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ABCC1 is a predictive biomarker for prognosis and therapy in hepatocellular carcinoma

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Abstract. – OBJECTIVE: Liver neoplasm is one of the most fatal malignancies worldwide, among which hepatocellular carcinoma (HCC) (MIM #114550, https://omim.org/) is the most prevalent type. ABCC1 (MIM *158343) is a membrane-bound protein that relies on ATP hydrolysis to transport substrates and is associated with tumour drug resistance and malignant potential. However, the relationship between *ABCC1*, HCC prognosis, and immune infiltration remains elusive.

MATERIALS AND METHODS: We analysed the mRNA expression of *ABCC1* using data from public databases. Immunohistochemistry staining was performed to identify ABCC1 expression in tumour samples. We further investigated the correlation between *ABCC1* and clinicopathological features. We investigated the connection between *ABCC1* and HCC prognosis using survival and Cox regression analyses. We investigated the underlying pathways of *ABCC1* in HCC using functional enrichment analysis and GSEA. We determine the relationship between *ABCC1* and immune cell infiltration via an integrated immune landscape analysis.

RESULTS: Our investigation revealed the upregulation of *ABCC1* expression in HCC ($p < 0.01$), which was verified in clinical samples ($p < 0.01$). In addition, *ABCC1* is adversely associated with HCC clinical features and prognosis ($p < 0.05$). GO/KEGG analysis and GSEA identified that *ABCC1* participates in multiple immune- and tumour-related pathways ($p < 0.05$). Immune cell infiltration analysis indicated that *ABCC1* was positively correlated with various immune cells, among which, the strongest correlation was with macrophages ($p < 0.001$). Furthermore, we observed significant variations in immune checkpoints between the *ABCC1*-low and ABCC1-high groups ($p < 0.01$). This indicated that patients with a high expression of *ABCC1* might respond poorly to immune checkpoint blockade (ICB) therapy ($p = 9.2e^{-07}$).

CONCLUSIONS: Our study identified *ABCC1* as a predictor of HCC prognosis and response to therapy.

Key Words:
HCC, *ABCC1*, Predictive biomarker, Prognosis, Therapy, Tumour immune microenvironment.

Introduction

Liver cancer is the fourth leading cause of cancer-related deaths worldwide, with the highest incidence and mortality of liver cancer occurring in Asian populations[1,2]. Hepatocellular carcinoma (HCC) is the main type of liver cancer, accounting for 75% of the cases. Chronic hepatitis B virus (HBV, MIM #610424) or hepatitis C virus (HCV, MIM #609532) infection, heavy episodic drinking, and non-alcoholic fatty liver disease (NAFLD, MIM #613282) are the main risk factors for HCC[3]. Despite numerous available treatments, including radiofrequency ablation (RFA), surgery, liver transplantation, and chemotherapy drugs, the overall survival rate of patients with HCC is unsatisfactory due to metastasis and a high recurrence rate[4]. With the advent of immunotherapy, immune checkpoint inhibitors (ICIs) have been a breakthrough in the management of HCC[5]. However, owing to the heterogeneity of tumors and the immune microenvironment, not all patients experience survival benefits[6]. Therefore, it is necessary to further study the molecular characteristics of HCC to identify new biomarkers, predict prognosis, and guide individualized clinical treatment[7].

ABCC1 is a member of the ATP-binding cassette (ABC) transporter superfamily and is

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expressed in several tissues, including the liver, kidneys, intestine, and brain. It functions as an exporter that mediates the translocation of various substrates, such as ions, sugars, amino acids, lipids, and drugs, across cell membranes[8]. Classically, *ABCC1* overexpression is associated with tumor resistance to multiple chemotherapeutic agents[9]. In HCC, the upregulation of *ABCC1* expression could lead to tumor resistance to sorafenib and enhance the capacity for metastasis[10,11]. However, the relationship between *ABCC1*, HCC prognosis, and immune infiltration remains elusive.

In this study, we demonstrated the upregulation of *ABCC1* in HCC using public databases and clinical samples. We investigated the correlation between *ABCC1* and the clinicopathological features of HCC and identified *ABCC1* as an independent prognostic indicator of HCC. Functional enrichment analysis and GSEA revealed that *ABCC1* is related to multiple immune- and tumor-related signaling pathways in HCC. Immune landscape analysis revealed that *ABCC1* was associated with various immune cells and immune checkpoints. Moreover, we analyzed the relationship between *ABCC1* expression and response to ICIs. Altogether, we expect that our research will provide insight into the development of new biomarkers for HCC prognosis and treatment.

Materials and Methods

Data Resources

We downloaded the pan-cancer and corresponding normal tissue RNA-seq data from the UCSC Xena database (https://xena.ucsc.edu/), which contains the data from the Cancer Genome Atlas (TCGA) and the Genotype-Tissue Expression (GTEx) project. Public microarray data from other cohorts were obtained from the GEO database, including GSE36376 and GSE45267.

Immunohistochemistry (IHC) Staining

We retrospectively included 35 patients with HCC who underwent surgical resection as the first treatment between 2018 and 2022 at the First Hospital of Shanxi Medical University (Taiyuan, China). Paraffin-embedded specimens were collected for IHC staining. All samples were obtained with patient consent and the patients signed informed consent forms. Paraffin sections were dewaxed, rehydrated, and subjected to heat-induced antigen retrieval using Tris-EDTA (pH 9.0) buffer (Solarbio, Beijing, China) for 10 min in a pressure cooker. Peroxidase inhibitors (Zhongshan Jinqiao, Beijing, China) were used to block the endogenous peroxidase activity. Next, the sections were incubated in ABCC1 primary antibody (Abcam, Cambridge, UK) at 4°C overnight and subsequently incubated with the secondary antibody (Zhongshan Jinqiao) at 37°C for 1 h. Colour was developed using freshly prepared DAB (KeyGen Biotech, Nanjing, China). Finally, the sections were counterstained with haematoxylin (Solarbio) and imaged under a bright-field microscope (Olympus, Tokyo, Japan). Each section was independently evaluated by two professional pathologists. The mean density (IOD/area) was calculated using Image-Pro Plus 6.0 (Media Cybernetics, Silver Spring, MD, USA).

Survival Analysis

The patients were divided into two groups based on the median expression of *ABCC1*. Kaplan-Meier analysis was performed to analyze the survival rate. Furthermore, the effect of *ABCC1* on survival was verified using an online Kaplan-Meier plotter[12].

Cox Regression Analysis

The possible risk factors for HCC prognosis among multiple elements were identified using univariate and multivariate regression analyses. Forest plots were used for visualisation.

Correlation Analysis and Enrichment Analysis

Correlations between *ABCC1* and co-expressed genes were described using Spearman's correlation analysis. Genes with $|r| \geq 0.5$ and $p < 0.001$ were chosen for Gene Ontology (GO) and Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathway analysis. The clusterprofiler and ggplot2 packages were used for enrichment analysis and visualisation.

Gene Set Enrichment Analysis (GSEA)

GSEA was performed to investigate the biological activities and predict the potential signaling pathways of *ABCC1* in HCC. The MSigDB collection database was used as the gene set database. The normalized enrichment score (NES) and *p*-values were used to classify the enriched pathways[13].

Immune Landscape Analysis

The CIBERSORT immune score was used to determine the ratio of 22 types of immune cells[14]. The correlation between *ABCC1* and the six immune cell types was investigated using Tumor Immune Estimation Resource (TIMER) database[15]. We further assess the association between *ABCC1* and 24 types of immune cells using ssGSEA. The differences of macrophage scores between the two groups were performed using differential analysis. Additionally, differences in immune checkpoint expression and tumor immune dysfunction and exclusion (TIDE) scores between the two groups were analyzed. Potential immunotherapeutic responses were predicted using the TIDE algorithm[16].

Anti-Cancer Drug Sensitivity Analysis

We downloaded the relevant data of 138 drugs from the Genomics of Drug Sensitivity in Cancer (GDSC) database. The link between *ABCC1* expression and inhibitory concentrations (IC_{50}) in each sample was predicted using the pRRophetic R package[17]. Ridge regression was used to determine the half-maximal IC_{50} of the samples. Correlation heat maps and scatter plots were used for visualization.

Statistical Analysis

We used R software (R Foundation for Statistical Computing, Vienna, Austria) to perform statistical analysis. The results of two independent samples were examined using the Wilcoxon rank-sum test. The relationship between *ABCC1* and other variables was analysed using univariate logistic regression analysis. We implemented Kaplan-Meier analysis using the survival and survminer R packages. Correlations among *ABCC1*, immune cells, and immune checkpoints were investigated using Spearman's correlation. A *p*-value lower than 0.05 was considered statistically significant.

Results

ABCC1 Expression Levels in Tumours vs. Normal Tissues

We first combined information from the TCGA database with the GTEx database to study *ABCC1* mRNA expression in cancerous and non-cancerous tissues. *ABCC1* was highly expressed in most cancer types, including HCC ($p < 0.001$, Figure 1A). Subsequently, we examined *ABCC1* expression in paired and unpaired samples and found that *ABCC1* expression was significantly elevated in the tumour tissues ($p < 0.001$, Figure 1B-C). Additionally, samples from GSE36376 and GSE45267 confirmed these findings ($p < 0.001$, Figure 1D and $p < 0.01$, Figure 1E). We further verified ABCC1 expression in clinical specimens, and IHC showed that tumour tissues exhibited higher levels of ABCC1 protein expression ($p < 0.01$, Figure 1F-H). In summary, our analysis demonstrated that *ABCC1* expression is significantly increased in HCC.

ABCC1 Related to Clinicopathological Characteristics of HCC

Owing to the small number of cases in some stages, certain classifications were combined for analysis. Our results indicated that the upregulation of *ABCC1* expression was significantly positively correlated with the T stage (all $p < 0.001$), pathologic stage (all $p < 0.01$), histologic stage ($p < 0.05$), residual tumours ($p < 0.01$), and OS events ($p < 0.01$) (Figure 2A-E). Nevertheless, *ABCC1* expression was not correlated with gender, age, or vascular invasion ($p > 0.05$, Figure 2F-H). Furthermore, univariate logistic regression analysis revealed that *ABCC1* was associated with T stage (all $p < 0.01$), pathologic stage (all $p < 0.01$), and histologic stage (G3&G4 *vs.* G1, $p = 0.022$) (Table I). Therefore, our findings imply that high *ABCC1* is associated with unfavourable clinicopathological features.

ABCC1 Identified as a Negative Prognostic Factor for HCC

First, we performed survival analysis to study the impact of *ABCC1* on the survival rate of HCC. We observed a poor OS ($p = 0.00119$) and DFS ($p = 0.0469$) rate in patients with high *ABCC1* expression (Figure 3A and 3B), indicating poor prognosis. Next, the results from the Kaplan-Meier Plotter further validated that *ABCC1* overexpression was associated with OS ($p = 8.9e^{-05}$) and DFS ($p = 0.0054$) (Figure 3C and 3D). In addition, univariate regression analysis indicated that *ABCC1* ($p < 0.001$), the pathologic stage ($p < 0.001$), and the T stage ($p < 0.001$) were significantly correlated with OS (Figure 3E). Based on multivariate regression analysis, *ABCC1* was the only parameter that was statistically associated with OS ($p = 0.019$, Figure 3F), which suggested that *ABCC1* was an independent predictor for HCC survival.

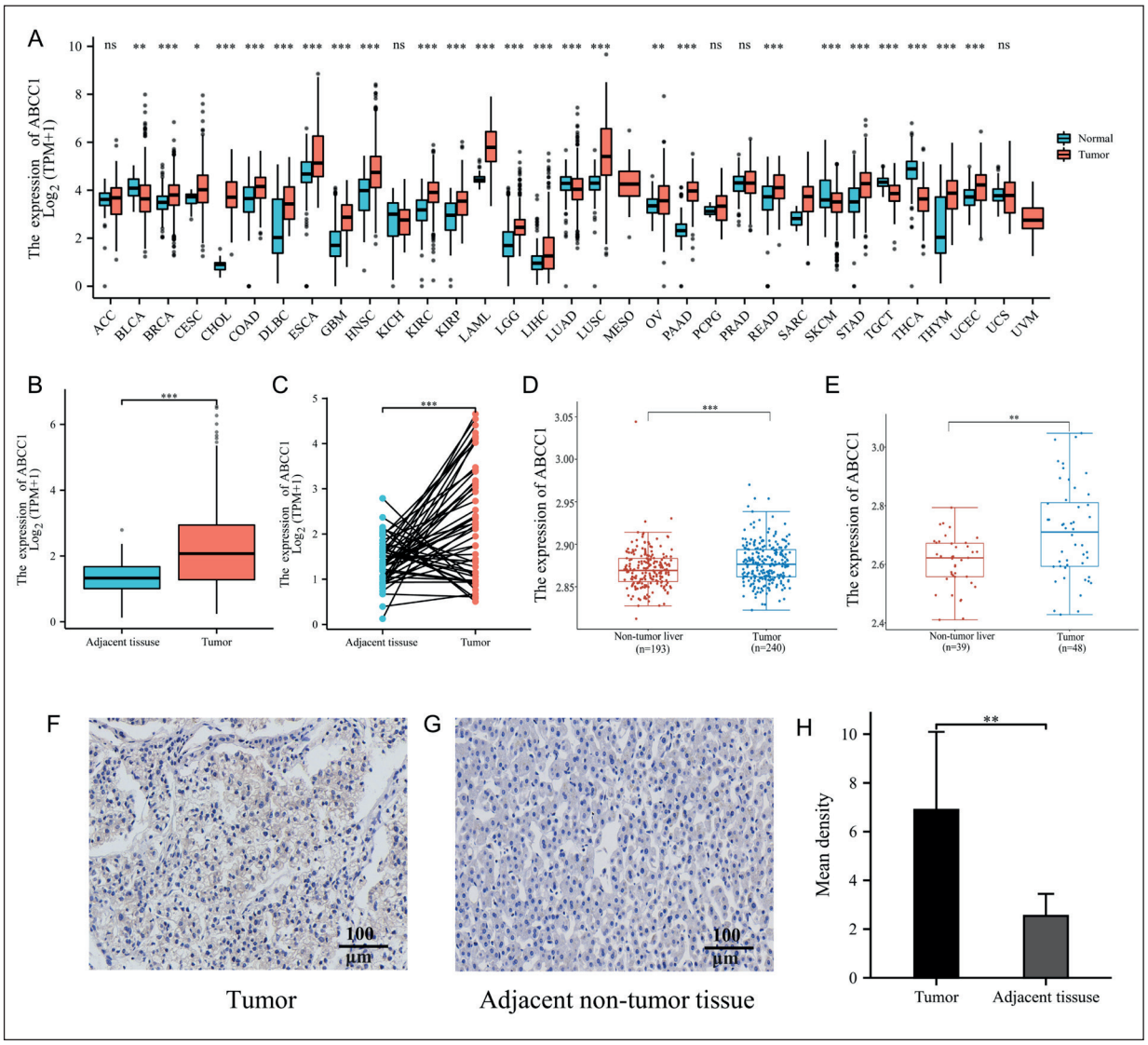

Figure 1. *ABCC1* expression in pan-cancer and HCC. **A,** *ABCC1* mRNA expression in multiple cancers and normal tissues from the TCGA and GTEx databases. **B,** Unpaired samples from the TCGA database showed different *ABCC1* mRNA expression in the tumour and adjacent tissues. **C,** Paired samples from the TCGA database showed different *ABCC1* mRNA expression in the tumour and adjacent tissues. **D-E,** Differential *ABCC1* mRNA expression between tumorous and non-tumorous liver tissues from the GSE36376 and GSE45267 datasets. **F-H,** IHC staining of ABCC1 in HCC and adjacent tissues. (*p < 0.05, **p < 0.01, ***p < 0.001).

Correlation Analysis and GO/KEGG Enrichment Analysis

We performed a correlation analysis of *ABCC1* and other co-expressed genes to gain insight into the underlying mechanisms of *ABCC1*-mediated HCC. For subsequent GO/KEGG enrichment analysis, we selected 469 genes that were co-expressed with *ABCC1* with $|r|>0.5$ and $p < 0.001$. The 50 top genes that were either positively or negatively correlated with *ABCC1* are depicted in Figure 4A and 4B. We identified 143 GO terms that were significantly enriched. The top ten GO terms of biological processes (BP), cellular components (CC), and molecular functions (MF) are represented in Figure 4C-E. GO annotations revealed that the genes co-expressed with *ABCC1* primarily participated in neutrophil-mediated immunity ($p < 0.001$), neutrophil activation involved in the immune response ($p < 0.001$), cell-substrate junctions ($p < 0.001$), and cell adhesion molecule binding ($p < 0.001$). Moreover, KEGG analysis indicated that regulation of the actin cytoskeleton

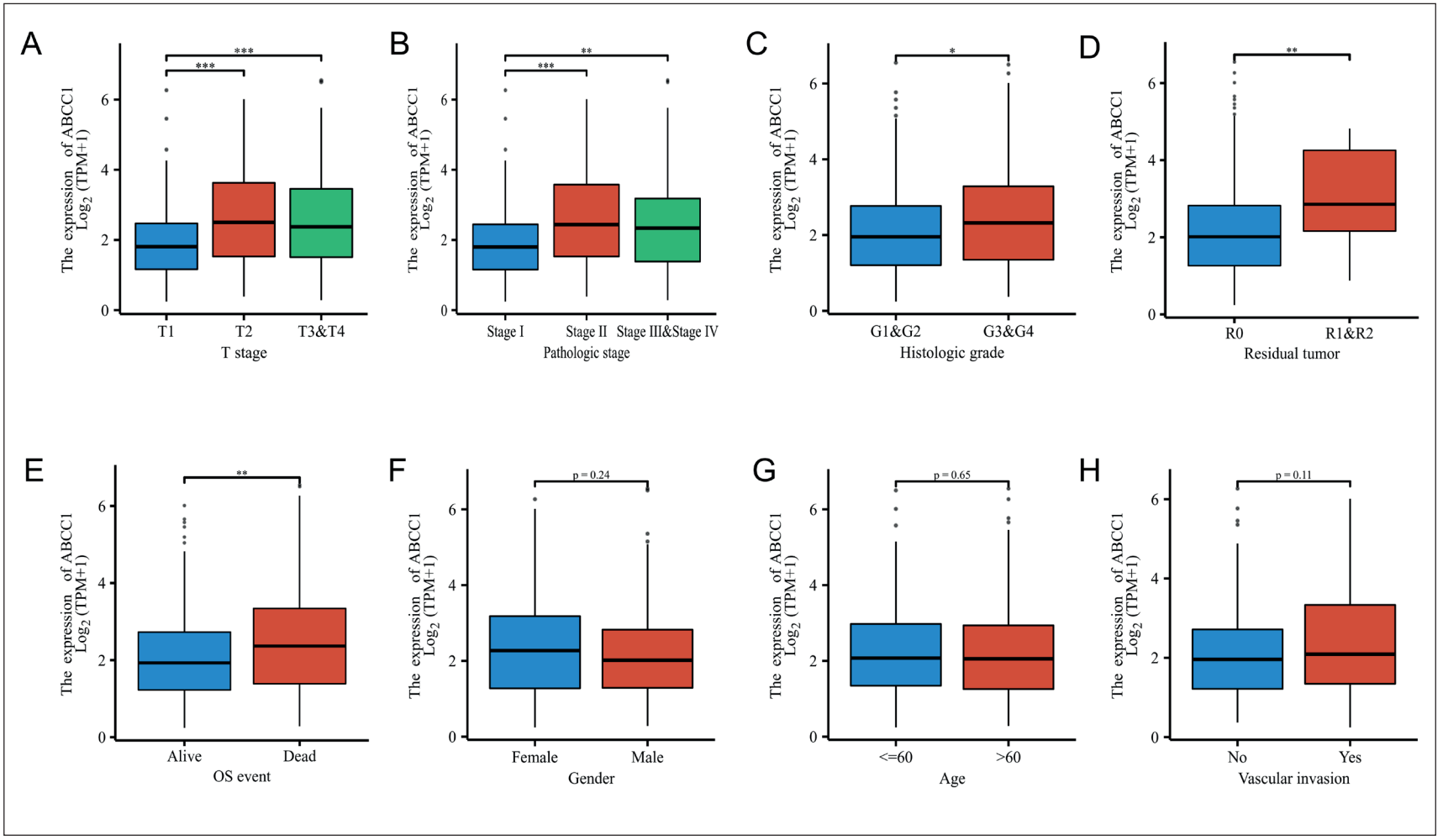

Figure 2. Relationship of *ABCC1* expression and clinical features in HCC. *ABCC1* mRNA expression level was significantly correlated with T stage (**A**), pathologic stage (**B**), histologic grade (**C**), residual tumour (**D**), and OS events (**E**). *ABCC1* mRNA expression levels had no significant relationship with gender (**F**), age (**G**), and vascular invasion (**H**). (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

Table I. Relationship between *ABCC1* expression and clinical characteristics using logistic regression.

Characteristics	Total (N)	Odds Ratio (OR)	*p*-value
Gender			
Male *vs.* Female	374	0.802 (0.519-1.238)	0.320
Age			
>60 *vs.* <=60	373	0.969 (0.645-1.455)	0.878
T stage			
T2 *vs.* T1	278	2.585 (1.560-4.335)	<0.001
T3&T4 *vs.* T1	276	2.283 (1.377-3.820)	0.001
N stage			
N1 *vs.* N0	258	3.298 (0.416-67.150)	0.304
M stage			
M1 *vs.* M0	272	1.062 (0.126-8.952)	0.953
Pathologic stage			
Stage II *vs.* Stage I	260	2.590 (1.533-4.427)	<0.001
Stage III&Stage IV *vs.* Stage I	263	2.274 (1.357-3.842)	0.002
Histologic grade			
G2 *vs.* G1	233	1.513 (0.821-2.841)	0.189
G3&G4 *vs.* G1	191	2.113 (1.121-4.058)	0.022
Weight			
>70 *vs.* <=70	346	1.071 (0.702-1.634)	0.752
Height			
>=170 *vs.* < 170	341	1.352 (0.878-2.087)	0.172
BMI			
>25 *vs.* <=25	337	0.986 (0.643-1.513)	0.950
Adjacent hepatic tissue inflammation			
Mild&Severe *vs.* None	237	1.206 (0.724-2.013)	0.472
AFP (ng/ml)			
400 *vs.* <=400	280	0.892 (0.509-1.556)	0.689
Albumin (g/dl)			
>=3.5 *vs.* <3.5	300	1.058 (0.617-1.824)	0.839
Child-Pugh grade			
B&C *vs.* A	241	0.839 (0.334-2.027)	0.700
Vascular invasion			
Yes *vs.* No	318	1.187 (0.747-1.887)	0.468
Prothrombin time			
>4 *vs.* <=4	297	0.947 (0.575-1.556)	0.829

($p < 0.001$), endocytosis ($p < 0.01$), proteoglycans in cancer ($p < 0.01$), platelet activation ($p < 0.01$), and the phosphatidylinositol signaling system ($p < 0.05$) were significantly enriched in HCC (Figure 4F).

Potential Signaling Pathways Meditated by ABCC1 in HCC

To explore the signaling pathways mediated by *ABCC1*, we performed GSEA between the *ABCC1*-low and *ABCC1*-high groups. The analysis indicated that various immune-related pathways and classic pathways implicated in cancer pathogenesis were enriched in the *ABCC1*-high group ($p < 0.05$, Figure 5A-L), suggesting that *ABCC1* may participate in pathways related to immunity and tumour development.

ABCC1 Expression Related to Immune Cells and Immune Checkpoints

We first assessed the infiltration of 22 types of immune cells in patients with HCC using

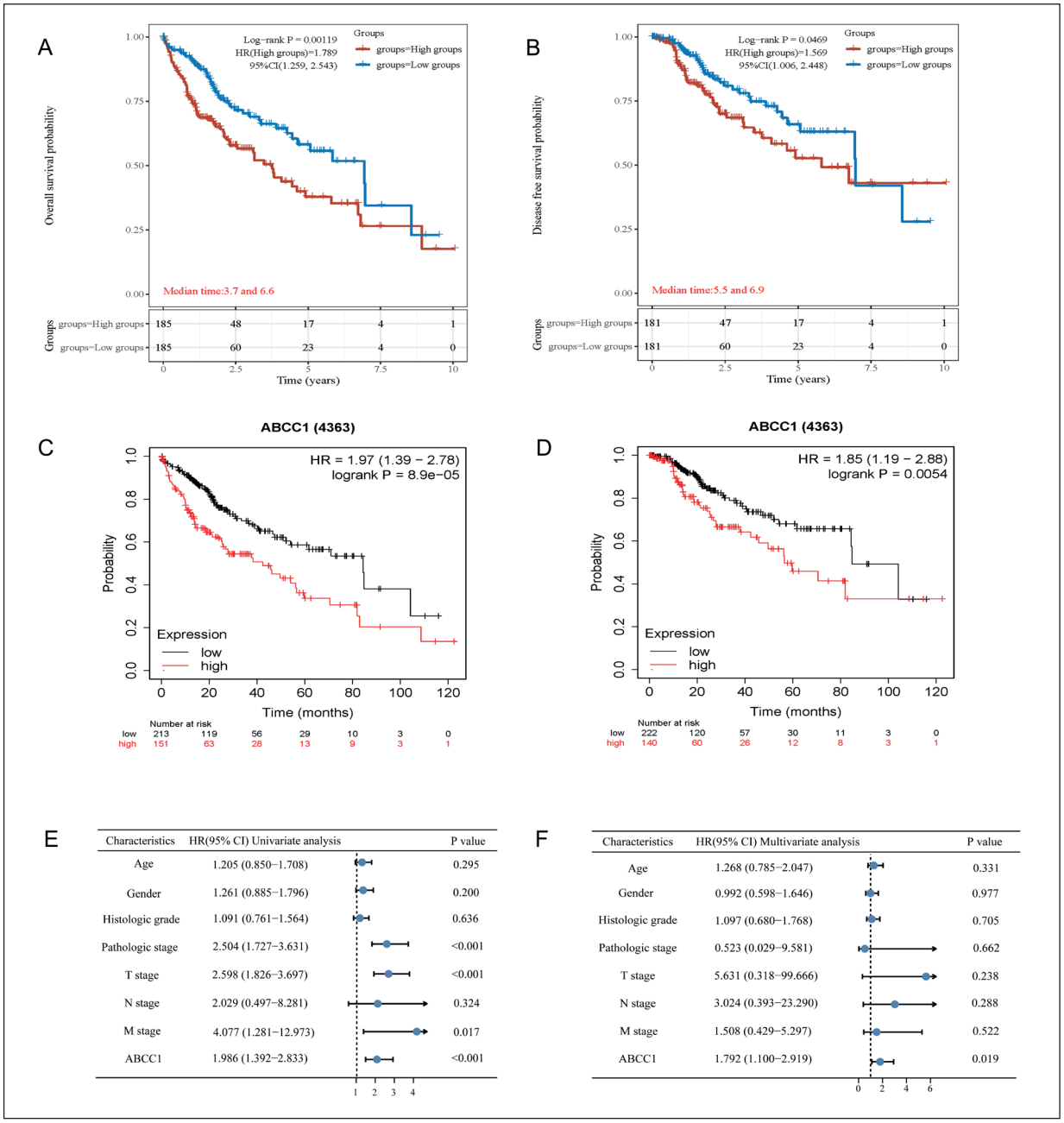

Figure 3. Effect of *ABCC1* expression and other clinical features on HCC survival. **A-B,** K-M analysis suggested that the high-*ABCC1* group was associated with unfavourable OS and DFS in the TCGA database. **C-D,** Survival analysis of OS and DFS performed by the K-M plotter website. **E-F,** Univariate and multivariate regression analysis of *ABCC1* and other factors. OS, overall survival; DFS, disease-free survival.

CIBERSORT analysis (Figure 6A). Subsequently, we calculated the correlation between *ABCC1* and the six types of immune cells using the TIMER database. We found that *ABCC1* expression was positively correlated with B cells ($p = 3.87e^{-11}$), CD8+ T cells ($p = 3.58e^{-11}$), CD4+ T cells ($p = 3.41e^{-19}$), macrophages ($p = 4.54e^{-35}$), neutrophils ($p = 1.39e^{-25}$), and dendritic cells ($p = 1.94e^{-18}$) (Figure 6B). Furthermore, ssGSEA was used to determine the correlation between *ABCC1* and 24 types of immune cells. The ssGSEA indicated that *ABCC1* was positively correlated with macrophages, TFH, Th1 cells, iDC, Th2 cells, T helper cells, Tem, NK CD56bright cells, aDC, NK cells,

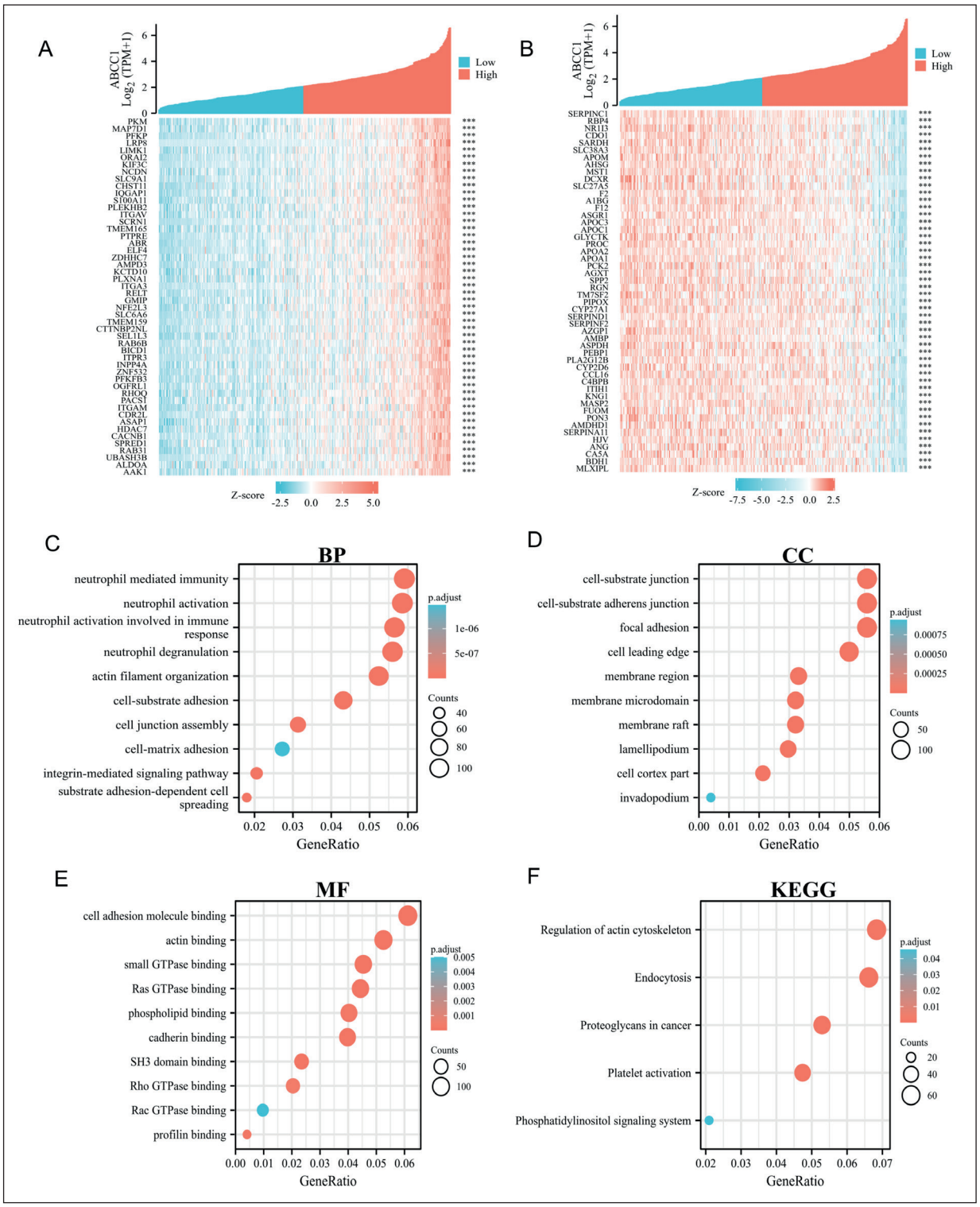

Figure 4. *ABCC1*-related genes and potential pathways enriched in HCC. **A,** The top 50 genes correlated positively with *ABCC1*. **B,** The top 50 genes correlated negatively with *ABCC1*. **C,** GO annotation: biological processes (BP). **D,** GO annotation: cellular component (CC). **E,** GO annotation: molecular functions (MF). **F,** KEGG terms.

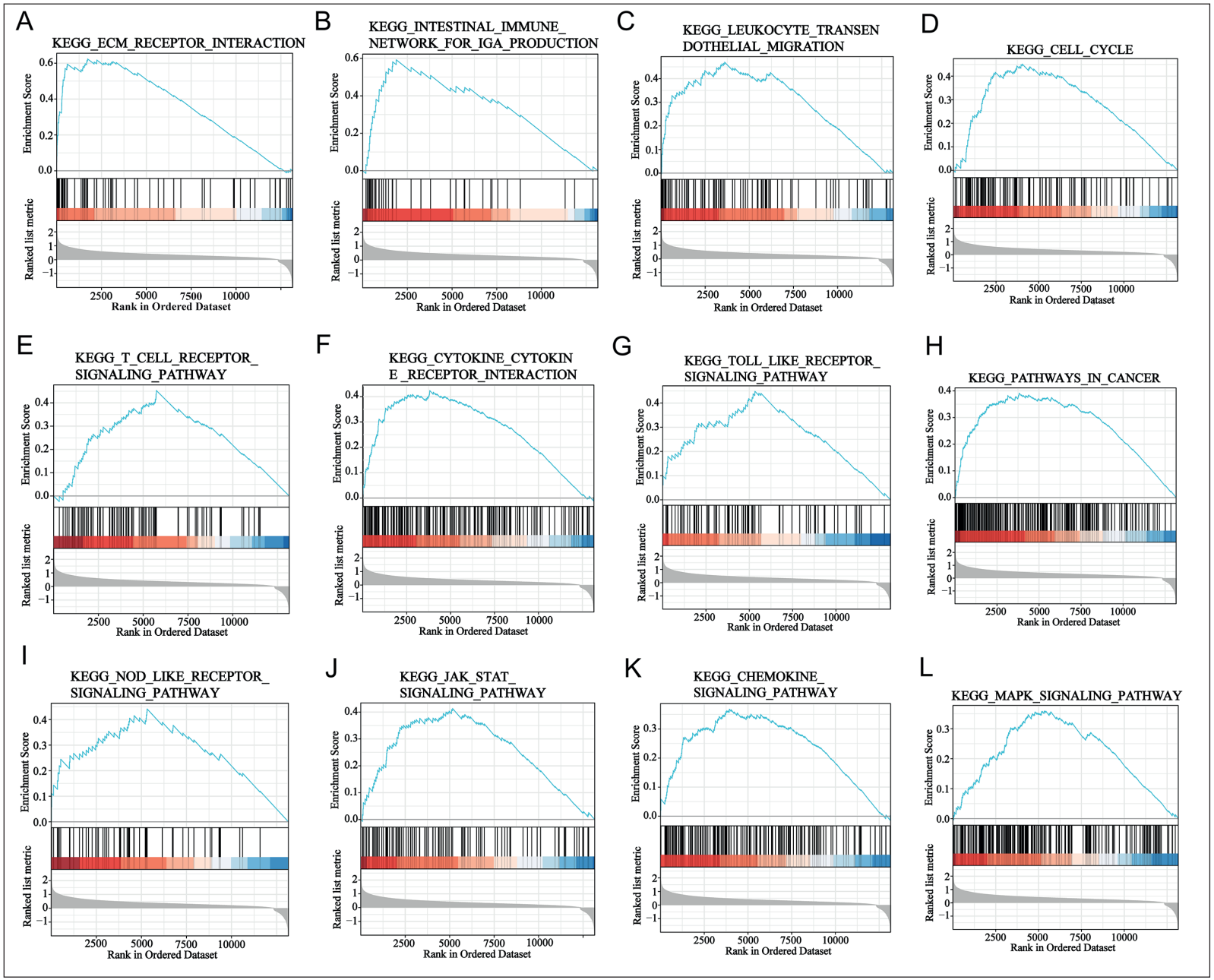

Figure 5. GSEA of the highly expressed *ABCC1* phenotype. **A,** ECM receptor interaction. **B,** Intestinal immune network for IgA production. **C,** Leukocyte transendothelial migration. **D,** Cell cycle. **E,** T cell receptor signaling pathway. **F,** Cytokine-cytokine receptor interaction. **G,** Toll-like receptor signaling pathway. **H,** Pathways in cancer. **I,** NOD-like receptor signaling pathway. **J,** JAK-STAT signaling pathway. **K,** Chemokine signaling pathway. **L,** MAPK signaling pathway.

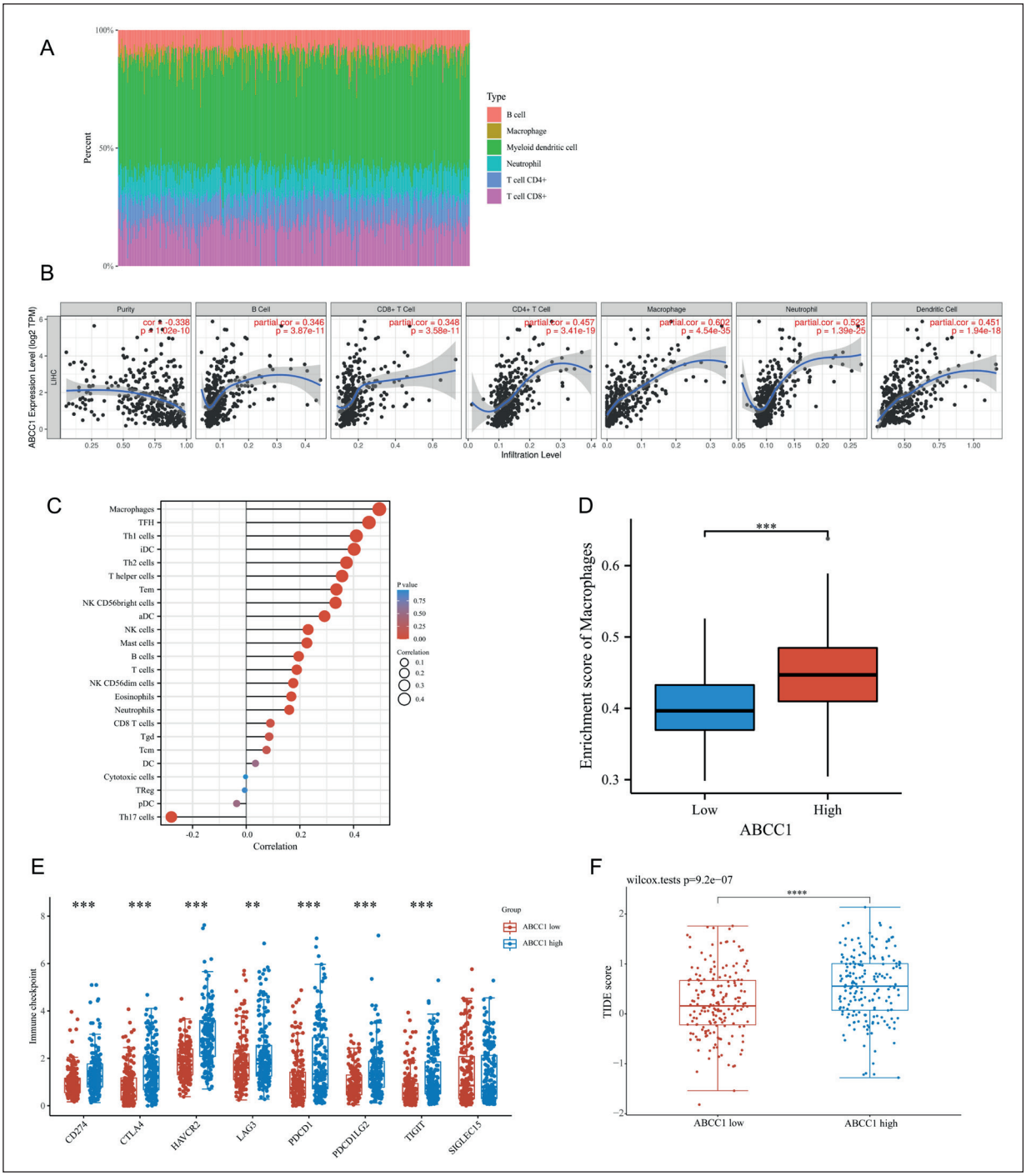

Figure 6. Relationship between *ABCC1*, TIICs and immune checkpoints. **A,** The proportion of 22 types of immune cells in patients with HCC. **B,** Correlation of *ABCC1*, tumour purity, and 6 kinds of immune cells from TIMER database. **C,** Correlation of *ABCC1* and 24 kinds of immune cells by ssGSEA. **D,** Differential analysis of macrophage enrichment score between *ABCC1*-low and *ABCC1*-high group. **E,** Differential analysis of immune checkpoint expression between *ABCC1*-low and *ABCC1*-high group. **F,** Differential analysis of TIDE scores between *ABCC1*-low and *ABCC1*-high group. (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

mast cells, B cells, T cells, NK CD56dim cells, eosinophils, and neutrophils; and negatively correlated with Th17 cells (Figure 6C, Spearman's correlation). The specific correlation coefficients and *p*-values of the Pearson's and Spearman's analyses are shown in Table II. Importantly, both the TIMER database and ssGSEA showed the strongest correlation was between *ABCC1* and

Table II. Correlation between *ABCC1* expression and 24 kinds of immune cells using ssGSEA analysis.

Immune cells	*r* (Pearson)	*p*-value	*r* (Spearman)	*p*-value
Macrophages	0.456	<0.001	0.496	<0.001
TFH	0.437	<0.001	0.458	<0.001
Th1 cells	0.396	<0.001	0.411	<0.001
iDC	0.373	<0.001	0.402	<0.001
Th2 cells	0.378	<0.001	0.374	<0.001
T helper cells	0.347	<0.001	0.357	<0.001
Tem	0.346	<0.001	0.336	<0.001
NK CD56bright cells	0.354	<0.001	0.333	<0.001
aDC	0.291	<0.001	0.292	<0.001
Th17 cells	-0.307	<0.001	-0.279	<0.001
NK cells	0.286	<0.001	0.231	<0.001
Mast cells	0.190	<0.001	0.226	<0.001
B cells	0.149	0.004	0.195	<0.001
T cells	0.170	<0.001	0.188	<0.001
NK CD56dim cells	0.210	<0.001	0.175	<0.001
Eosinophils	0.137	0.008	0.168	0.001
Neutrophils	0.112	0.030	0.160	0.002
CD8 T cells	0.116	0.024	0.090	0.082
Tgd	0.073	0.158	0.085	0.099
Tcm	0.080	0.121	0.076	0.144
pDC	-0.081	0.117	-0.036	0.493
DC	-0.031	0.555	0.034	0.507
TReg	-0.078	0.133	-0.006	0.913
Cytotoxic cells	-0.027	0.598	-0.003	0.962

macrophages; therefore, we investigated the difference in macrophage levels between the *ABCC1*-low and *ABCC1*-high groups. The group with high *ABCC1* levels had a higher macrophage enrichment score ($p < 0.001$, Figure 6D). Thus, these results suggest that *ABCC1* is significantly associated with immune cell infiltration, especially of macrophages.

Immune checkpoints serve as modulatory molecules in the control of the immune system, suppressing the activation of T cells and promoting T cell depletion, resulting in tumour immune escape[18]. We examined the differences in immune checkpoint expression between the *ABCC1*-low and *ABCC1*-high groups. Differential analysis showed that there were significant differences in the expression of the seven immune checkpoints between the two groups, and the *ABCC1*-high group had higher levels of immune checkpoint expression than the *ABCC1*-low group (all $p < 0.01$, Figure 6E). Additionally, we examined whether the reactions of the two groups to immune checkpoint blockade (ICB) varied. The results showed that the *ABCC1*-high group had a considerably higher ICB score than the *ABCC1*-low group ($p = 9.2e^{-07}$), indicating that individuals with elevated *ABCC1* expression did not respond well to immune checkpoint blockades (Figure 6F). Therefore, our results indicate that *ABCC1* is a predictive biomarker for the efficacy of ICIs.

Anti-Cancer Drug Sensitivity Analysis

We further investigated the correlation between *ABCC1* expression and drug sensitivity by analysing the drug response data for 138 compounds. We found an inverse correlation between IC_{50} and *ABCC1* expression in 65 drugs, suggesting that the chemotherapeutic response to these medications may be improved in samples with high *ABCC1* expression. The IC_{50} values of 30 drugs were positively correlated with *ABCC1* expression, suggesting that these drugs may have a worse curative effect on samples with high *ABCC1* expression (Figure 7A). We selected six drugs with IC_{50} values that were best associated with high *ABCC1* expression among the total 95 drugs (Figure 7B, nilotinib, $r = –0.60$, $p = 3.37e^{-37}$; PD.173074, $r = –0.53$, $p = 8.55e^{-28}$; ABT.263,

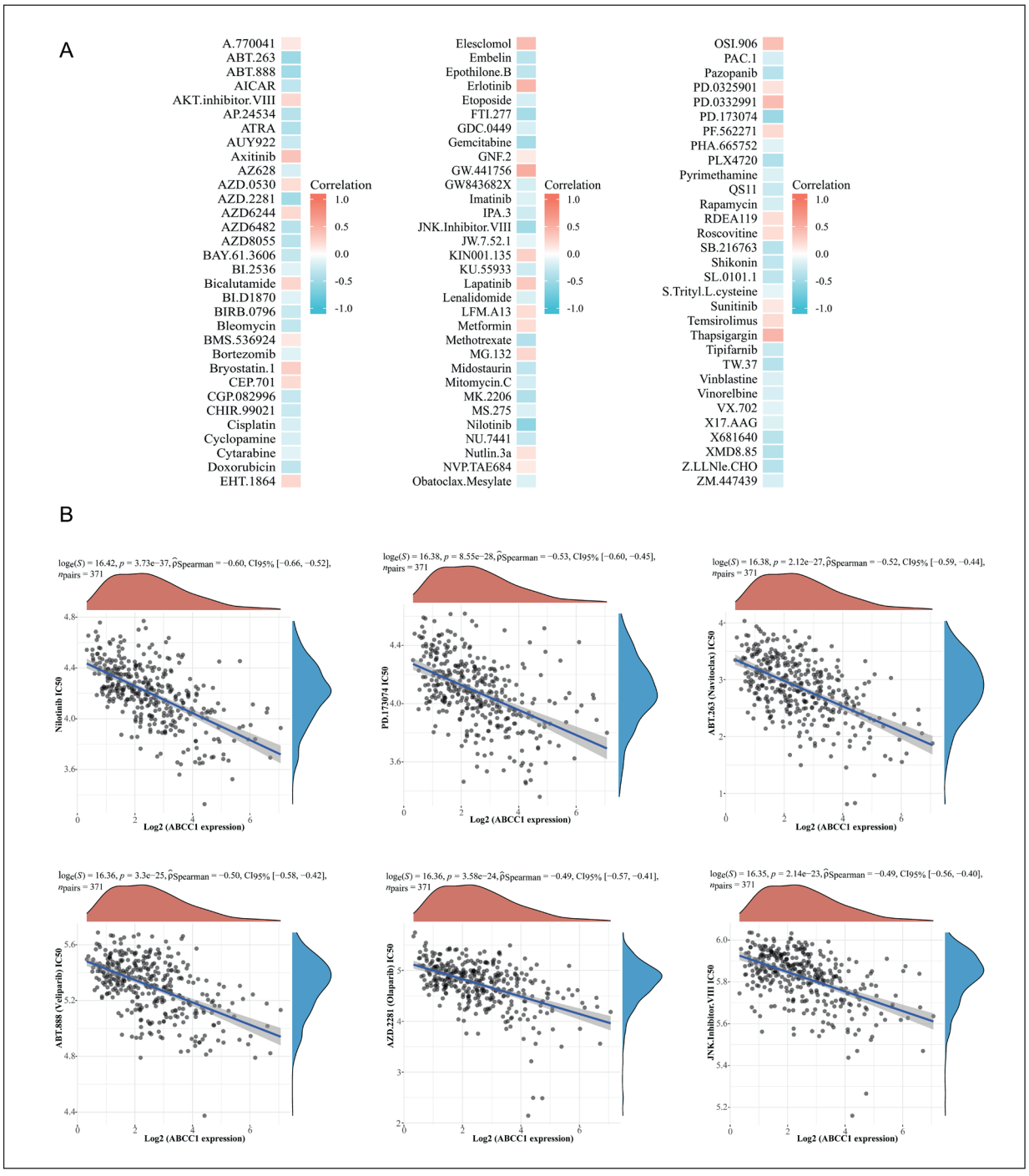

Figure 7. Correlation of drug IC_{50} and *ABCC1* expression. **A,** The heatmap of the correlation between *ABCC1* expression and drug IC_{50}. **B,** The scatter plot of the correlation between *ABCC1* expression and drug IC_{50} values.

$r = -0.52$, $p = 2.12e^{-27}$; ABT.888, $r = -0.50$, $p = 3.3e^{-25}$; AZD2281, $r = -0.49$, $p = 3.58e^{-24}$; JNK. Inhibitor.VIII, $r = -0.49$, $p = 2.14e^{-23}$). For patients with HCC exhibiting high *ABCC1* expression, these findings may be clinically beneficial.

Discussion

Although increasing numbers of therapeutic strategies aimed at HCC have been developed, their clinical efficacy and the outcomes of HCC

remain unsatisfactory[1,4]. Hence, it is crucial to identify new prognostic and predictive biomarkers to improve HCC prognosis.

ABCC1 plays a key role in cellular drug excretion. The upregulation of *ABCC1* can promote chemotherapy drug resistance[9]. Furthermore, *ABCC1* has a direct impact on tumor progression[19]. However, the prognostic value of *ABCC1* and its role in the tumor immune microenvironment are still not fully understood.

In our study, we first characterized the *ABCC1* expression in pan-cancer and found that *ABCC1* is overexpressed in a variety of tumors. Next, we combined the TCGA and GEO databases with clinical samples and confirmed the upregulation of *ABCC1* mRNA and ABCC1 protein expression in HCC. Furthermore, high levels of *ABCC1* expression are associated with worse clinical and pathological features. Survival analysis further indicated that patients with HCC and high *ABCC1* levels had worse OS and DFS. Consistent with our findings, high *ABCC1* expression in intrahepatic cholangiocarcinoma (MIM 615619) and acute myeloid leukemia (MIM #601626) was also associated with poor prognosis[20,21]. Moreover, Cox regression analysis showed that *ABCC1* expression had a better performance in predicting the survival of HCC than the T stage and pathologic stage, which indicated that *ABCC1* is an independent predictor of HCC prognosis. However, the specific mechanisms of *ABCC1* in the progression and prognosis of HCC remain to be elucidated. Hence, we performed GO/KEGG analysis and GSEA to investigate the underlying regulatory pathways in which *ABCC1* might be involved. We observed that *ABCC1* and its co-expressed genes participate in immune progression by regulating neutrophil-mediated immunity, neutrophil activation, and neutrophil degranulation. Moreover, pathway analysis showed significant enrichment of endocytosis, proteoglycans in cancer, and platelet activation. Immune, inflammatory, and cancerous signaling pathways were considerably enriched in the group with high *ABCC1* expression, according to GSEA.

The application of ICI has been a major breakthrough in the history of cancer immunotherapy, which shed new light on the treatment of many advanced cancers[22]. The tumor microenvironment (TME) includes a wide range of immune cells, endothelial cells, and fibroblasts. The composition of the TME may influence the response to ICB therapy, and the TME is of great importance in guiding the selection and combination of immune therapy[23]. HCC is mediated by a strong immune mechanism, making immunotherapy a promising method for the systemic management of HCC. However, the existence of numerous immunosuppressive mechanisms in the TME of HCC combined with a lack of reliable predictive biomarkers limits the clinical application of immunotherapy[24,25]. Therefore, we investigated how *ABCC1* expression affects tumor-infiltrating immune cells (TIICs) and immune checkpoints. Results showed that *ABCC1* was significantly positively associated with a variety of immune cells, suggesting a higher degree of tumor immune cell infiltration in patients with HCC with high *ABCC1* expression. Moreover, macrophages had the strongest correlation with high *ABCC1*. Differential analysis revealed that macrophage infiltration varied between the *ABCC1*-low and *ABCC1*-high groups. Macrophages are a major component of TIICs that differentiate into two functionally distinct subtypes: M1 and M2 macrophages. M1 macrophages have the capacity for anti-tumor function, whereas M2 macrophages promote tumor progression[26]. M2 macrophages also express programmed death receptor-1 (PD-1), which plays a critical role in inducing tumor immune escape[27]. Moreover, the infiltration of macrophage was associated with poor prognosis in patients with solid tumours[28]. In HCC, tumor-associated macrophages are correlated with either negative or positive outcomes which specifically depend on the subtype of macrophage infiltration[29]. Regardless, targeting macrophage reprogramming is a promising strategy in cancer immunotherapy[30]. Our results indicate that *ABCC1* may be involved in macrophage infiltration in HCC, which could partially explain the association between *ABCC1* and poor HCC prognosis. However, whether *ABCC1* regulates macrophage polarization and affects tumor progression requires further investigation.

During the last decade, impressive advances have been made in immune checkpoint blockade therapy. As in other cancers, inhibitory receptors or their ligands play a crucial role in HCC[31]. Several clinical trials[32,33] have explored the efficacy of ICI as an alternative therapy for advanced HCC, and anti-PD-1 drugs have been approved as a second option for people who are sorafenib refractory. However, for reasons currently unknown, most patients with HCC do not achieve a complete response to ICI[6,34]. Therefore, we investigated the differential expression of immune checkpoints between patients with low and high

ABCC1 expression and predicted their response to ICB therapy. We observed that the levels of immune checkpoints were higher in the *ABCC1*-high group, indicating that *ABCC1* may promote the expression of immune checkpoint in HCC. Moreover, the *ABCC1*-high group had a higher TIDE score, which suggests that the *ABCC1*-high group experienced poor efficacy of ICB treatment and short survival after ICB treatment. This partly explains the heterogeneous response to ICIs among patients with HCC. Finally, we studied the correlation between *ABCC1* expression and drug sensitivity and identified six drugs that were most sensitive to *ABCC1* overexpression. Our research may help improve the precision of antitumor therapy among different individuals.

Conclusions

In the present study, we analysed *ABCC1* expression, its association with clinical characteristics and prognosis, and its potential biological function in HCC. Furthermore, our study demonstrated that *ABCC1* contributes to the TME of HCC and may serve as a predictor for the efficacy of immunotherapy. We hope that our results provide a basis for predicting HCC prognosis and guiding precise treatment.

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Conflict of Interest
The authors declare no conflicts of interest in this study.

Authors' Contributions
YH, YG, and LL conceived of and designed the study. YH, YW, and JB performed data analysis, conducted the experiments, and wrote the manuscript. QZ participated in performing of the experiment. The experiments were reviewed by LL, who also edited the manuscript. The submission and publication of this article were approved by all authors.

Data Availability
The datasets for this study can be found in the UCSC Xena database and GEO database (https://www.ncbi.nlm.nih.gov/geo/).

Ethics Approval
This study was performed in accordance with the principles of the Declaration of Helsinki and approved by the Ethics Review Committee of the First Hospital of Shanxi Medical University (2021 K018).

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