings of this study suggest that APOE can serve as a prognostic biomarker for predicting the prognosis of various types of cancer. Furthermore, the observed correlation between APOE and TMB, MSI, as well as various components of TME, highlights the significant predictive value of APOE in determining efficacy of immunotherapy. These findings may provide valuable insights for early cancer diagnosis and precise treatment, and contribute to our understanding of the role of APOE in tumorigenesis and progression.

Acknowledgments

The authors would like to thank Home for Researchers (https://www.home-for-researchers.com), for editing the manuscript.

Funding

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article. This work was supported by the National Natural Science Foundation of China (NO. 81860142).

Disclosure

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

References

1. Bray F, Laversanne M, Weiderpass E, Soerjomataram I. The ever-increasing importance of cancer as a leading cause of premature death worldwide. *Cancer*. 2021;127(16):3029–3030.
2. Kalbasi A, Ribas A. Tumour-intrinsic resistance to immune checkpoint blockade. *Nat Rev Immunol*. 2019;20(1):25–39.
3. Chen F, Fan Y, Cao P, et al. Pan-Cancer Analysis of the Prognostic and Immunological Role of HSF1: a Potential Target for Survival and Immunotherapy. *Oxid Med Cell Longev*. 2021;2021:5551036.
4. Li B, Chan HL, Chen P. Immune Checkpoint Inhibitors: basics and Challenges. *Curr Med Chem*. 2019;26(17):3009–3025.
5. Slattery ML, Sweeney C, Murtaugh M, et al. Associations between apoE genotype and colon and rectal cancer. *Carcinogenesis*. 2005;26 (8):1422–1429.
6. Basit A, Cho MG, Kim EY, Kwon D, Kang SJ, Lee JH. The cGAS/STING/TBK1/IRF3 innate immunity pathway maintains chromosomal stability through regulation of p21 levels. *Exp Mol Med*. 2020;52(4):643–657.
7. Ostendorf BN, Bilanovic J, Adaku N, et al. Common germline variants of the human APOE gene modulate melanoma progression and survival. *Nat Med*. 2020;26(7):1048–1053.
8. Zheng P, Luo Q, Wang W, et al. Tumor-associated macrophages-derived exosomes promote the migration of gastric cancer cells by transfer of functional Apolipoprotein E. *Cell Death Dis*. 2018;9(4):434.
9. Huang J, Sun W, Wang Z, et al. FTO suppresses glycolysis and growth of papillary thyroid cancer via decreasing stability of APOE mRNA in an N6-methyladenosine-dependent manner. *J Exp Clin Cancer Res*. 2022;41(1):456.
10. Su WP, Chen YT, Lai WW, Lin CC, Yan JJ, Su WC. Apolipoprotein E expression promotes lung adenocarcinoma proliferation and migration and as a potential survival marker in lung cancer. *Lung Cancer*. 2011;71(1):28–33.
11. Kim YW, Bae SM, Kim IW, et al. Multiplexed bead-based immunoassay of four serum biomarkers for diagnosis of ovarian cancer. *Oncol Rep*. 2012;28(2):585–591.
12. Bancaro N, Cali B, Troiani M, et al. Apolipoprotein E induces pathogenic senescent-like myeloid cells in prostate cancer. *Cancer Cell*. 2023;41 (3):602–619 e611.
13. Talmadge JE, Gabrilovich DI. History of myeloid-derived suppressor cells. *Nat Rev Cancer*. 2013;13(10):739–752.
14. Tavazoie MF, Pollack I, Tanqueco R, et al. LXR/ApoE Activation Restricts Innate Immune Suppression in Cancer. *Cell*. 2018;172(4):825–840. e818.
15. Bejarano L, Jordao MJC, Joyce JA. Therapeutic Targeting of the Tumor Microenvironment. *Cancer Discov*. 2021;11(4):933–959.
16. Kanehisa M, Goto S. KEGG: Kyoto encyclopedia of genes and genomes. *Nucleic Acids Res*. 2000;28(1):27–30.
17. Kanehisa M, Furumichi M, Sato Y, Ishiguro-Watanabe M, Tanabe M. KEGG: integrating viruses and cellular organisms. *Nucleic Acids Res*. 2021;49(D1).
18. Kanehisa M. Toward understanding the origin and evolution of cellular organisms. *Protein Sci*. 2019;28(11):1947–1951.
19. Gotwals P, Cameron S, Cipolletta D, et al. Prospects for combining targeted and conventional cancer therapy with immunotherapy. *Nat Rev Cancer*. 2017;17(5):286–301.
20. Schaub FX, Dhankani V, Berger AC, et al. Pan-cancer Alterations of the MYC Oncogene and Its Proximal Network across the Cancer Genome Atlas. *Cell Syst*. 2018;6(3):282–300 e282.

21. Shi R, Wang X, Wu Y, et al. APOBEC-mediated mutagenesis is a favorable predictor of prognosis and immunotherapy for bladder cancer patients: evidence from pan-cancer analysis and multiple databases. *Theranostics*. 2022;12(9):4181–4199.
22. Krishnamurthy N, Goodman AM, Barkauskas DA, Kurzrock R. STK11 alterations in the pan-cancer setting: prognostic and therapeutic implications. *Eur J Cancer*. 2021;148:215–229.
23. Xu B, Lu M, Yan L, et al. A Pan-Cancer Analysis of Predictive Methylation Signatures of Response to Cancer Immunotherapy. *Front Immunol*. 2021;12:796647.
24. Gui X, Deng M, Song H, et al. Disrupting LILRB4/APOE Interaction by an Efficacious Humanized Antibody Reverses T-cell Suppression and Blocks AML Development. *Cancer Immunol Res*. 2019;7(8):1244–1257.
25. He L, Shi M, Ren S, et al. Jun-APOE-LRP1 axis promotes tumor metastasis in colorectal cancer. *Biomol Biomed*. 2023;23(6):1026–1037.
26. Mrkonjic M, Chappell E, Pethe VV, et al. Association of apolipoprotein E polymorphisms and dietary factors in colorectal cancer. *Br J Cancer*. 2009;100(12):1966–1974.
27. Obradovic A, Chowdhury N, Haake SM, et al. Single-cell protein activity analysis identifies recurrence-associated renal tumor macrophages. *Cell*. 2021;184(11):2988–3005 e2916.
28. Lin A, Zhang J, Luo P. Crosstalk Between the MSI Status and Tumor Microenvironment in Colorectal Cancer. *Front Immunol*. 2020;11:2039.
29. Liu L, Bai X, Wang J, et al. Combination of TMB and CNA Stratifies Prognostic and Predictive Responses to Immunotherapy Across Metastatic Cancer. *Clin Cancer Res*. 2019;25(24):7413–7423.
30. Hutarew G. PD-L1 testing, fit for routine evaluation? From a pathologist's point of view. *Memo*. 2016;9(4):201–206.
31. Postow MA, Callahan MK, Wolchok JD. Immune Checkpoint Blockade in Cancer Therapy. *J Clin Oncol*. 2015;33(17):1974–1982.
32. Le DT, Uram JN, Wang H, et al. PD-1 Blockade in Tumors with Mismatch-Repair Deficiency. *N Engl J Med*. 2015;372(26):2509–2520.
33. Lange M, Joly F, Vardy J, et al. Cancer-related cognitive impairment: an update on state of the art, detection, and management strategies in cancer survivors. *Ann Oncol*. 2019;30(12):1925–1940.
34. Jardim DL, Goodman A, de Melo Gagliato D, Kurzrock R. The Challenges of Tumor Mutational Burden as an Immunotherapy Biomarker. *Cancer Cell*. 2021;39(2):154–173.
35. Hui B, Lu C, Li H, et al. Inhibition of APOE potentiates immune checkpoint therapy for cancer. *Int J Biol Sci*. 2022;18(14):5230–5240.
36. Wu T, Dai Y. Tumor microenvironment and therapeutic response. *Cancer Lett*. 2017;387:61–68.
37. Hinshaw DC, Shevde LA. The Tumor Microenvironment Innately Modulates Cancer Progression. *Cancer Res*. 2019;79(18):4557–4566.
38. Zang X, Sullivan PS, Soslow RA, et al. Tumor associated endothelial expression of B7-H3 predicts survival in ovarian carcinomas. *Mod Pathol*. 2010;23(8):1104–1112.
39. Rodig N, Ryan T, Allen JA, et al. Endothelial expression of PD-L1 and PD-L2 down-regulates CD8+ T cell activation and cytolysis. *Eur J Immunol*. 2003;33(11):3117–3126.
40. Berntsson J, Nodin B, Eberhard J, Micke P, Jirstrom K. Prognostic impact of tumour-infiltrating B cells and plasma cells in colorectal cancer. *Int, J, Cancer*. 2016;139(5):1129–1139.
41. Lianyuan T, Dianrong X, Chunhui Y, Zhaolai M, Bin J. The predictive value and role of stromal tumor-infiltrating lymphocytes in pancreatic ductal adenocarcinoma (PDAC). *Cancer Biol Ther*. 2018;19(4):296–305.
42. Powell DR, Huttenlocher A. Neutrophils in the Tumor Microenvironment. *Trends Immunol*. 2016;37(1):41–52.
43. Dysthe M, Parihar R. Myeloid-Derived Suppressor Cells in the Tumor Microenvironment. *Adv Exp Med Biol*. 2020;1224:117–140.
44. Gajewski TF, Schreiber H, Fu YX. Innate and adaptive immune cells in the tumor microenvironment. *Nat Immunol*. 2013;14(10):1014–1022.
45. Turley SJ, Cremasco V, Astarita JL. Immunological hallmarks of stromal cells in the tumour microenvironment. *Nat Rev Immunol*. 2015;15(11):669–682.
46. Buss LA, Mandani A, Phillips E, Scott NJA, Currie MJ, Dachs GU. Characterisation of a Mouse Model of Breast Cancer with Metabolic Syndrome. *Vivo*. 2018;32(5):1071–1080.
47. Kemp SB, Carpenter ES, Steele NG, et al. Apolipoprotein E Promotes Immune Suppression in Pancreatic Cancer through NF-kappaB-Mediated Production of CXCL1. *Cancer Res*. 2021;81(16):4305–4318.
48. Celhay O, Bousset L, Guy L, et al. Individual Comparison of Cholesterol Metabolism in Normal and Tumour Areas in Radical Prostatectomy Specimens from Patients with Prostate Cancer: results of the CHOMECAP Study. *Eur Urol Oncol*. 2019;2(2):198–206.