Journal of Hepatocellular Carcinoma

Dovepress
Taylor & Francis Group

Open Access Full Text Article

ORIGINAL RESEARCH

Apolipoprotein E: A Potential Prognostic and Diagnostic Biomarker for Hepatocellular Carcinoma

Yuxia Li, Ruijiao Lu, Xieyidai Abuduhailili, Yangchun Feng

Department of Clinical Laboratory Center, Cancer Hospital Affiliated to Xinjiang Medical University, Urumqi, 830000, People's Republic of China

Correspondence: Yangchun Feng, Email paopao1987123@163.com

Purpose: The apolipoprotein E (APOE) gene is one of the strongest genetic determinants of the risk of developing late-onset Alzheimer's disease (AD) and may also increase the risk of cancer. However, its importance goes far beyond this. The aim of this study was to comprehensively analyze the potential role and prognostic value of APOE in hepatocellular carcinoma (HCC) using bioinformatics and multiplex fluorescence immunohistochemistry (mIHC).

Methods: Clinicopathologic samples from 90 hCC patients enrolled between April 2007 and June 2012 were included in this study. Researchers used tissue microarrays (HLiv180Su09) and multiple fluorescent immunohistochemical analyses to validate APOE protein expression and patient prognosis. Several online databases were used to investigate APOE expression and prognosis in HCC, followed by a comprehensive analysis of correlations between APOE and clinicopathologic features, immune cell infiltration levels, immune checkpoint genes, mutations, and functional enrichment analysis. The distribution of APOE in immune cell populations was also determined using a single-cell database.

Results: APOE mRNA was significantly overexpressed in HCC at both transcriptional and translational levels. Survival analysis suggested that APOE might be a favorable prognostic indicator for HCC patients. In addition to its involvement in immune cell infiltration, immune checkpoint gene expression, genetic variation, immunomodulatory genes, and methylation alterations in HCC, enrichment analysis showed that APOE was involved in multiple cancer-related signaling pathways.

Conclusion: This study comprehensively examines the critical role of APOE in HCC and highlights its significant potential as a biomarker and therapeutic target. This finding not only paves the way for new avenues of research in HCC, but also provides valuable insights into clinical diagnosis and treatment strategies.

Keywords: hepatocellular carcinoma, APOE, survival prognosis, tumor immunity, biomarker

Introduction

Hepatocellular carcinoma (HCC) is a prevalent malignancy that affects numerous individuals worldwide. Prominent risk factors associated with hepatocellular carcinoma include excessive body weight and obesity, both of which are acknowledged as contributing factors. The liver, which synthesizes most cholesterol and fatty acids, serves as the center of lipid metabolism. Aberrant lipid metabolism, a prominent hallmark of metabolic reprogramming in hepatocellular carcinoma (HCC), profoundly impacts patient prognosis through the modulation of inflammatory responses and alteration of the immune microenvironment. The incidence of liver cancer progressively increases annually, with approximately 910,000 new cases and 8.3 million deaths projected globally in 2020; liver cancer ranks sixth and third, respectively, in terms of patients and fatalities.[1] The predominant form of primary liver cancer is hepatocellular carcinoma, which constitutes approximately 90% of all cases.[2] The development of HCC is associated with various risk factors, including viral hepatitis, alcoholic hepatitis, and certain genetic metabolic disorders.[3,4] HCC is characterized by its insidious onset, challenging early diagnosis, rapid progression, and high rates of recurrence and metastasis. Standard treatment options for HCC include surgical resection, chemotherapy, radiation therapy, radiofrequency ablation, vascular embolization or liver transplantation. In recent years, immunotherapy has made significant advances in the treatment of HCC, offering new hope to patients. In particular, immune checkpoint inhibitors have emerged as a promising therapeutic approach with

Received: 18 November 2024
Accepted: 5 February 2025
Published: 17 February 2025

significant potential in the treatment of HCC.[5–9] However, effective treatments for advanced HCC remain limited.[10] Therefore, the identification of novel diagnostic genes and prognostic markers for hepatocellular carcinoma (HCC) is crucial to improve the accuracy of early detection and refine treatment strategies. Apolipoprotein E (ApoE) is a fragile glycoprotein consisting of 299 amino acids and has a molecular weight of 34 kDa. Its main function is as a lipid transporter, carrying phospholipids and cholesterol throughout the body. In peripheral tissues, APOE is primarily secreted by hepatocytes and macrophages and plays a pivotal role in lipid metabolism. It has been extensively investigated for its association with cardiovascular diseases[11] and Alzheimer's disease (AD).[12] Recent evidence suggests that APOE is closely implicated in diverse human malignancies, including brain tumors,[13] bladder cancer,[14] and breast cancer.[15] In the context of cancer, APOE exhibits multifaceted functions with specific roles that vary depending on the circumstances. For example, it regulates T-cell suppression in acute myeloid leukemia (AML)[16] while mediating cytotoxic T-cell responses in melanoma,[17] and its high expression in gastric cancer is associated with a shortened survival duration,[18] particularly when APOE is closely linked to muscle damage. In recent years, considerable progress has been made in elucidating the role of apolipoprotein E (APOE) and its variants in hepatocellular carcinoma (HCC) and the immune microenvironment. Two key studies have highlighted important aspects: one study uncovered the relationship between different APOE isoforms and the risk of developing HCC;[19] the other investigated how APOE methylation affects the tumour immune microenvironment, suggesting that APOE methylation may influence HCC progression by altering immune cell function and further investigating its influence on patient prognosis.[20] These studies have not only laid a strong scientific foundation for HCC research, but also provided new perspectives on the biological mechanisms driving HCC. Based on these findings, we performed an in-depth analysis of the role of APOE in HCC and its prognostic value using bioinformatics methods and multiplex fluorescence immunohistochemistry. Our findings not only underscore the prognostic significance of APOE in HCC, but also open up new possibilities for personalised diagnostic and therapeutic approaches for HCC patients.

Supplies and Procedures

Analysis of APOE Expression

I downloaded RNA-seq data for 33 tumors and obtained expression profile data for LIHC from the TCGA+GTEx database. Moreover, the CCLE database (https://portals.broadinstitute.org/ccle) was used to study APOE expression in tumor cell lines. The expression of APOE in LIHC was analysed by the Wilcoxon rank sum test. The TCGA database contains 424 cancer tissue samples, while among the LIHC samples used in this investigation, 50 paracancerous tissue samples from the GTEx database and 110 normal tissue samples were used. The expression difference data were collected and visually presented using the ggplot2 package in R software. Furthermore, the validation of APOE expression in LIHC involved the integration of data from the GEPIA (http://gepia.cancer-pku.cn/index.html) database and the GSE25097 dataset downloaded from the GEO (https://www.ncbi.nlm.nih.gov/geo/) database. Furthermore, data from the GSE125449 dataset were extracted from the scTIME portal, enabling an analysis of APOE expression across different cell types within liver tissue.

Protein Expression Level

According to the information retrieved from the CPTAC database, we acquired the protein expression profile of hepatocellular carcinoma (HCC) and subsequently categorized the samples into two groups based on the median value of APOE across all samples. Next, a chi-square test was performed to investigate the protein expression pattern of APOE in HCC, and box plots were obtained from the UALCAN (http://ualcan.path.uab.edu/) database. Subsequently, we validated the expression of APOE protein in liver cancer tissue using the Human Protein Atlas (http://ualcan.path.uab.edu/) database and demonstrated its subcellular localization in cancer cells through immunofluorescence staining images obtained from two human cancer cell lines (SK-MEL-30 and U2OS).

Prognostic Analysis of APOE

By utilizing the Kaplan–Meier Plotter (http://kmplot.com/analysis/) database and employing the Log rank test, we investigated the associations between APOE and overall survival (OS), progression-free survival (PFS), disease-specific survival (DSS), and recurrence-free survival (RFS) in patients with hepatocellular carcinoma within the "liver cancer mRNA" module. To further assess the clinical diagnostic value of the APOE gene, we performed receiver operating characteristic (ROC) analysis. An area under the curve (AUC) exceeding 0.7 indicates heightened accuracy associated with this gene. Subsequently, OS univariate and multivariate Cox regression analysis were performed to assess how APOE influences prognosis in HCC patients.

Gene Mutation and Methylation Analysis

By querying the cBioPortal database (http://www.cbioportal.org), we retrieved mutation information for the APOE gene from the Liver Hepatocellular Carcinoma (TCGA, Firehose, Legacy) dataset and investigated its potential correlation with patients' clinical survival prognosis. Furthermore, utilizing the Sangerbox online database, we examined the frequency of gene mutations in both the high-expression group (n=184) and low-expression group (n=185) among HCC patients. We assessed differences in gene mutation frequencies between each sample group using a chi-square test. MuTarget (https://www.mutarget.com/) is an online tool utilized for linking the mutation status of solid tumors with gene expression alterations. By selecting "all somatic mutations" as the specific type of somatic mutation and setting a minimum mutation frequency of 2%, we investigated the mutated genes (n = 358) that impact APOE expression in LIHC. The level of APOE promoter DNA methylation is determined through the utilization of an online platform available at https://grswsci.top/analyse, which provides comprehensive visualization capabilities. Additionally, we used the MethSurv database (https://biit.cs.ut.ee/methsurv/) to evaluate the methylation levels of APOE CpG sites and explored their correlation with prognosis. Finally, we applied Spearman correlation analysis to explore the relationships between APOE and m6A, m5C, and m1A regulatory proteins.

Relationships Between APOE and MMR, Methyltransferases, Immune Regulatory Genes, and Immune Checkpoints

Using TCGA expression profile data, we systematically examined the correlation between APOE expression and the expression of five mature mismatch repair genes (including MLH1, MSH2, MSH6, PMS2, and EPCAM). TCGA transplantation data were also used to analyse the translation of the APOE14 enzyme. By employing the "Immune Regulator Genes" module from the Sangerbox database, our study revealed significant associations between APOE expression levels and major histocompatibility complex (MHC), immunostimulatory factors, immunoinhibitory factors, chemokines, and chemokine receptors across diverse tissue types. Furthermore, employing Spearman analysis to investigate correlations between APOE expression and immune checkpoints enabled us to visualize these findings via coexpression heatmaps. To further validate our observations in hepatocellular carcinoma (HCC), we utilized both the GEPIA2 and TCGA databases to confirm strong correlations between APOE expression levels and specific immune checkpoint genes. Finally, sensitive small molecule drugs were predicted by the Cancer Therapy Response Portal (CTRP) (https://portals.broadinstitute.org). The correlation between APOE mRNA expression and the semi-inhibitory concentration was detected by Pearson correlation analysis. p values were adjusted by FDR; blue bubbles indicate negative correlations, red bubbles indicate positive correlations, and the darker the color is, the greater the correlation. Bubble size was positively correlated with FDR.

Analysis of Immune Cell Infiltration

The ESTIMATE algorithm was used to estimate the immune infiltration score, including the stromal score, immune score and ESTIMATEScore, of the HCC samples. Simultaneously, the R package "GSVA" was used to investigate the correlation between APOE and 24 immune infiltrating cells through single-sample gene set enrichment analysis (ssGSEA) using the Spearman method. The comparative enrichment scores for the high and low APOE expression groups were determined by utilizing characteristic genes of 24 previously validated immune cell types and gene

expression profiles of each tumor sample. Furthermore, TIMER (http://timer.comp-genomics.org/timer/) analysis was employed to investigate the association between APOE expression and the infiltration of immune cells (including B cells, CD4+ T cells, CD8+ T cells, neutrophils, macrophages, and dendritic cells) in LIHC. The "correlation" module was utilized to evaluate the correlation between APOE expression and immune marker genes in LIHC. Additionally, the "SCNA" module was used to compare different CNV variations of APOE with immune cell infiltrations.

Single-Cell Analysis

The Tumor Immune Single-cell Hub (TISCH) database (http://tisch.comp-genomics.org) serves as a valuable resource for evaluating the expression levels of APOE in distinct cell types within the tumor microenvironment.

GO, KEGG, and GSEA of APOE in Hepatocellular Carcinoma Patients

To investigate the potential role of APOE in hepatocellular carcinoma, we performed correlation analysis with other genes. The significance threshold was set at $|cor|>0.4$ and a false discovery rate (FDR)<0.05, and a Circos plot was generated to visualize genes highly correlated with APOE. APOE data were obtained from the TCGA database LIHC dataset and categorized into high expression and low-expression groups based on their expression levels. Differential expression analysis was conducted using the DESeq2 package, and differentially expressed genes (DEGs) with adjusted P values < 0.05 and $|log2FC|>1$ were selected and visualized using a volcano plot generated by the ggplot2 package in R software. Finally, we processed the enrichment analysis using the R package "ClusteProfiler", which covers GO terms and KEGG pathways as well as GSEA enrichment analysis. The GSEA analysis was considered to be significantly enriched when $|NES|>1$, NOM p.adjust<0.05 and FDR qvalue<0.25.

Multiple Fluorescence Immunohistochemistry (mIHC)

Using bioinformatics data, we procured liver hepatocellular carcinoma tissue microarrays (HLiv180Su09, K16-027, and TMA1-4) from Shanghai XinChao Biotechnology Co., Ltd., to validate APOE protein expression in liver cancer tissues. The microarray underwent dewaxing treatment at 63°C in an oven. Subsequently, the samples underwent pretreatment using commercial H2O2 and TBST washing steps. Various reagents, including anti-APOE rabbit monoclonal antibody and secondary antibodies, the fluorescent dyes Opal480 and Opal780, and DAPI working solution, were applied to the glass slides. Multiple spectral analyses were subsequently employed to assess the expression of APOE in hepatocellular carcinoma tissues. The comprehensive cellular organization and image processing were validated using Phenochart software and inForm software. To validate the prognostic value of APOE in hepatocellular carcinoma (HCC) patients, we conducted a survival analysis using comprehensive clinical and pathological data. Clinical patient information was extracted from 90 HCC tissue microarray samples (follow-up period: 0.5–6 years; surgery time: February 2006–May 2007; follow-up time: April 2007–June 2012) obtained from HLiv180Su09 liver cancer patients. After excluding two patients lost to follow-up, we categorized APOE patients into a high-expression group and a low-expression group using an optimal grouping method. We explored the impact of APOE on overall survival (OS) in HCC patients by calculating 95% confidence intervals (CIs) and log-rank p values. Finally, univariate and multivariate Cox regression analyses were performed to evaluate the influence of age, sex, pathological stage, T stage, and APOE status on patient survival risk/protective factors among the cohort of 88 patients. Informed consent forms were signed by all the participants, and the study was approved by the Ethics Committee of the Affiliated Cancer Hospital of Xinjiang Medical University for the purpose of the present study (K-S2023001). All methods strictly comply with the relevant standards and regulations as required by the Declaration of Helsinki.

Statistical Methods

Statistical analysis was conducted using default parameters from various databases. The R software package used was version 4.1.1 of R. Kaplan–Meier survival curves were plotted using the survival R package. The data were analysed for receiver operating characteristic (ROC) curves using the pROC package. The results were visualized and subjected to correlation analysis using ggplot2, a visualization package in R. Spearman correlation analysis was employed, with statistical significance defined as $p < 0.05$.

Results

APOE Is Overexpressed in Hepatocellular Carcinoma (HCC)

The study flowchart is depicted in Figure 1. Analysis of APOE mRNA expression in various tumor types and their corresponding normal tissues from the TCGA and GTEx databases revealed upregulation of APOE mRNA in 10 tumor tissues, including breast invasive carcinoma (BRCA), esophageal carcinoma (ESCA), glioblastoma multiforme (GBM), neck squamous cell carcinoma (HNSC), renal clear cell carcinoma (KIRC), liver hepatocellular carcinoma (LIHC), prostate adenocarcinoma (PRAD), gastric adenocarcinoma (STAD), thyroid carcinoma (THCA) and uterine corpus endometrial carcinoma (UCEC) (Figure 2A). According to the CCLE database, APOE is also highly expressed in various tumor cell lines, especially in LIHC (Figure 2B). Notably, the levels of APOE mRNA were significantly greater in LIHC tumor tissues than in paracancerous tissues (Figure 2C–E). These findings were consistent across multiple datasets, including those from the GEPIA2 database analysis, which included 369 LIHC tissues versus 160 normal tissues, and from the GEO database GSE25097 dataset, which included 249 normal tissues, 40 cirrhotic tissues, and 268 LIHC tissues (Figure 2F and G). Single-cell sequencing analysis revealed that the liver tissue mainly contained malignant cells, macrophages, CAFs, T cells and other types of cells (Figure 3A), and APOE may be expressed primarily in malignant tumor cells in liver tissue (Figure 3B and C).

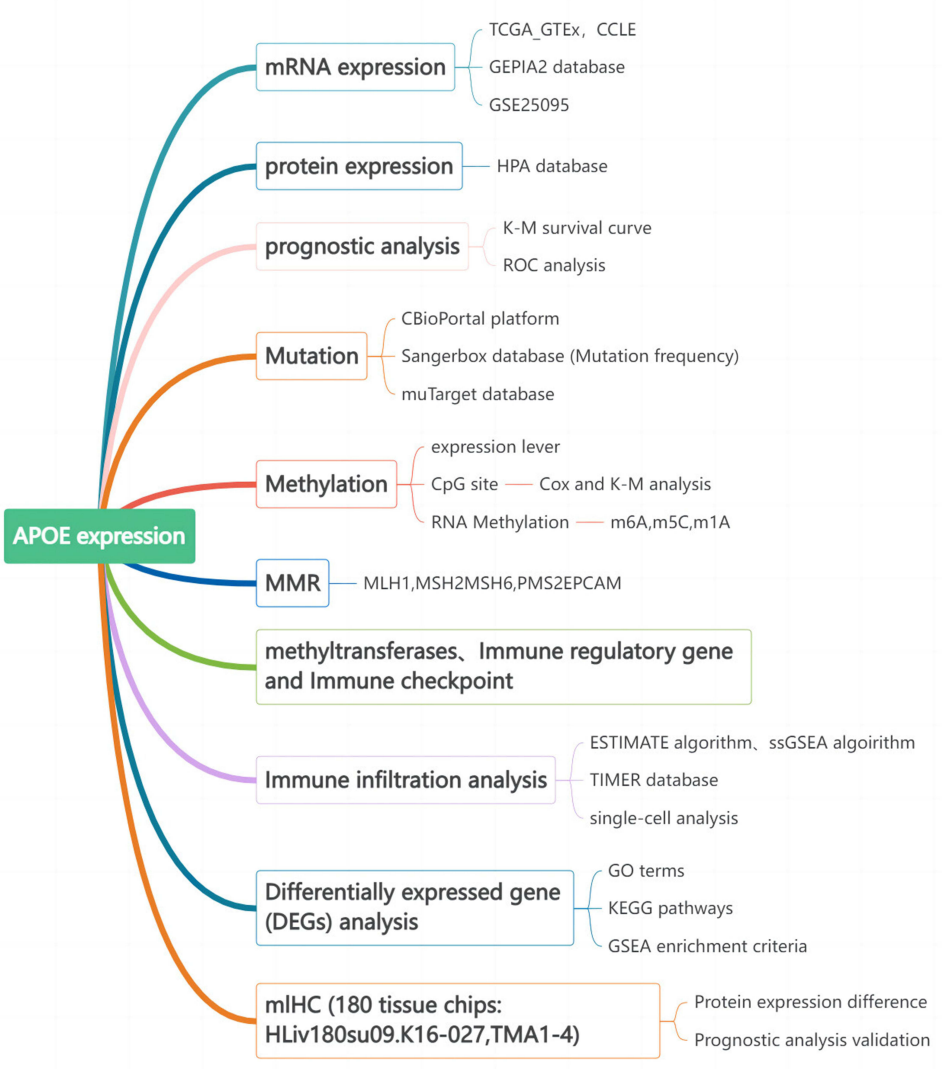

Figure 1 Research process diagram.

https://doi.org/10.2147/JHC.S504078

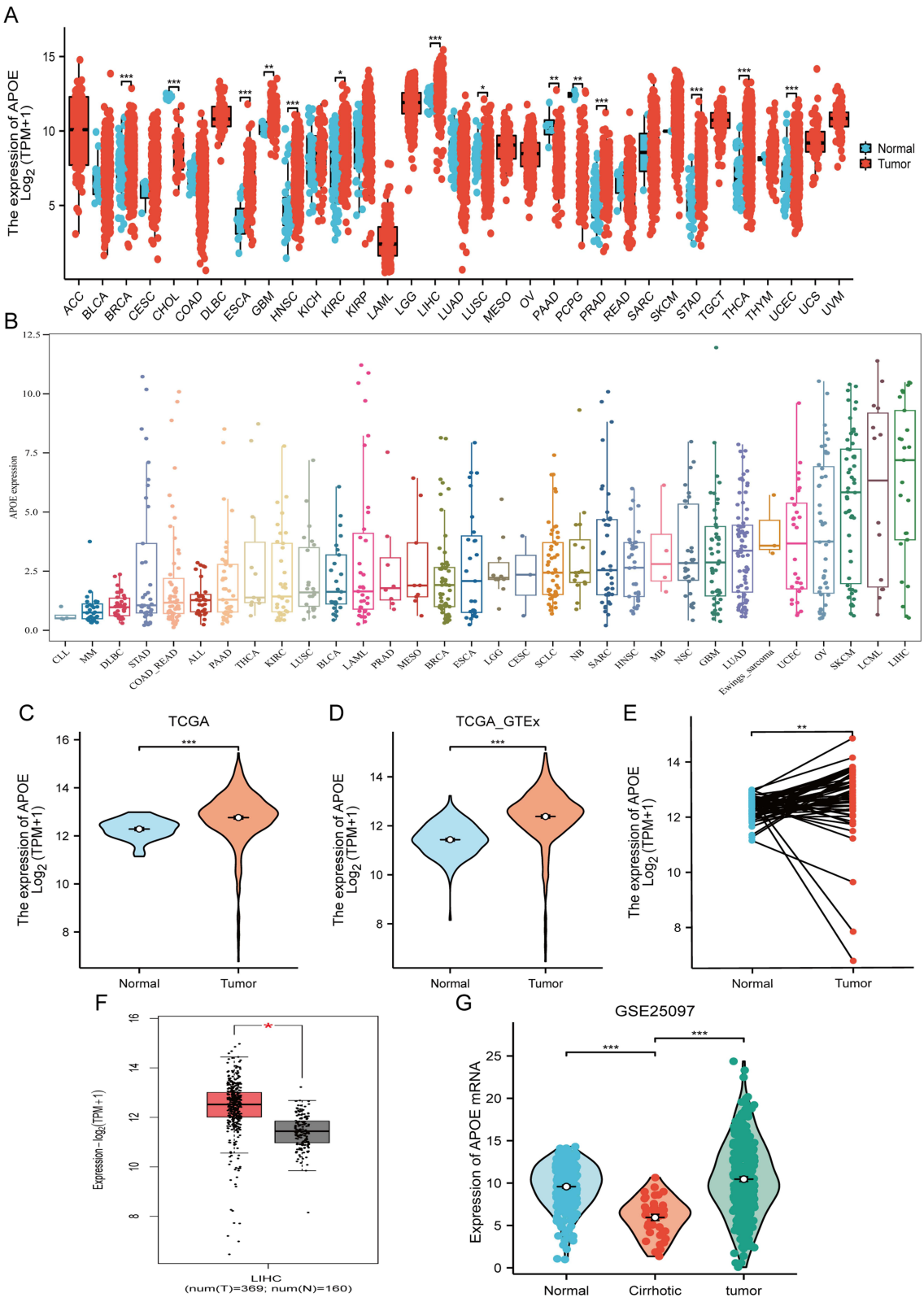

Figure 2 Expression of APOE. (**A**) Expression profile of APOE across cancers; (**B**) APOE expression levels in different cancer cell lines based on CCLE; (**C**) APOE expression in LIHC samples in the TCGA database; (**D**) APOE expression in LIHC samples in the TCGA combined with the GTEx database; (**E**) APOE expression in LIHC paired samples in the TCGA database; (**F**) APOE expression in LIHC in the GEPIA2 database; (**G**) APOE expression in LIHC in the GSE25097 dataset of the GEO database. *p < 0.05, **p < 0.01, ***p < 0.001.

https://doi.org/10.2147/JHC.S504078

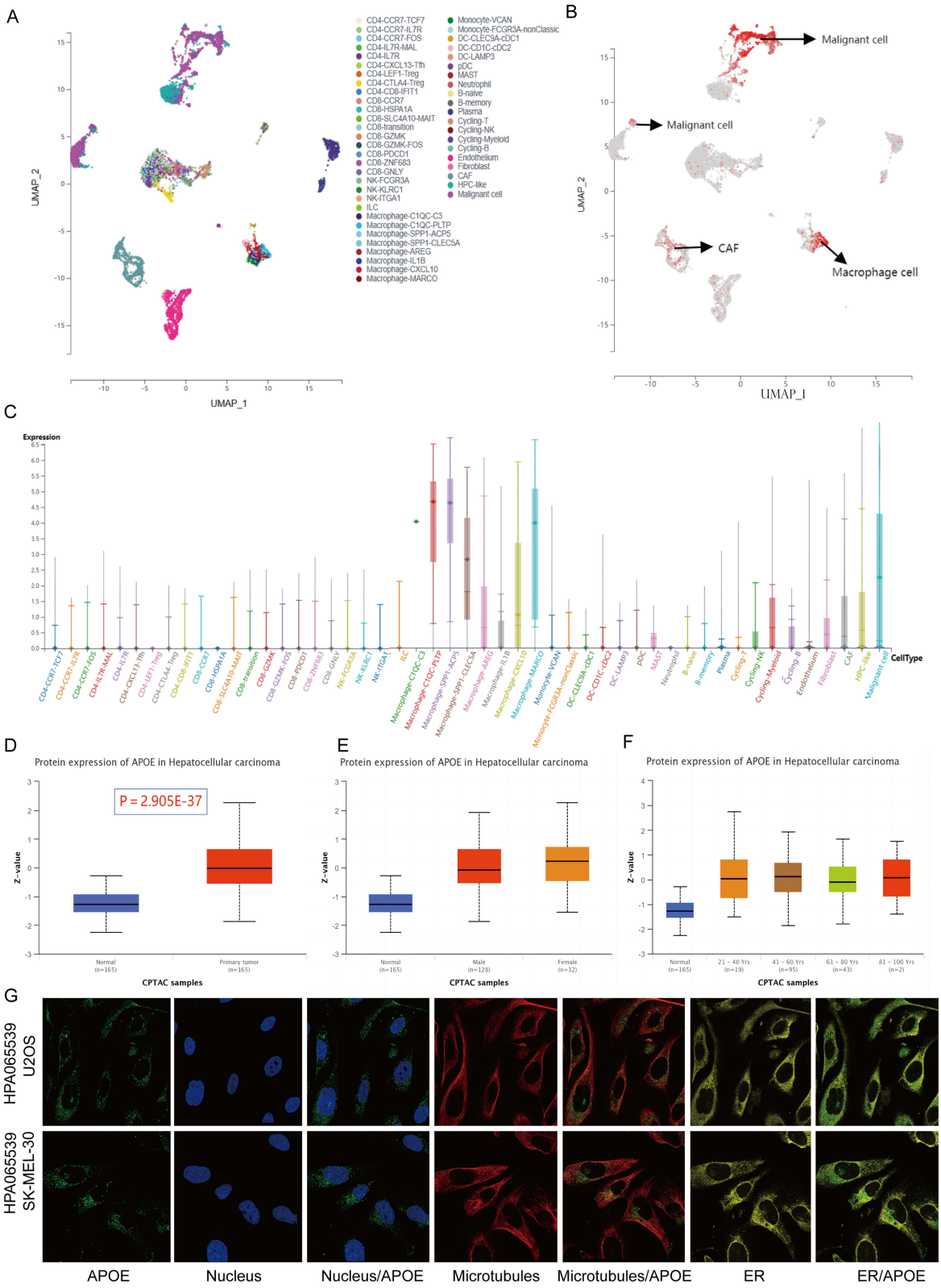

Figure 3 Protein expression of APOE in HCC patients. (**A–C**) Single-cell sequencing analysis of APOE expression in cells of different liver tumor types. (**D**) Differential expression of APOE protein in normal tissues and HCC tissues; (**E**) Differential expression of APOE protein in HCC tissues of different sexes (male and female) and normal tissues; (**F**) Differential expression of APOE protein in HCC tissues of patients of different ages (21–40 years; 41–60 years; 61–80 years) versus normal tissues; (**G**) Subcellular localization of APOE in SK-MEL-30 and U2OS cell lines.

https://doi.org/10.2147/JHC.S504078

Protein Expression Levels of APOE in Hepatocellular Carcinoma

To test the disparity in APOE protein expression levels between normal liver tissue and liver tumors, we conducted a validation using the CPTAC database. In the CPTAP samples, APOE protein expression was significantly elevated in hepatocellular carcinoma (HCC) (Figure 3D). However, no significant differences were found among HCC patients of different sexes (Figure 3E) or ages (Figure 3F). Moreover, the subcellular localization of APOE was determined by immunofluorescence localization in SK-MEL-30 and U2OS cell lines, revealing the predominant cytoplasmic distribution of the APOE protein (Figure 3G).

The Impact of the APOE Gene on the Prognosis of Hepatocellular Carcinoma Patients

The prognosis analysis revealed significant differences in overall survival (OS) (HR=0.59, P=0.0026), disease-specific survival (DSS) (HR=0.71, P=0.041), and progression-free survival (PFS) (HR=0.58, P=0.018) between the high and low-expression groups of APOE in HCC patients (Figure 4A–C). These findings suggest that increased APOE expression is related to a favourable prognosis for HCC patients and imply that the expression level of APOE may impact the survival prognosis of HCC patients to some extent. To further probe the clinical diagnostic value of APOE in HCC prognosis, we conducted ROC analysis by integrating the TCGA and GTEx databases. The results demonstrated an AUC of 0.829 with a 95% confidence interval [0.794, 0.864] when utilizing APOE as an adjunctive diagnostic indicator for HCC (Figure 4D). This indicates that APOE expression may be valuable for assisting in diagnosing HCC. The univariate Cox regression analysis confirmed that T stage, M stage, pathological stage, tumor status, and APOE expression were significant prognostic factors for the overall survival of HCC patients. Multivariate Cox analysis showed that tumour status and APOE expression were independent prognostic factors (Table 1). In general, the expression of APOE has certain reference value in the diagnosis and prognosis of HCC patients.

Differences in APOE Gene Mutations in Hepatocellular Carcinoma

To determine the presence of APOE gene mutations or copy number amplifications in HCC tissues, we used the cBioPortal database and observed that the mutation frequency and copy number increase of APOE in HCC were 0.28% and 1.69%, respectively (Figure 5B). Among 366 hCC patients, seven exhibited alterations in APOE, resulting in a mutation rate of 2% (Figure 5A). Notably, missense mutations were found to be the predominant type of genetic alteration in APOE, with the R56H mutation being detected in one HCC patient (Figure 5C). The three-dimensional structure of the APOE protein highlighting the R56H locus is depicted in Figure 5D. Furthermore, our analysis did not reveal any significant associations between APOE gene mutations and the survival outcomes of patients (Figure 5E). Finally, we explored the distribution of mutations associated with APOE expression in the LIHC cohort from the TCGA database. The waterfall map shows the distribution of the top 15 mutated genes, with significant differences between the high-expression and low-expression APOE groups. The five mutated genes with the greatest differences between the two groups included CTNNB1, ALB, PCDH15, KMT2D, and AHNAK (Figure 5F).

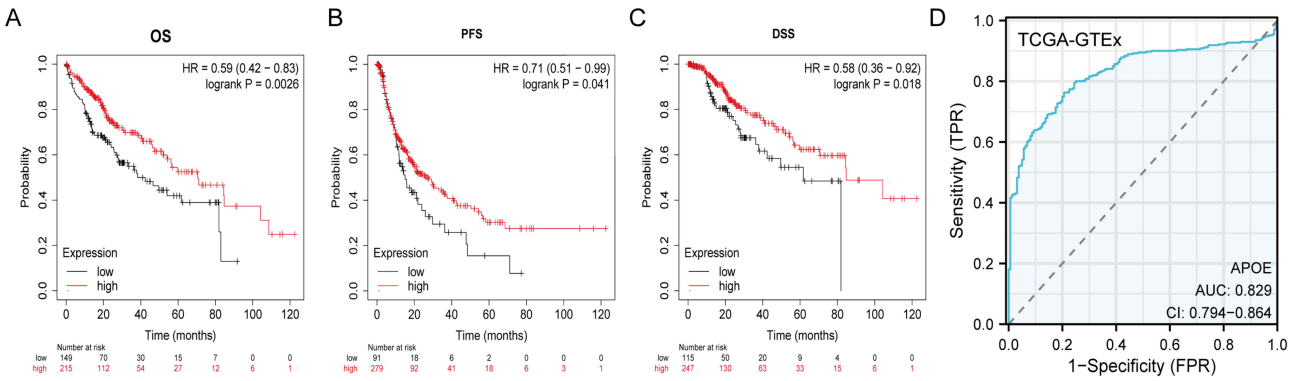

Figure 4 Prognostic analysis of APOE in HCC. (**A–C**) Kaplan–Meier survival curves comparing APOE expression in hepatocellular carcinoma patients by OS (**A**), PFS (**B**) and DSS (**C**); (**D**) APOE expression and diagnostic value in patients with HCC.

Table 1 Univariate and Multivariate Cox Analysis of APOE With Clinical Factors

Characteristics	N	Univariate Analysis		Multivariate Analysis	
		HR(95% CI)	P value	HR(95% CI)	P value
Gender	373				
Female	121	Reference			
Male	252	0.793 (0.557–1.130)	0.200		
Age	373				
<= 60	177	Reference			
> 60	196	1.205 (0.850–1.708)	0.295		
Pathologic T stage	370				
T1	183	Reference		Reference	
T2	94	1.428 (0.901–2.264)	0.129	0.000 (0.000 - Inf)	0.996
T3&T4	93	2.949 (1.982–4.386)	**< 0.001**	0.796 (0.107–5.912)	0.823
Pathologic N stage	258				
N0	254	Reference			
N1	4	2.029 (0.497–8.281)	0.324		
Pathologic M stage	272				
M0	268	Reference		Reference	
M1	4	4.077 (1.281–12.973)	**0.017**	1.591 (0.373–6.792)	0.530
Pathologic stage	349				
Stage I	173	Reference		Reference	
Stage II	86	1.416 (0.868–2.312)	0.164	30,355,992.5799 (0.000 - Inf)	0.996
Stage III&Stage IV	90	2.823 (1.862–4.281)	**< 0.001**	3.555 (0.470–26.868)	0.219
Tumor status	354				
Tumor free	202	Reference		Reference	
With tumor	152	2.317 (1.590–3.376)	**< 0.001**	1.849 (1.158–2.952)	**0.010**
APOE	373	0.867 (0.756–0.994)	**0.041**	0.820 (0.685–0.981)	**0.030**

Note: Bold black indicates p≤0.05.

Relationships Between APOE Expression and Mutated Genes and Methylation Levels

According to the results of the muTarget database, in CESC (n = 358), the mutated genes affecting APOE expression included the first 4 genes, KMT2D, RGPD4, KIF19 and MYH7 (Figure 6A). In other words, APOE expression in these gene mutant groups was significantly different from APOE expression in the wild-type group. Subsequently, we explored the DNA methylation level of APOE in LIHC and found that the methylation level of APOE in LIHC was lower than that in normal tissues (Figure 6B), and the expression of APOE mRNA was negatively correlated with the DNA methylation level in LIHC patients ($r = -0.29$, $p < 0.001$; Figure 6C). MethSur analysed the DNA methylation levels and prognostic significance of CpG islands within the APOE gene. The analysis revealed 12 methylated CpG islands, with cg16471933, cg05501958, cg18799241, and cg21879725 exhibiting high levels of DNA methylation (Figure 6D). Additionally, Cox regression and KM analyses demonstrated a close association between DNA methylation at the cg26190885 and cg08955609 loci and HCC prognosis (Figure 6E–G; $P < 0.05$). Moreover, positive correlations were detected between APOE and most m6A-, m5C-, and m1A-regulatory genes (Figure 6H). This finding suggested that APOE may exert oncogenic effects by influencing the expression levels of genes involved in methylation regulation.

Correlations Between APOE Expression and the Expression of Mature MMR Genes, Methyltransferases, Immune Regulatory Genes, and Immune Checkpoints in Hepatocellular Carcinoma

Mismatch repair genes play a crucial role in maintaining genomic stability, thereby influencing cancer development and progression. Therefore, we further investigated this correlation using the GEPIA2 website and consistently found that APOE exhibited a significant negative correlation with five mature mismatch repair genes across TCGA datasets

 https://doi.org/10.2147/JHC.S504078

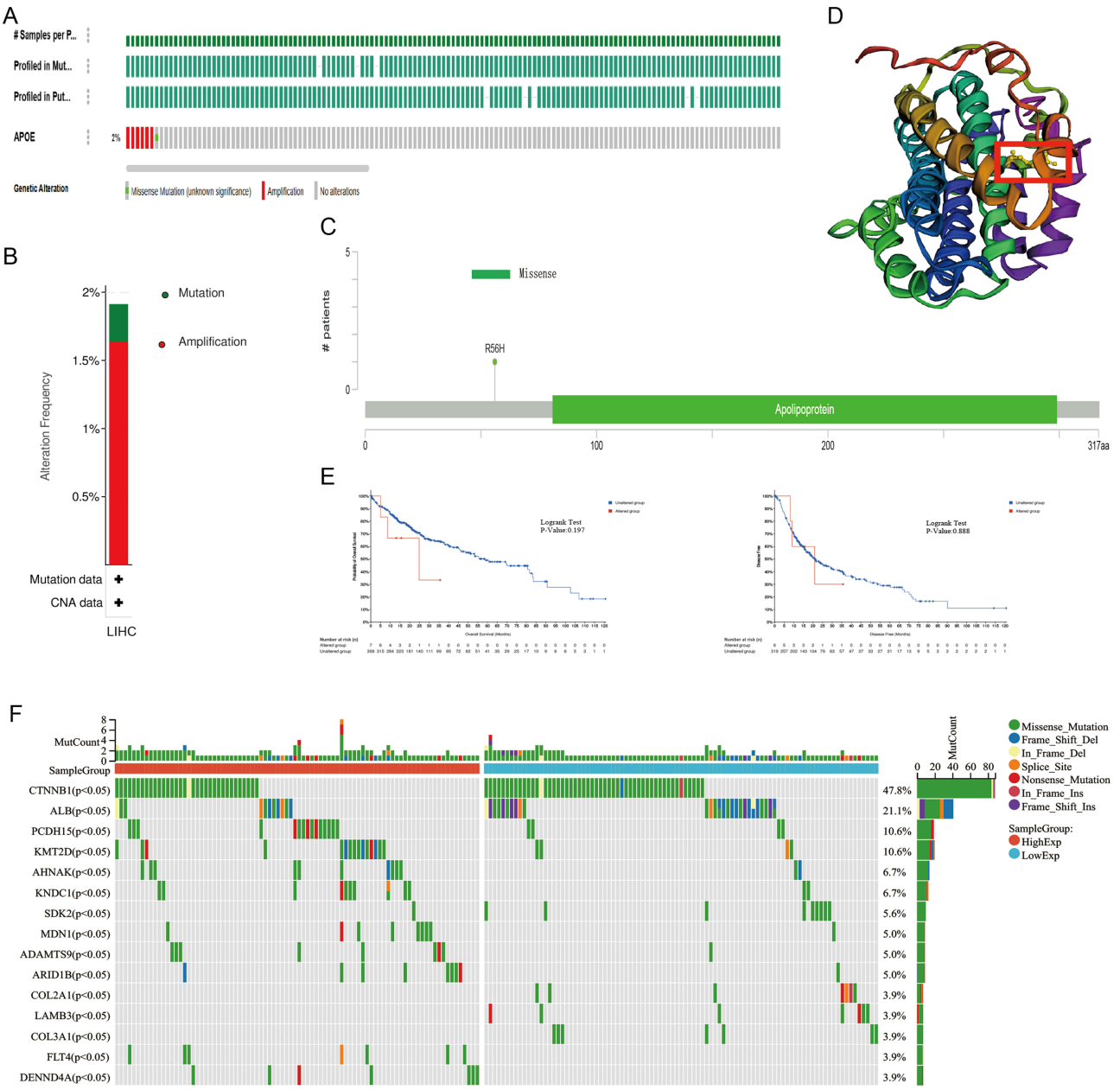

Figure 5 Mutation information for the APOE gene. (**A–C**) APOE alteration frequency (**A**), alteration type (**B**) and mutation site (**C**); (**D**) Mutation site (R56H) with the highest alteration frequency in the three-dimensional structure of APOE; (**E**) Correlation between the APOE mutation status and patients' overall survival (OS) and disease-free survival (DFS); (**F**) HCC patients in the APOE high-expression and APOE low-expression groups in the 15 genes with the highest mutation frequency.

(Figure 7A and B). Additionally, we explored the relationships between APOE and fourteen methyltransferases and discovered consistent negative correlations among them (Figure 7C and D). In our study, we also investigated the relationship between APOE and immunomodulatory genes, including major histocompatibility complex (MHC), immunostimulants, immunosuppressants, chemokines, and chemokine receptors. The results showed a negative correlation between APOE expression and the expression of most immune regulatory genes (Figure 7E–I). Immune checkpoint genes play a key role in immune cell invasion and are important targets for immunotherapy. To explore the potential of APOE in immunotherapy for HCC, we collected data on 20 common immune checkpoint genes and analysed their relationship with APOE expression. According to the coexpression heatmap, APOE expression exhibited a significant negative correlation with the majority of immune checkpoint genes (Figure 8A). Moreover, additional validation analysis was performed using the GEPIA2 website, which confirmed substantial negative associations between APOE and

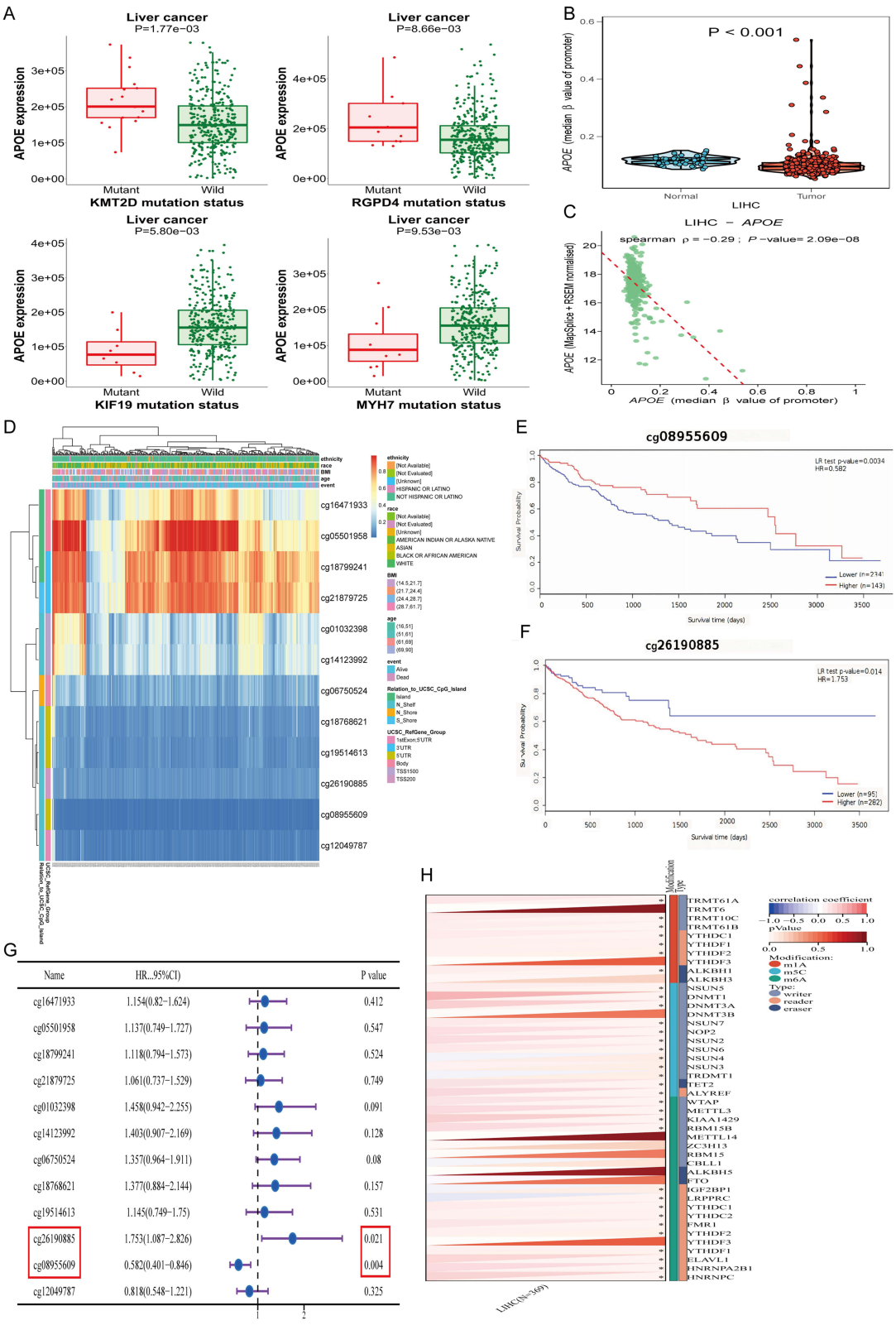

Figure 6 DNA methylation level of APOE and its prognostic value in HCC. (**A**) The top 4 mutated genes affecting the upregulation or downregulation of APOE expression in the muTarget database; (**B**) DNA methylation levels of LIHC and APOE in normal tissues; (**C**) The relationship between APOE expression and DNA methylation; (**D**) Heatmap of DNA methylation at the CpG site of the APOE gene in the Methsurv database; (**E** and **F**) K–M curves were used to compare the overall survival of HCC patients with high and low DNA methylation levels at cg26190885 (**E**) and cg08955609 (**F**); (**G**) Cox regression analysis of the correlation between the APOE DNA methylation locus and the prognosis of HCC patients (forest plot); (**H**) Correlations between APOE expression and m1A, m5C, and m6A regulatory genes.

https://doi.org/10.2147/JHC.S504078

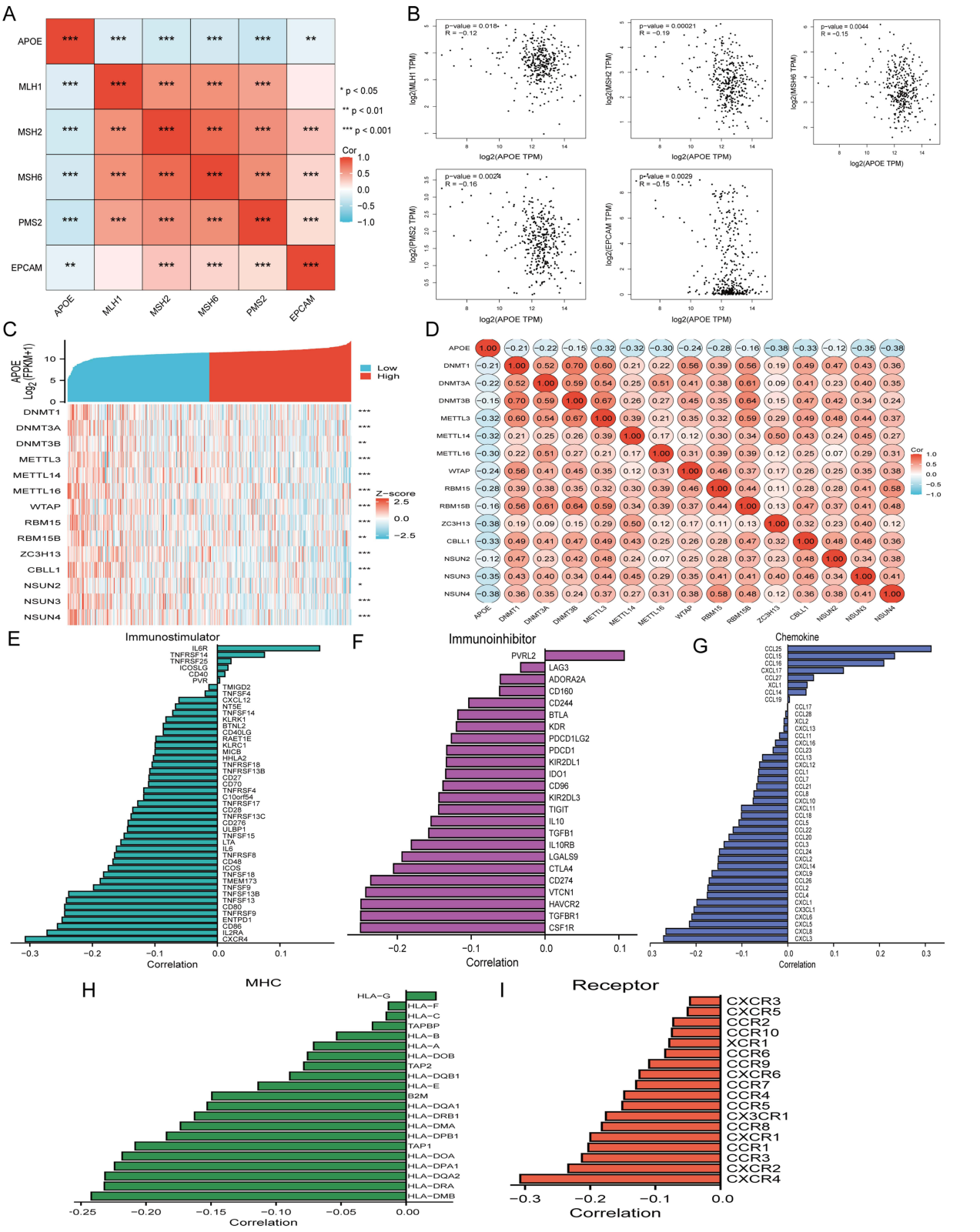

Figure 7 (**A** and **B**) Correlations between APOE expression and MMR genes; (**C** and **D**) Correlations with methyltransferases; (**E–I**) Correlations between APOE expression and immune stimulatory genes (**E**), immune inhibitory genes (**F**), chemokines (**G**), MHC (**H**) and chemokine receptors (**I**). *p < 0.05, **p < 0.01, ***p < 0.001.

https://doi.org/10.2147/JHC.S504078

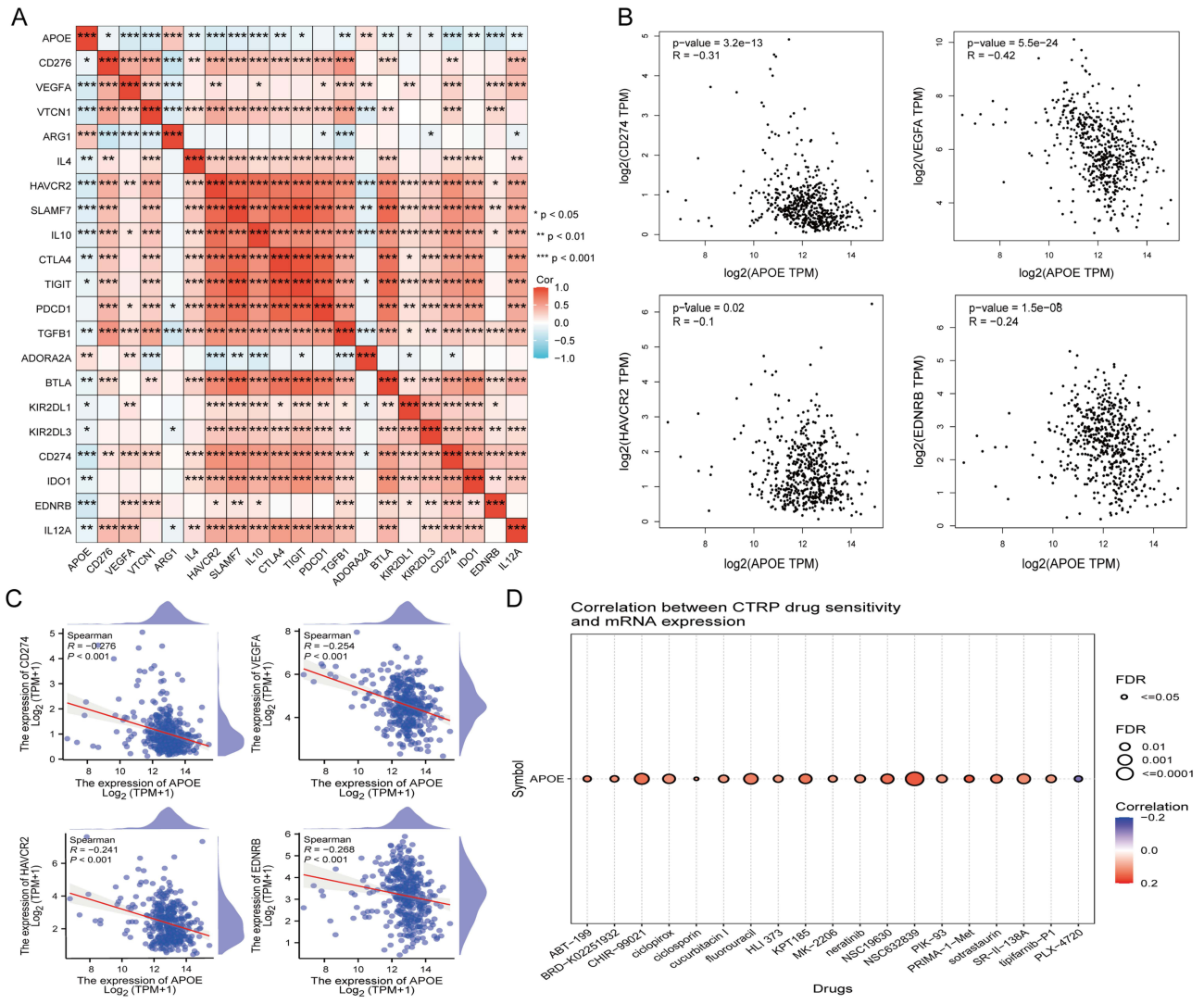

Figure 8 Correlation analysis of APOE expression with immune checkpoints and drug sensitivity in hepatocellular carcinoma. (**A**) Heatmap of coexpression of APOE and immune checkpoint genes. (**B**) Validation of the correlation between APOE and immune checkpoint genes in the GEPIA2 database. (**C**) Validation of the correlation between APOE and immune checkpoint genes in the TCGA database. (**D**) Correlation analysis of APOE mRNA expression and CTRP drug sensitivity. *p < 0.05, **p < 0.01, ***p < 0.001.

CD274, VEGFA, HAVCR2, and EDNRB (Figure 8B), consistent with the findings from the TCGA database (Figure 8C). Through drug sensitivity analysis, we observed a positive correlation between the expression of APOE and the 50% inhibitory concentration (IC50) values of drugs such as ABT-199, BRD-K02251932, CHIR-99021, ciclopirox, cyclosporin, cucurbitacin I, fluorouracil, HLI 373, KPT185, MK-2206, neratinib, NSC19630, NSC632839 PIK-93, PRIMA-1-Met, sotrastaurin, SR-II-138A and tipifarnib-P1 (Figure 8D). These results suggest that tumor cells with high expression of APOE are prone to drug resistance.

Immunoinfiltration Analysis of APOE

The ESTIMATE algorithm was used to calculate the ESTIMATEScore (r=−0.11, P=0.03), stromal score (r=−0.08, P=13), and immune score (r=−0.12, P=0.02). Our analysis revealed a significant negative correlation between APOE and the immune score, stromal score, and estimated score (Figure 9A–C). Subsequently, the Spearman method was used to investigate the correlation between APOE expression and the infiltration of 24 immune cell types. The findings revealed a negative association between APOE expression and Tcm, CD8+ T cell, Th1 cell, neutrophil, macrophage, aDC and iDC infiltration. Conversely, a positive correlation was observed with the Th17 infiltration level (Figure 9E). Finally, ssGSVA

https://doi.org/10.2147/JHC.S504078

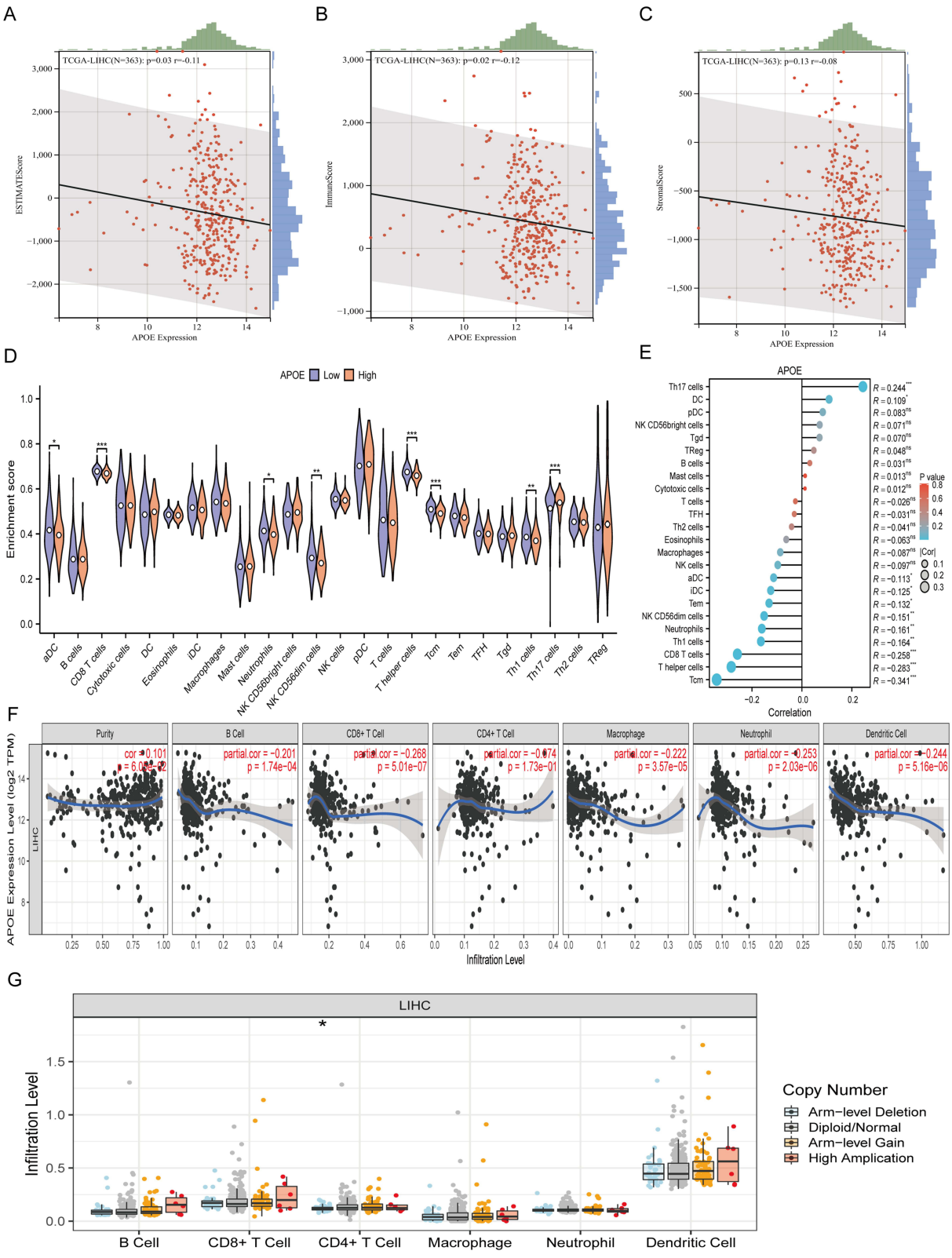

Figure 9 Correlation between APOE expression and immune infiltration. (**A–C**) Association between APOE expression and the ESTIMATEScore, immunescore, and stromalscore in hepatocellular carcinoma; (**D**) Enrichment scores of the NSUN7 high-expression group and low-expression group; (**E**) Correlation between NSUN7 expression and 24 immune cells; (**F**) Correlation analysis of APOE expression with infiltration of different immune cells using the TIMER database; (**G**) Comparison of immune infiltration levels based on copy number variations of the APOE gene in different somatic cells in the TIMER database. *P<0.05; **P<0.01; ***P<0.001; ns: no significant difference.

was employed to quantify the relative abundance of tumor-infiltrating immune cells in CESC patients stratified into high and low APOE expression groups. Our findings revealed that compared to the NSUN7 low-expression group, the NSUN7 high-expression group exhibited reduced enrichment scores for aDCs, CD8+ T cells, neutrophils, NK CD56dim cells, T helper cells, and Th1 cells (Figure 9D). The TIMER database also confirmed a negative correlation between the abundance of APOE and B cells, CD8+ T cells, CD4+ T cells, macrophages, neutrophils, and dendritic cells (Figure 9F). Furthermore, we investigated the associations between APOE and various immune cell markers in LIHC using the TIMER dataset. After adjusting for tumor purity, the results demonstrated a significant association between APOE expression and gene markers of M1/M2 macrophages, dendritic cells, natural killer (NK) cells, monocytes, and TAMs (Table 2). Furthermore, no significant correlation was detected between APOE CNV and the infiltration levels of B cells, CD8+ T cells, CD4+ T cells, macrophages, neutrophils or DCs (Figure 9G).

Table 2 Correlation Analysis Between APOE and Immune Cell Gene Markers in TIMER

Description	Gene Markers	LIHC			
		None		Purity	
		Cor	P	Cor	P
CD8 +T-cell	CD8A	−0.125	*	−0.088	0.103
	CD8B	−0.136	**	−0.115	*
T-cell (general)	CD3D	−0.122	*	−0.091	0.093
	CD3E	−0.129	*	−0.088	0.101
	CD2	−0.110	*	−0.073	0.175
B-cell	CD19	−0.124	*	−0.090	0.096
	CD79A	−0.070	0.178	−0.033	0.538
Monocyte	CD86	−0.272	***	−0.263	***
	CD115 (CSF1R)	−0.278	***	−0.267	***
TAM	CCL2	−0.190	***	−0.135	*
	CD68	−0.214	***	−0.186	***
	IL10	−0.215	***	−0.188	***
M1 Macrophage	INOS (NOS2)	−0.023	0.658	−0.026	0.635
	IRF5	−0.113	*	−0.115	*
	COX2 (PTGS2)	−0.277	***	−0.263	***
M2 Macrophage	CD163	−0.213	***	−0.188	***
	VSIG4	−0.209	***	−0.190	***
	MS4A4A	−0.234	***	−0.211	***
Neutrophils	CD66b(CEACAM8)	−0.065	0.212	−0.058	0.284
	CD11b(ITGAM)	−0.186	***	−0.157	**
	CCR7	−0.145	**	−0.100	0.064
Natural killer cell	KIR2DL1	−0.128	*	−0.103	0.056
	KIR2DL3	−0.134	**	−0.129	*
	KIR3DL1	−0.144	**	−0.155	**
	KIR3DL2	−0.081	0.120	−0.085	0.116
	KIR2DS4	−0.047	0.371	−0.052	0.338
Dendritic cell	HLA-DPB1	−0.205	***	−0.180	***
	HLA-DQB1	−0.160	**	−0.137	*
	HLA-DPA1	−0.251	***	−0.226	***
	CD1C	−0.134	*	−0.081	0.133
	NRP1	−0.445	***	−0.414	***
	ITGAX	−0.187	***	−0.179	***

(*Continued*)

https://doi.org/10.2147/JHC.S504078

Table 2 (Continued).

Description	Gene Markers	LIHC			
		None		Purity	
		Cor	P	Cor	P
Th1	TBX21	−0.114	*	−0.079	0.141
	STAT4	−0.091	0.080	−0.073	0.175
	STAT1	−0.232	***	−0.229	***
	IFN-γ (IFNG)	−0.081	0.118	−0.062	0.248
	TNF-α (TNF)	−0.226	***	−0.217	***
Th2	GATA3	−0.193	***	−0.178	***
	STAT6	−0.178	***	−0.161	**
	STAT5A	−0.192	***	−0.165	**
	IL13	0.038	0.470	0.051	0.347

Notes: *p<0.05,**p<0.01,***p<0.001.

Single-Cell Level Analysis of APOE

To investigate the associations between APOE expression and immune cell distribution and proportions at the single-cell level, we acquired eight independent LIHC datasets (LIHC_GSE_aPDL1aCTLA4, LIHC_GSE146115, LIHC_GSE140228_10X, LIHC_GSE140228_Smartseq2, LIHC_GSE146409, LIHC_GSE166635, LIHC_GSE179795 and LIHC_GSE98638) (Figure 10A). As depicted in Figure 10B–E, we successfully identified the expression pattern of APOE across the LIHC_GSE_aPDL1aCTLA4, LIHC_GSE146115, LIHC_GSE166635, and LIHC_GSE146409 datasets. Markedly elevated levels of APOE were observed in both malignant cells and macrophages. These findings strongly suggest a pivotal role for APOE in immune infiltration within the context of liver hepatocellular carcinoma.

Identification of APOE-Related Genes and Cellular Functions in Hepatocellular Carcinoma

According to the coexpression analysis of RNA sequencing data from the TCGA database, a total of 490 genes were strongly associated with APOE. Among these genes, 469 genes were negatively correlated, while 21 genes were positively correlated. Figure 11A illustrates the interactions between APOE and a set of highly correlated genes (12 in total). Moreover, to explore the underlying mechanism underlying the role of APOE in HCC, we identified 1398 DEGs (| log2FoldChange| >1, p adj <0.05) between samples with high and low APOE expression within the TCGA-LIHC cohort (Figure 11B). Among these DEGs, 242 were upregulated, and 1156 were downregulated. To visualize this information effectively, we employed a heatmap to present the top 16 up-/downregulated genes (Figure 11C). The functional enrichment analysis of differentially expressed genes (DEGs) revealed that APOE was primarily involved in synaptic assembly and regulation, with predominant enrichment in synaptic membranes. Additionally, it was associated with signaling receptor activator activity and passive transmembrane transporter protein activity (Figure 11D and E). KEGG functional enrichment analysis revealed that APOE plays a vital role in classical pathways such as the Wnt signaling pathway and cAMP signaling pathway (Figure 11F). Finally, GSEA demonstrated that in the respiratory electron transport chain, ATP synthesis-coupled electron transport, oxidative phosphorylation, oxidoreduction-driven active transmembrane transporter activity, and other reactome pathways were significantly enriched (Figure 11G). The Wiki pathway exhibited notable enrichment in nonalcoholic fatty liver disease and malignant pleural mesothelioma (Figure 11H).

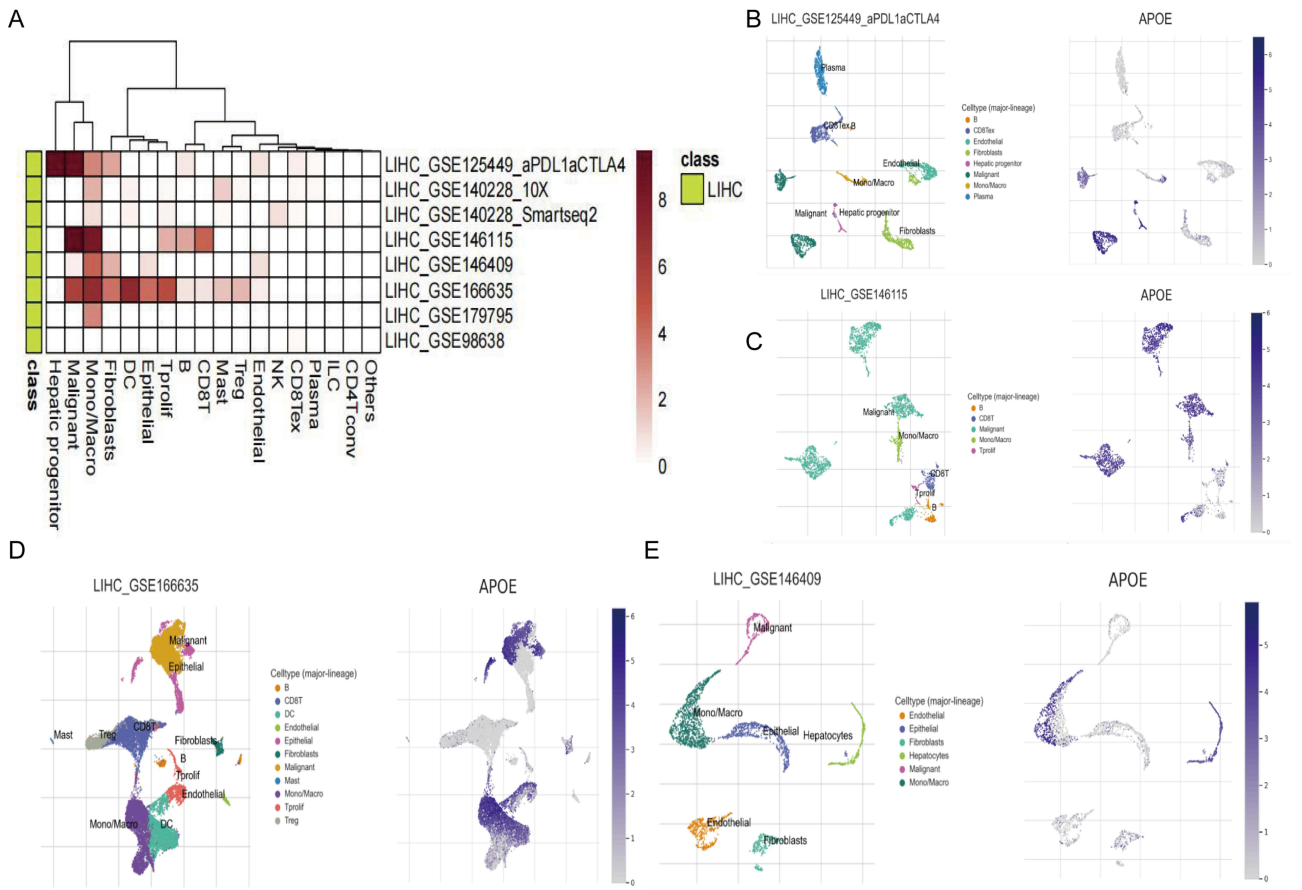

Figure 10 Analysing the expression of APOE in different types of cells at the single-cell level. (**A**) Heatmaps correlating APOE with levels of immune cell infiltration in 8 independent GEO datasets. (**B–E**) Single-cell transcriptomics revealed the distribution of APOE in different types of immune cells, including LIHC_GSE_aPDL1aCTLA4 (**B**), LIHC_GSE146115 (**C**), LIHC_GSE166635 (**D**) and LIHC_GSE146409 (**E**).

Validating the Overexpression and Prognostic Value of APOE Based on the Clinicopathologic Data of HCC Patients

To further elucidate the differences in APOE expression between HCC tissues and normal tissues, 90 human hepatocellular carcinoma tissues and corresponding normal tissue samples were selected for multiplex fluorescence immunohistochemistry (Figure 12A). The mIHC-positive rate results showed that APOE was significantly over-expressed in HCC, mainly in the cytoplasm (Figure 12B–D). The visual examination revealed an average intensity of APOE and CK8, as depicted in Figure 12F, with a detection rate of 64.85% for APOE in these patients. Therefore, the establishment of APOE overexpression as a promising therapeutic target for hepatocellular carcinoma treatment solidifies its potential in the realm of medical intervention. To prove the prognostic value of APOE expression in patients with hepatocellular carcinoma, we evaluated APOE expression using detailed clinical and pathological data from 88 liver cancer tissue microarrays (HLiv180Su09). The results revealed a good correlation between the proportion of APOE-positive liver cancer cells in HCC and patient survival, indicating that the number of APOE-positive cells has a significant impact on patient prognosis (Figure 12E). To verify whether APOE is an independent factor influencing the prognosis of hepatocellular carcinoma patients, we conducted univariate and multivariate Cox regression analyses. The findings revealed that APOE serves as an independent predictive indicator (Table 3). Therefore, based on the KM analysis and multivariate Cox regression analysis results, APOE may be a protective prognostic indicator in HCC patients.

https://doi.org/10.2147/JHC.S504078

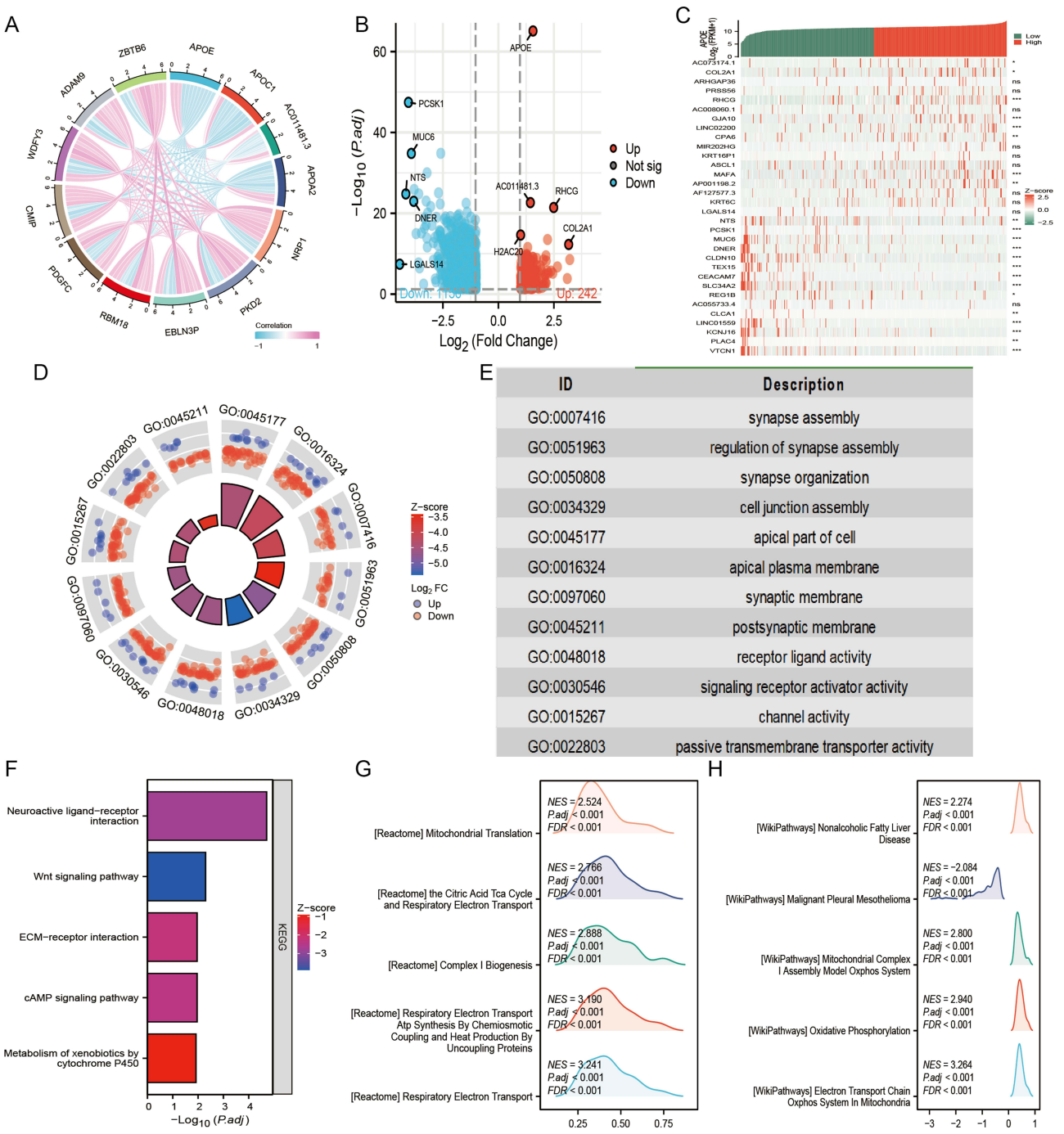

Figure 11 APOE-related genes, pathways and functional identification. (**A**) Circos plot showing the coexpression network of APOE with 12 genes in hepatocellular carcinoma samples; (**B**) Volcano plots of DEGs between the high-APOE expression group and low-APOE expression group; (**C**) Heatmaps showing the top 16 up- and downregulated genes; (**D–F**) Circle plots and bar plots of DEGs significantly enriched for GO terms and KEGG pathways; (**G** and **H**) GESA enrichment analysis revealed significant enrichment of the REACTOME pathway (**G**) and WiKi pathway (**H**). *p < 0.05, **p < 0.01, ***p < 0.001, ns: no significant difference.

Discussion

Hepatocellular carcinoma (HCC) is a type of liver malignancy with unique metabolic characteristics in which lipoproteins play an essential role in lipid metabolism and homeostasis. Damage to hepatocytes leads to dysregulated lipoprotein metabolism, resulting in lipid imbalances and accumulation that accelerate the development of HCC. HCC cells adapt their metabolism to generate more energy for rapid proliferation, making them particularly resistant to treatment. Apolipoprotein E (APOE) has been identified as a key player in several cancers, influencing lipid transport (such as

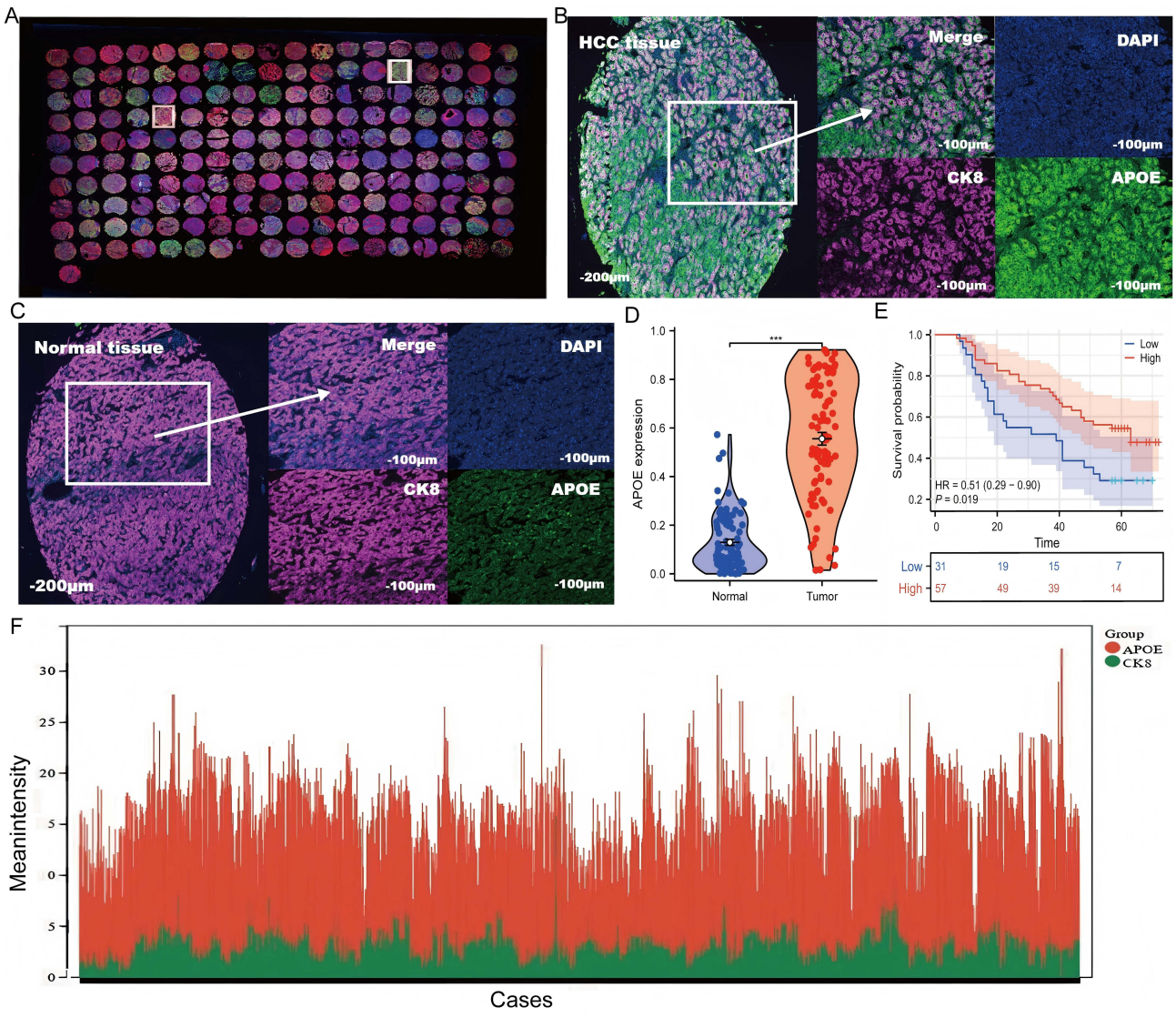

Figure 12 Protein expression and survival analysis of APOE in hepatocellular carcinoma tissue and corresponding normal tissue were detected using multiple immunofluorescence techniques. (**A**) Immunofluorescence images of 90 hCC tissue microarray samples depicting APOE (green), DAPI (blue), and CK8 (purple); (**B** and **C**) Representative immunofluorescence images illustrating APOE (green), DAPI (blue), and CK8 (purple) in tumor tissues and adjacent normal tissues of HCC patients. (**D**) Comparative protein expression analysis between 90 hCC tissues and corresponding normal liver tissues was performed. (**E**) Survival analysis demonstrated the association between APOE expression level and overall patient survival among 88 hCC patients. (**F**) Distribution of average intensity scores for APOE and CK8 in HCC.

cholesterol and fatty acids) and cardiovascular health, and research has shown that nuclear APOE expression in ovarian cancer patients significantly correlates with prognosis in peritoneal fluid,[21] highlighting its potential importance in predicting ovarian cancer outcomes. In lung cancer, APOE promotes cell proliferation and migration, with higher expression associated with more aggressive forms of lung adenocarcinoma.[22] Animal studies have shown that pro-inflammatory and pro-fibrotic genes are upregulated in ApoE-deficient mice during hepatitis, suggesting a critical role for APOE in inflammation and fibrosis.[23] In acute myeloid leukaemia (AML), APOE binds to the LILRB4 receptor and contributes to immune evasion.[24] Elevated APOE mRNA levels in colorectal cancer are associated with shorter overall and progression-free survival, and promote cell migration and invasion via LRP1 binding.[25] In breast cancer, APOE inhibits tumour cell proliferation and migration by modulating cholesterol metabolism, suggesting that it may be a valuable target for improving patient prognosis.[26] Overall, APOE exhibits diverse and complex functions in different cancers, affecting cancer initiation, progression and response to therapy. Its involvement in lipid metabolism, cell proliferation, migration and immune escape highlights its potential as a biomarker and therapeutic target.

https://doi.org/10.2147/JHC.S504078

Table 3 Univariate and Multivariate Analyses of Prognostic Factors for OS

Characteristics	Total(N)	Univariate Analysis		Multivariate Analysis	
		HR (95% CI)	P value	HR (95% CI)	P value
Age	88	1.012 (0.986–1.038)	0.365	1.019 (0.991–1.047)	0.190
Sex	88				
Male	72	Reference		Reference	
Female	16	0.581 (0.261–1.294)	0.184	0.559 (0.235–1.328)	0.187
Grade	88				
I-II	58	Reference		Reference	
III	30	0.954 (0.525–1.735)	0.878	0.991 (0.539–1.821)	0.977
T stage	88				
T1	59	Reference		Reference	
T2	29	1.893 (1.071–3.345)	**0.028**	1.617 (0.900–2.904)	0.108
APOE	88				
Low	31	Reference		Reference	
High	57	0.510 (0.290–0.897)	**0.019**	0.546 (0.304–0.981)	**0.043**

Note: Bold black indicates p≤0.05.

In recent years, there has been an increasing focus on merging genomic information with clinical data to improve disease modelling and prediction through enhanced data analysis.[27] In this context, the role of APOE as a key gene in HCC has attracted increasing interest from researchers. While APOE has been extensively studied in other diseases, its investigation in HCC has remained relatively limited. Li et al found that APOE is overexpressed in HCC and correlates with better patient outcomes.[20] To validate these observations, we performed bioinformatic analyses and multiple immunofluorescence experiments. Our results showed a significant upregulation of APOE expression in HCC tissues, particularly in the cytoplasmic compartment. By measuring the average intensity values of APOE and CK8 at the single cell level, we obtained reliable quantitative data on APOE protein levels, providing a solid basis for our conclusions. Kaplan-Meier survival analysis showed that patients with higher APOE expression had improved survival, which is consistent with previous studies and reinforces the potential prognostic significance of APOE in HCC. In addition, ROC curve analysis highlighted the substantial diagnostic value of APOE in the detection of HCC. Finally, by combining APOE expression data from HCC samples with patient clinicopathological information, we performed Cox univariate and multivariate analyses. The results suggest that APOE may serve as a valuable tool for the assessment of HCC prognosis. Future research should further investigate the role of APOE in HCC and explore its potential clinical applications.

Mutations result from changes in gene sequences that influence tumour development.[28] Several studies have shown that APOE gene mutations are closely associated with the progression of various diseases.[29,30] In this study, APOE gene mutations were detected in 2% of HCC patients, with "missense" being the predominant type of mutation. However, our study did not find any associations between these gene mutations and patient prognosis. Among the 15 genes analysed in HCC, CTNNB1, ALB, PCDH15, KMT2D and AHNAK exhibited a greater frequency of mutations in both the high-expression group and low-APOE-expression group. Moreover, mutations in the KMT2D, RGPD4, KIF19, and MYH7 genes exerted an impact on the expression level of APOE. Research has shown that these mutated genes are closely associated with the development of tumors. DNA methylation is a crucial factor in the epigenetic regulation of gene expression, impacting both cancer cells and components of the immune and stromal microenvironment. Research has demonstrated that APOE methylation status is closely tied to the tumor microenvironment (TME) in HCC.[20] Observations indicate that as APOE transcription increases, the methylation level of its promoter region decreases, suggesting that APOE demethylation is linked to immune evasion and may offer novel perspectives on the immune microenvironment in HCC. Methylation analysis revealed two CpG sites significantly correlated with patient outcomes, highlighting the potential of APOE DNA methylation as a prognostic biomarker for clinical applications. Consequently, APOE shows promise as a diagnostic tool for identifying mutations and epigenetic changes in HCC. Mismatch repair (MMR) genes primarily ensure DNA stability by correcting base mismatches that occur during replication. Damage to

these genes can lead to microsatellite instability and an accumulation of mutations, which can ultimately contribute to the development of malignant tumors. In our investigation, we explored the relationship between APOE and several critical MMR genes, such as MLH1, MSH2, MSH6, PMS2, and EPCAM. Our results indicated a negative correlation between APOE expression and the expression levels of these MMR genes in patients with HCC. This suggests that APOE may play a role in the progression of HCC by influencing DNA repair processes.

The tumor microenvironment (TME) represents a complex network integral to tumor development and progression, encompassing various cell types and extracellular matrix elements. Studies have demonstrated that the TME not only impacts patient prognosis but also serves as a biomarker for assessing the effectiveness of immunotherapy. In pancreatic ductal adenocarcinoma (PDAC), higher levels of APOE have been linked to immunosuppression and reduced patient survival. Notably, APOE knockout mice exhibited a significant increase in CD8+ T-cell counts, indicating that APOE may play a role in immune suppression.[31] In gastric cancer, exosome-mediated transfer of APOE proteins from tumor-associated macrophages to tumor cells enhances GC cell migration, highlighting the intricate interplay between APOE and the gastric cancer TME.[32] APOE drives macrophage polarization toward the M2 phenotype, promoting tumor angiogenesis and suppressing anti-tumor immune responses.[33,34] Single-cell analysis has shown that APOE is highly expressed in macrophages and strongly correlated with marker genes of diverse immune cells, such as M1/M2 macrophages, neutrophils, natural killer (NK) cells, and tumor-associated macrophages, underscoring its critical role in immune suppression. However, the impact of APOE varies across different cell types and tumor types. LXR/APOE activation therapy modulates the abundance of myeloid-derived suppressor cells (MDSCs) and enhances anti-tumor immune responses and T-cell activation during immunotherapy,[17] making it a promising target for cancer immunotherapy. Previous research has indicated that APOE inhibits T-cell proliferation, alters macrophage function, and facilitates lipid antigen presentation to NK T cells.[35] In HCC, APOE expression correlates positively with NK cell infiltration and negatively with effector memory $CD4^+$ T cell infiltration, aligning with earlier findings. ESTIMATE algorithm analysis revealed a significant association between APOE and immune infiltration scores in HCC tissues, suggesting that APOE may influence the regulation of the immunosuppressive microenvironment in HCC. While existing studies highlight the significance of APOE in the tumor microenvironment, further investigation is necessary to fully understand its mechanisms of action. This will deepen our comprehension of APOE's role in tumor immunomodulation and identify new targets for cancer immunotherapy.

Immunotherapies, especially immune checkpoint inhibitors (ICIs), present new opportunities for treating hepatocellular carcinoma. Therapies targeting immune checkpoint molecules like PD-1 and CTLA-4 can reinvigorate anti-tumor immune responses.[36] Recently, two drugs targeting PD-L1/PD-1—nivolumab and pembrolizumab—have been approved for use in subsequent HCC treatments.[37] APOE, a member of the low-density lipoprotein (LDL) receptor family, plays a role in lipoprotein clearance and helps reduce the risk of atherosclerosis. Research has shown that APOE expression is significantly elevated in patients resistant to αPD-1 therapy compared to those who respond positively,[38] suggesting that APOE may contribute to resistance mechanisms. By modulating tumor macrophage infiltration, APOE may enhance the effectiveness of checkpoint immunotherapy. Additionally, APOE shows potential as a biomarker for predicting αPD-1 responsiveness, and its inhibitors could improve sensitivity to αPD-1/αTIGIT checkpoint blockade. This opens up new avenues for personalized therapy and may help identify patient groups more responsive to immunotherapy. In this study, APOE was found to be strongly associated with many immune checkpoint genes, particularly demonstrating a significant negative correlation ($|r| \geq 0.3$, $p < 0.001$) with PD-L1/CD274 and VEGFA. Furthermore, APOE exhibited close relationships with numerous immunomodulatory genes. Drug susceptibility analysis revealed a positive correlation between APOE expression and various medications (eg, ABT-199, BRD-K02251932, CHIR-99021, ciclopirox and ciclosporin), indicating that tumor cells with high APOE expression may be more susceptible to drug resistance. These findings suggest that APOE could be a promising target for developing novel immunosuppressive agents. Further investigation into the specific mechanisms of APOE in HCC will be an important direction for future research. However, studies have demonstrated that knocking down the ApoE gene in mouse models leads to spontaneous atherosclerotic lesions,[39] indicating that ApoE is not only essential for lipid metabolism but also closely linked to cardiovascular health. Therefore, when considering ApoE as a therapeutic target for HCC, its potential side effects on the cardiovascular system must be carefully evaluated. Given the lack of sufficient in vivo experimental data and the complex mechanisms of action in the human body, further studies are currently necessary.

In the enrichment analysis of DEGs, APOE was found to be primarily involved in synaptic assembly and regulation, passive transmembrane protein activity, and classical pathways, including the Wnt signaling pathway and cAMP signaling pathway. The Wnt signaling pathway and cAMP signaling pathway play vital roles in hepatocellular carcinoma. Abnormal regulation of Wnt signal transduction is a key mechanism in the development of HCC.[40] In particular, the Wnt/β-catenin signaling pathway is heavily activated in qualitative liver and kidney lesions and can be used as a marker of pathological injury, and its activation plays a key role in maintaining the dynamic balance of liver and kidney tissues.[41] In most tumor cells, including HCC cells, cAMP acts as a controller of cell proliferation. A negative correlation between cAMP levels and malignancy was found in these cells. Studies have shown that increasing cAMP levels may help counteract the development and effects of HCC.[42] Through KEGG enrichment analysis, we found that the APOE gene is closely related to the Wnt and cAMP signaling pathways, which revealed that APOE may regulate the occurrence and development of hepatocellular carcinoma through several mechanisms. Gene set enrichment analysis (GSEA) further revealed associations between APOE and diseases related to the respiratory electron transport chain, oxidative phosphorylation, Alzheimer's disease, and Parkinson's disease. Additionally, previous research has demonstrated significant links between ApoE expression regulation and apoE gene polymorphisms in neurodegenerative disorders such as Alzheimer's disease (AD), Parkinson's disease (PD), ischaemic stroke, and other neurological conditions.[43]

In summary, this study further confirmed the expression patterns of APOE in HCC using bioinformatics analysis and multiplex immunofluorescence techniques, underscoring its potential significance in immunotherapy and prognosis prediction for HCC. Moreover, an initial investigation into the signaling pathways associated with APOE offers new perspectives on its role in HCC development. However, several limitations should be acknowledged. First, the study predominantly utilized retrospective analyses of existing datasets without additional experimental validation from independent cohorts, which may limit the robustness of our conclusions. Second, the retrospective nature of the data could introduce bias, and the relatively small sample size may not fully support the generalizability of the findings. Third, constraints related to time, sample collection, funding, and technical challenges prevented us from conducting further basic experiments to substantiate our conclusions and explore underlying mechanisms. Notwithstanding these limitations, our research corroborated the link between elevated APOE expression and improved patient outcomes, as evidenced by multiple immunofluorescence techniques and clinicopathological data. Future large-scale prospective studies are essential to comprehensively validate these observations and to investigate the specific mechanisms through which APOE affects HCC.

Conclusion

In hepatocellular carcinoma (HCC), APOE expression is markedly increased at both mRNA and protein levels. Studies have shown that APOE is strongly associated with immunosuppression and could be a promising target for the development of new immunotherapeutic approaches. Additionally, research indicates that higher APOE expression correlates with better patient outcomes, suggesting its potential as a prognostic marker for HCC. Further analysis has revealed that APOE expression is closely tied to the Wnt and cAMP signaling pathways, indicating that it may affect the onset and progression of HCC through various mechanisms. Our results highlight the need for further investigation into the prognostic significance and specific roles of APOE in HCC. To achieve this, multi-center, large-scale clinical trials are essential to establish a solid scientific basis for considering APOE as a therapeutic target in HCC treatment. If future studies can confirm the mechanisms by which APOE functions and validate its effectiveness as a prognostic marker, APOE could become a key factor in the clinical management of HCC. This would aid physicians in tailoring more personalized treatment plans for patients.

Data Sharing Statement

This study utilized publicly available datasets. The databases and corresponding websites used included TCGA (https://portal.gdc.cancer.gov/), GTEx (https://commonfund.nih.gov/GTEx), CCLE (https://portals.broadinstitute.org/ccle), GEO (https://www.ncbi.nlm.nih.gov/geo/), GEPIA (http://gepia.cancer-pku.cn/index.html), UALCAN (http://ualcan.path.uab.edu/), The Human Protein Atlas (https://www.proteinatlas.org/), Kaplan–Meier Plotter (http://kmplot.com/analysis/), cBioPortal database (http://www.cbioportal.org), MuTarget (https://www.mutarget.com/), MethSurv (https://biit.cs.ut.ee/methsurv/), Therapy Response Portal (https://portals.broadinstitute.org), TIMER (http://timer.comp-genomics.org/timer/), TISCH (http://tisch.comp-genomics.org).

Ethical Approval and Consent to Participate

This study was approved by the Ethics Committee of the Affiliated Cancer Hospital of Xinjiang Medical University (Approval No. K-S2023001; Urumqi, China), and the study was conducted in accordance with the Declaration of Helsinki. All participants provided written informed consents to participate in this study.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

This work was supported by the Tianchi Young Talent - Youth Doctor Program of Xinjiang Uygur Autonomous Region (2023TCYCFYC).

Disclosure

The authors declare that there are no conflicts of interest in the publication of this article.

References

1. Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA*. 2021;71(3):209–249. doi:10.3322/caac.21660
2. Vogel A, Meyer T, Sapisochin G, Salem R, Saborowski A. Hepatocellular carcinoma. *Lancet*. 2022;400(10360):1345–1362. doi:10.1016/S0140-6736(22)01200-4
3. Yang JD, Hainaut P, Gores GJ, Amadou A, Plymoth A, Roberts LR. A global view of hepatocellular carcinoma: trends, risk, prevention and management. *Nat Rev Gastroenterol Hepatol*. 2019;16(10):589–604. doi:10.1038/s41575-019-0186-y
4. McGlynn KA, Petrick JL, El-Serag HB. Epidemiology of hepatocellular carcinoma. *Hepatology*. 2021;73(Suppl 1):4–13. doi:10.1002/hep.31288
5. Rizzo A, Ricci AD. Challenges and future trends of hepatocellular carcinoma immunotherapy. *Int J mol Sci*. 2022;23(19):11363. doi:10.3390/ijms231911363
6. Rizzo A, Ricci AD, Brandi G. Trans-arterial chemoembolization plus systemic treatments for hepatocellular carcinoma: an update. *J Pers Med*. 2022;12(11):1788. doi:10.3390/jpm12111788
7. Guven DC, Erul E, Kaygusuz Y, et al. Immune checkpoint inhibitor-related hearing loss: a systematic review and analysis of individual patient data. *Support Care Cancer*. 2023;31(12):624. doi:10.1007/s00520-023-08083-w
8. Sahin TK, Ayasun R, Rizzo A, Guven DC. Prognostic value of Neutrophil-to-Eosinophil Ratio (NER) in cancer: a systematic review and meta-analysis. *Cancers*. 2024;16(21):3689. doi:10.3390/cancers16213689
9. Rizzo A, Santoni M, Mollica V, et al. Peripheral neuropathy and headache in cancer patients treated with immunotherapy and immuno-oncology combinations: the MOUSEION-02 study. *Expert Opin Drug Metab Toxicol*. 2021;17(12):1455–1466. doi:10.1080/17425255.2021.2029405
10. Reig M, Forner A, Rimola J, et al. BCLC strategy for prognosis prediction and treatment recommendation: the 2022 update. *J Hepatol*. 2022;76(3):681–693. doi:10.1016/j.jhep.2021.11.018
11. Kulminski AM, Culminskaya I, Arbeev KG, Ukraintseva SV, Arbeeva L, Yashin AI. Trade-off in the effect of the APOE gene on the ages at onset of cardiocascular disease and cancer across ages, gender, and human generations. *Rejuvenation Res*. 2013;16(1):28–34. doi:10.1089/rej.2012.1362
12. Zhao N, Ren Y, Yamazaki Y, et al. Alzheimer's risk factors age, APOE genotype, and sex drive distinct molecular pathways. *Neuron*. 2020;106(5):727–742.e6. doi:10.1016/j.neuron.2020.02.034
13. Butterbrod E, Sitskoorn M, Bakker M, et al. The APOE ε4 allele in relation to pre- and postsurgical cognitive functioning of patients with primary brain tumors. *Eur J Neurol*. 2021;28(5):1665–1676. doi:10.1111/ene.14693
14. Urquidi V, Goodison S, Ross S, Chang M, Dai Y, Rosser CJ. Diagnostic potential of urinary α1-antitrypsin and apolipoprotein E in the detection of bladder cancer. *J Urol*. 2012;188(6):2377–2383. doi:10.1016/j.juro.2012.07.094
15. Carroll JE, Small BJ, Tometich DB, et al. Sleep disturbance and neurocognitive outcomes in older patients with breast cancer: interaction with genotype. *Cancer*. 2019;125(24):4516–4524. doi:10.1002/cncr.32489
16. 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. doi:10.1158/2326-6066.CIR-19-0036
17. Tavazoie MF, Pollack I, Tanqueco R, et al. LXR/ApoE activation restricts innate immune suppression in cancer. *Cell*. 2018;172(4):825–840.e18. doi:10.1016/j.cell.2017.12.026T
18. Sakashita K, Tanaka F, Zhang X, et al. Clinical significance of ApoE expression in human gastric cancer. *Oncol Rep*. 2008;20(6):1313–1319.
19. Gao B, Zhou P, Wang L, et al. Effects of the subtypes of apolipoprotein E on immune inhibition and prognosis in patients with Hepatocellular Carcinoma. *J Cancer Res Clin Oncol*. 2024;150(7):341. doi:10.1007/s00432-024-05856-6
20. Li J, Tian S, Liu Q, Peng P. Apoprotein E methylation is correlated with immune microenvironment in hepatocellular carcinoma. *Acta oncologica*. 2023;62(6):550–559. doi:10.1080/0284186X.2023.2225703

21. Chen YC, Pohl G, Wang TL, et al. Apolipoprotein E is required for cell proliferation and survival in ovarian cancer. *Cancer Res*. 2005;65(1):331–337. doi:10.1158/0008-5472.331.65.1
22. 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. doi:10.1016/j.lungcan.2010.04.009
23. Ferré N, Martínez-Clemente M, López-Parra M, et al. Increased susceptibility to exacerbated liver injury in hypercholesterolemic ApoE-deficient mice: potential involvement of oxysterols. *Am J Physiol Gastrointest Liver Physiol*. 2009;296:G553–62. doi:10.1152/ajpgi.00547.2007
24. Deng M, Gui X, Kim J, et al. LILRB4 signalling in leukaemia cells mediates T cell suppression and tumour infiltration. *Nature*. 2018;562(7728):605–609. doi:10.1038/s41586-018-0615-z
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. doi:10.17305/bb.2023.9248
26. Ben Hassen C, Gutierrez-Pajares JL, Guimaraes C, et al. Apolipoprotein-mediated regulation of lipid metabolism induces distinctive effects in different types of breast cancer cells. *Breast Cancer Res*. 2020;22:38–45. doi:10.1186/s13058-020-01276-9
27. Yousefi S, Amrollahi F, Amgad M, et al. Predicting clinical outcomes from large scale cancer genomic profiles with deep survival models. *Sci Rep*. 2017;7:11707. doi:10.1038/s41598-017-11817-6
28. Martincorena I, Campbell PJ. Somatic mutation in cancer and normal cells. *Science*. 2015;349(6255):1483–1489. doi:10.1126/science.aab4082
29. Yang M, Weng Q, Pan X, et al. Clinical and genetic analysis of lipoprotein glomerulopathy patients caused by APOE mutations. *mol Genet Genomic Med*. 2020;8(8):e1281. doi:10.1002/mgg3.1281
30. Wintjens R, Bozon D, Belabbas K, et al. Global molecular analysis and APOE mutations in a cohort of autosomal dominant hypercholesterolemia patients in France. *J Lipid Res*. 2016;57(3):482–491. doi:10.1194/jlr.P055699
31. Kemp SB, Carpenter ES, Steele NG, et al. Apolipoprotein E promotes immune suppression in pancreatic cancer through NF-κB-mediated production of CXCL1. *Cancer Res*. 2021;81(16):4305–4318. doi:10.1158/0008-5472.CAN-20-3929
32. 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. doi:10.1038/s41419-018-0465-5
33. Baitsch D, Bock HH, Engel T, et al. Apolipoprotein E induces antiinflammatory phenotype in macrophages. *Arteriosclerosis Thrombosis Vasc Biol*. 2011;31(5):1160–1168. doi:10.1161/ATVBAHA.111.222745
34. Zhang W, Wang M, Ji C, Liu X, Gu B, Dong T. Macrophage polarization in the tumor microenvironment: emerging roles and therapeutic potentials. *Biomed Pharmacother*. 2024;177:116930. doi:10.1016/j.biopha.2024.116930
35. Zhang H, Wu LM, Wu J. Cross-talk between apolipoprotein E and cytokines. *Mediators Inflammation*. 2011;2011:949072. doi:10.1155/2011/949072
36. Choi C, Yoo GS, Cho WK, Park HC. Optimizing radiotherapy with immune checkpoint blockade in hepatocellular carcinoma. *World J Gastroenterol*. 2019;25:2416–2429. doi:10.3748/wjg.v25.i20.2416
37. Longo V, Brunetti O, Gnoni A, et al. Emerging role of immune checkpoint inhibitors in hepatocellular carcinoma. *Medicina*. 2019;55:698. doi:10.3390/medicina55100698
38. 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. doi:10.7150/ijbs.70117
39. Li Y, Zhang CG, Wang XH, Liu DH. Progression of atherosclerosis in ApoE-knockout mice fed on a high-fat diet. *Eur Rev Med Pharmacol Sci*. 2016;20(18):3863–3867.
40. Liu LJ, Xie SX, Chen YT, Xue JL, Zhang CJ, Zhu F. Aberrant regulation of Wnt signaling in hepatocellular carcinoma. *World J Gastroenterol*. 2016;22(33):7486–7499. doi:10.3748/wjg.v22.i33.7486
41. Russell JO, Monga SP. Wnt/β-catenin signaling in liver development, homeostasis, and pathobiology. *Annu Rev Pathol*. 2018;13:351–378. doi:10.1146/annurev-pathol-020117-044010
42. Massimi M, Ragusa F, Cardarelli S, Giorgi M. Targeting cyclic AMP signalling in hepatocellular carcinoma. *Cells*. 2019;8(12):1511. doi:10.3390/cells8121511
43. Yin Y, Wang Z. ApoE and neurodegenerative diseases in aging. *Adv Exp Med Biol*. 2018;1086:77–92. doi:10.1007/978-981-13-1117-8_5