

# Identification of ABCC5 Among ATP-Binding Cassette Transporter Family as a New Biomarker for Hepatocellular Carcinoma Based on Bioinformatics Analysis

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**Purpose:** Liver cancer is the fifth most common type of cancer worldwide, and the ATP-binding cassette (ABC) transporter family has been widely accepted as a cause of multidrug resistance. This study was conducted to explore the potential value and mechanisms of the ABC transporter gene family in the liver hepatocellular carcinoma (LIHC).

**Materials and Methods:** Data were collected from different public databases. UALCAN, ONCOMINE, and GEPIA were used to retrieve a selection of differently expressed and pathological stage-related genes among the ABC family. Principal component analysis (PCA) was utilized for grouping, and its prognostic value was evaluated by univariate and multivariate Cox analyses. The co-expression pattern was constructed with UALCAN, and the functional analyses were carried out with DAVID. The correlation between the biomarker and immune infiltration, genetic alteration frequency, and drug sensitivity were explored with TIMER, cBioPortal, GDSC and CTRP, respectively. Finally, tSNE algorithm was used to explore the distribution of ABCC5 expressed cells.

**Results:** Among the ABC transporter family members, ABCC5 was differently expressed and strongly related to the pathological stage of LIHC. PCA divided patients of LIHC into two groups, and Cox analyses demonstrated that ABCC5 was an independent risk factor of LIHC. Functional analyses indicated that the genes were enriched in the pathways of transmembrane transporter, ATPase activity, and bile secretion. ABCC5 is also associated with immune infiltration of cells like macrophages, neutrophils, and dendritic cells. The genetic alteration frequency of ABCC5 confirmed its potential value in LIHC. In addition, several drugs were explored and found to be relevant to LIHC. The t-SNE showed that expression of ABCC5 was most concentrated in macrophages, followed by hepatocytes.

**Conclusion:** ABCC5 may facilitate LIHC progression through different mechanisms and be a potential biomarker and target for diagnosis, prognosis, and therapy of LIHC.

**Keywords:** LIHC, bioinformatics analysis, ATP-binding cassette transporter family, ABCC5

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## Introduction

Liver cancer is the fifth most common type of cancer worldwide. According to statistics, the incidence of liver cancer accounted for 4.7% of cancers in 2018, while the number of deaths caused by liver cancer accounted for 8.2%, ranking it the fourth leading cause of cancer-related mortality worldwide.<sup>1</sup> Liver cancer tends to occur in elderly males with a male-to-female incidence ratio of approximately 2–2.5:1. Histopathology subtyping has shown that liver hepatocellular carcinoma



(LIHC) represents about 90% of primary hepatic carcinomas.<sup>2</sup> As a result, LIHC is a serious and challenging global health problem. The major causes of LIHC include hepatitis C or B, alcoholic and non-alcoholic fatty liver disease (NAFLD), and metabolic syndrome (MS), which is a pathological cluster of metabolic disorders that may also contribute to LIHC.<sup>3,4</sup> One of the reasons for its high mortality is the difficulty in detecting LIHC in an early stage. Currently, the treatment for LIHC consists of radical liver resection, liver transplantation, transcatheter arterial chemoembolization, radiofrequency ablation, radioembolization, and tyrosine-kinase inhibitors.<sup>4</sup>

Despite the great amount of attention for biomarkers, no effective new biomarkers for diagnosis and surveillance of LIHC have been discovered. The recommended biomarkers for clinical usage such as Alpha-Fetoprotein (AFP), Alpha-Fetoprotein Lens Culinaris Agglutinin-3 (AFP-L3), and Des- $\gamma$ -carboxy prothrombin (DFP) were insufficient in sensitivity and specificity.<sup>5</sup> Therefore, exploring and confirming new biomarkers is of great significance for early diagnosis and prognosis of LIHC.

Transporters have an essential role to play in various physiological processes. The human adenosine triphosphate (ATP)-binding cassette (ABC) transporter family is one of the largest gene families of transporters.<sup>6</sup> The ABC transporters are a type of ATP-powered pump and include 49 ABC genes which are divided into seven sub-families (ABCA, ABCB, ABCC, ABCD, ABCE, ABCF, and ABCG). In humans, the ABC transporter family is mainly expressed in the liver, gut, kidney, placenta, and blood-brain and blood-testis barrier. They contribute to the transport of many endogenous substances, such as peptides, metal ions, and inorganic anions across the plasma as well as intracellular membranes.<sup>7</sup> It has been reported that various mutations in genes of the ABC family can lead to different genetic diseases. For instance, mutations in the ABCA4 gene contribute to Stargardt macular degeneration, progressive familial intrahepatic cholestasis is caused by ABCB11 deficiency, and ABCC7, which is also known as cystic fibrosis transmembrane conductance regulator, is linked to cystic fibrosis.<sup>8–10</sup> ABC transporters are also involved in absorbing, distributing, and excreting the drugs and their metabolites,<sup>11</sup> which is associated with drug resistance of cancer cells. For example, ABCB1 is confirmed to be involved in multidrug resistance (MDR) and it can also be named MDR.<sup>6</sup> In addition, recent studies have discovered other functions of the ABC family in cancers. For example, ABCB6 was observed to be over-

expressed in LIHC and accelerate tumor progression by inhibiting ferroptosis.<sup>12</sup> They may be responsible for the spread and metastasis of tumor cells as well, which presents great potential for treatment purposes.<sup>13</sup>

The ABCC transporter family comprises the ABC sub-families and consists of 13 transporters, most of which belong to a large group of multidrug resistance-associated proteins (MRPs).<sup>14</sup> It has been reported that MRPs are related to tumor resistance to many kinds of anti-cancer drugs and their metabolites.<sup>14,15</sup> The ABCC transporters act as ion channels for ion transport and have diverse functions, including toxin secretion and signal transduction.<sup>7</sup>

In the study, we made a comparison of the expression of all members of the ABC transporter family between LIHC and normal tissues and selected one or several potential biomarkers for LIHC. Then we explored the potential function and value of these biomarkers by several public databases. We analyzed the differences in expression of biomarkers between tumor and normal liver tissue, and further explored their prognostic value, co-expression pattern, functional pathways, correlation with immune infiltration, genetic alteration frequency, drug sensitivity, and distribution in LIHC. This study evaluates the potential of the ABC transporter family in diagnosis and surveillance of LIHC, and being targets for anti-cancer therapy in LIHC.

## Materials and Methods

### Selection of the Potential Biomarker for LIHC

On one hand, ONCOMINE (<https://www.oncomine.org/resource/main.html>) is a comprehensive web resource providing practical RNA expression analyses,<sup>16</sup> and UALCAN (<http://ualcan.path.uab.edu/>) is a comprehensive network resource that supplies The Cancer Genome Atlas (TCGA)-based analysis.<sup>17</sup> The transcriptional levels of each gene in the ABC transporter family are firstly compared between LIHC tumor and normal tissues with data from ONCOMINE, and the differentially expressed genes are selected with  $p < 0.05$ . Then, transcriptional levels of these genes are further compared between the two kinds of tissues with UALCAN to confirm the differentially expressed genes.

On the other hand, GEPIA (<http://gepia.cancer-pku.cn/index.html>) is designed by Peking University and is a database that is used to analyze the expression of RNA

sequences.<sup>18</sup> It is used to evaluate the relationship between the expression of the members in ABC transporter gene family and the LIHC patients' pathological stage. The related genes and the correlation are determined.

Combining the results of the two steps, the genes which are both differentially expressed between LIHC tumor and normal tissues, and related to LIHC patients' pathological stage are defined as our potential biomarkers for LIHC.

## Preliminary Validation of the Potential Biomarker for LIHC

The expression of the selected potential biomarkers was compared between various kinds of cancers and the corresponding normal tissues to evaluate their value in tumors. The genes are thought to be valuable in tumors with significant differential expression ( $p < 0.0001$ ). Finally, the expression level of the eventually selected genes were compared between LIHC tumor and normal tissues once again in GEPIA database.

## PCA Dimension Reduction Analysis

The expression of ABCC5 varies between individual patients of LIHC. Principal component analysis (PCA) dimension reduction was carried out to distinguish them based on the expression of ABCC5. The number of PCs is set to 2, and the clusters are presented in uMAP format. We differentiate these clusters into high-ABCC5 expression subgroup and low-ABCC5 expression subgroup.

## The Prognostic Prediction Value of ABCC5 in LIHC

On the basis of high- and low-ABCC5 expression subgroups, GEPIA was applied to evaluate the prognostic prediction value of ABCC5 in LIHC. We conducted a Kaplan–Meier (KM) analysis in order to explore the difference in overall survival (OS) and disease-free survival (DFS) of patients between the two subgroups. Cox regression analyses were used to tell whether the expression level of ABCC5 is an independent risk factor for OS among LIHC patients.

## Co-Expression Pattern and Functional Enrichment Analyses of ABCC5 in LIHC

Since the ABCC5 expression may also be influenced by other genes, we constructed a co-expression network with UALCAN to clarify its function and clinical value in LIHC development. DAVID (<http://david.ncifcrf.gov>) is a comprehensive bioinformatics tool in which the

biological functions of provided genes are clarified.<sup>19</sup> ABCC5 and other genes in the ABC transporter family were put into Gene Ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis.

## Correlation Between Immune Infiltration and Expression of ABCC5 in LIHC

TIMER (<https://cistrome.shinyapps.io/timer/>) is a reliable and intuitive resource to systematically evaluate the infiltration and clinical effects of immune cells.<sup>20</sup> In our study, the “SCNA module” was used to analyze the relationship between immune cell infiltration levels and the number of ABCC5 gene copies; additionally, the “Gene module” was applied to assess the correlation between ABCC5 and immune infiltration. The data from the cancer genome atlas (TCGA) were taken as a training group while the data from GSE112790 were used for validation.

## Genetic Alteration Frequency of ABCC5 Across Cancers

cBioPortal ([www.cbioportal.org](http://www.cbioportal.org)), a bioinformatics service, can perform the visualization and analysis of multidimensional cancer genomics data.<sup>21</sup> On the basis of TCGA, genetic alterations and co-expression of ABCC5 were obtained from cBioPortal. Three hundred and sixty LIHC samples from Firehose legacy and 348 LIHC samples from PanCancer Atlas are analyzed, respectively, to examine the genetic alteration of ABCC5 across LIHC.

## Prediction of Drug Sensitivity Associated with ABCC5 in LIHC

To confirm the relationship between our gene and drug responses, GDSC (<https://www.cancerrxgene.org/>) and CTRP (<http://portals.broadinstitute.org/ctrp/>) database were used to analyze the relationship between half inhibitory concentration (IC50) data of different molecules and the gene expression levels. The criteria were set as  $FDR < 0.05$ .

## Distribution of ABCC5 Expressed Cells in LIHC Tissues

LIHC tumor tissues were composed of different cells. The t-SNE algorithm was employed to explore the distribution of cells which express ABCC5 in LIHC tissues. The criteria were set as  $\log_2$  [fold change (FC)]  $> 0.5$  and  $FDR < 0.05$ .

## Results

### Expression of ABC Transporter Family in LIHC

Firstly, we probed the transcriptional levels of the ABC transporter family in LIHC and normal liver tissues with ONCOMINE, and twelve members of the ABC transporter family were retrieved (Figure 1A). Then the same analysis of transcriptional levels of these genes was performed with another database, UALCAN. Eight genes selected from ONCOMINE were as well differently expressed in UALCAN. In detail, ABCB2, ABCB3, ABCC4, and ABCC5 in LIHC samples were drastically elevated while the levels of ABCA6, ABCA8, ABCB4, and ABCD3 were significantly reduced in LIHC compared to normal liver tissues ( $p < 0.05$ , Figure 1B). Then, we evaluated the relationship between the expression of the ABC transporter family members and the LIHC patients' pathological stage. A significant correlation between the level of ABCC1, ABCC5, ABCE1, ABCF2, and the pathological stage was found ( $p < 0.05$ , Figure 1C). As the tumor progressed, the level of ABCC1, ABCC5, and ABCF2 increased.

Notably, ABCC5 showed significant differences in LIHC patients compared to healthy samples and seemed to have an important part to play in LIHC progression; therefore, we presumed that ABCC5 may be a potential disease biomarker for LIHC.

### Expression of ABCC5 Across Cancers and in LIHC

UALCAN was used to analyze ABCC5 expression across various types of cancers. The results indicated that ABCC5 was significantly up-regulated in multiple cancers, including LIHC. Furthermore, compared with different kinds of cancers, there is a relatively more significant difference between the tumor and relevant controls in LIHC (Figure 2A). Then the specific analysis of ABCC5 expression in LIHC revealed that the expression of ABCC5 was exactly elevated in LIHC samples in contrast to normal liver samples ( $p < 0.0001$ , Figure 2B), and the results of "Gene Comparison" demonstrated that the expression of ABCC5 in tumor tissues was approximately 2.2 times higher than that in healthy controls (Figure 2C).

### The Prognostic Prediction Value of ABCC5 in LIHC

The PCA divided patients of LIHC into high- and low-expression of ABCC5 subgroups (Figure 3A). Then we conducted a KM analysis in order to explore the prognostic prediction value of ABCC5 in LIHC. We found that LIHC patients in low-expression subgroup had a more prolonged OS ( $p = 0.000049$ , Figure 3B), while their DFS was not affected ( $p = 0.072$ , Figure 3C). Cox regression analyses showed that the expression level of ABCC5 was an independent risk factor for OS among LIHC patients as well (Table 1).

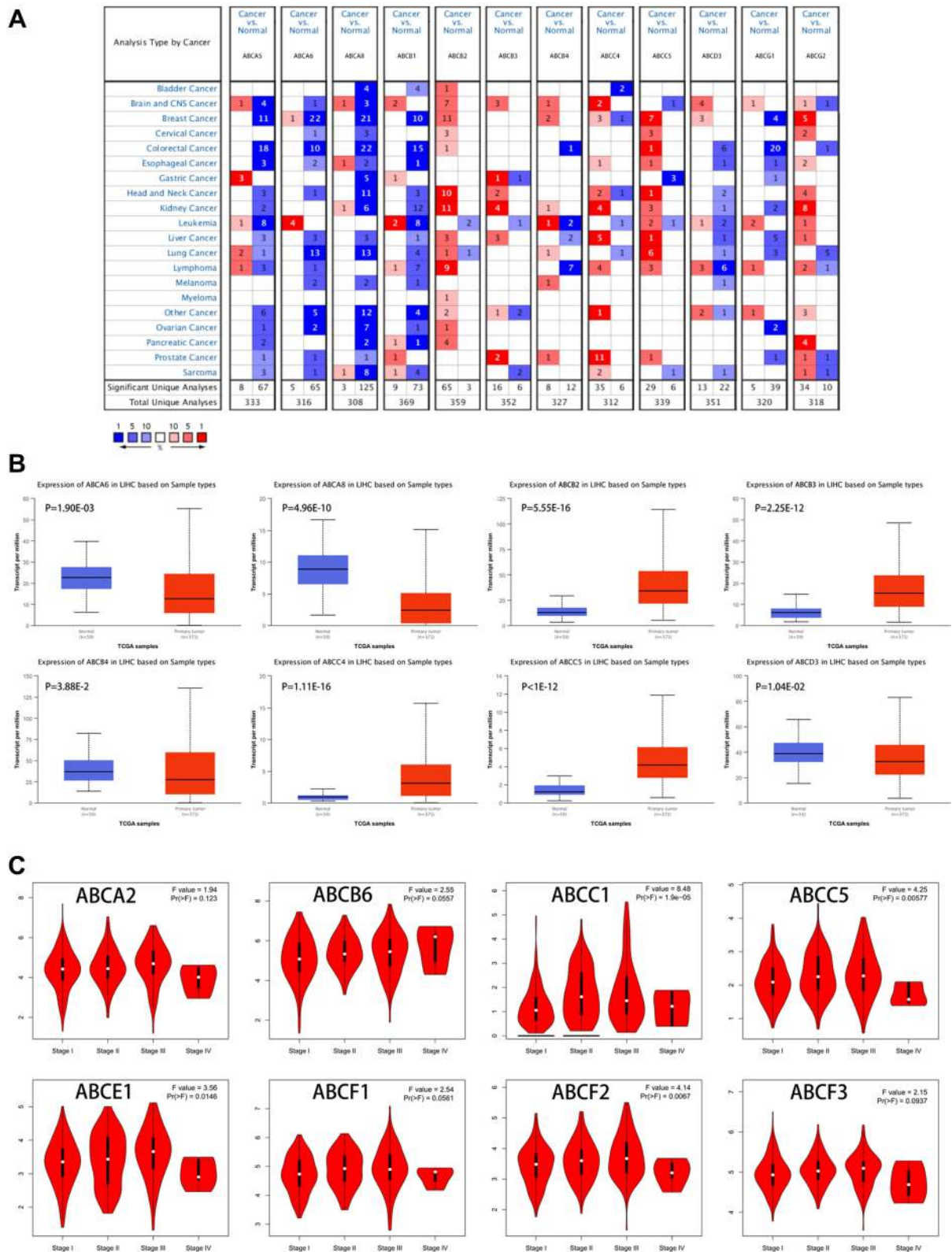
### Co-Expression Pattern of ABCC5 in LIHC

The co-expression network of ABCC5 is constructed with the application of UALCAN. The top 100 genes co-expressed with ABCC5 were displayed in heat maps, of which ABCC5, YEATS2, SLC36A1, KIAA0226, PLXNA1, VPS8, MTMR2, ACTR3, SIN3B, and ARPC2 rank the top ten (Figure 4A, Supplementary Figure 1A-C). The pattern may reveal the function of ABCC5 in the development of LIHC and can be utilized in further functional studies of ABCC5.

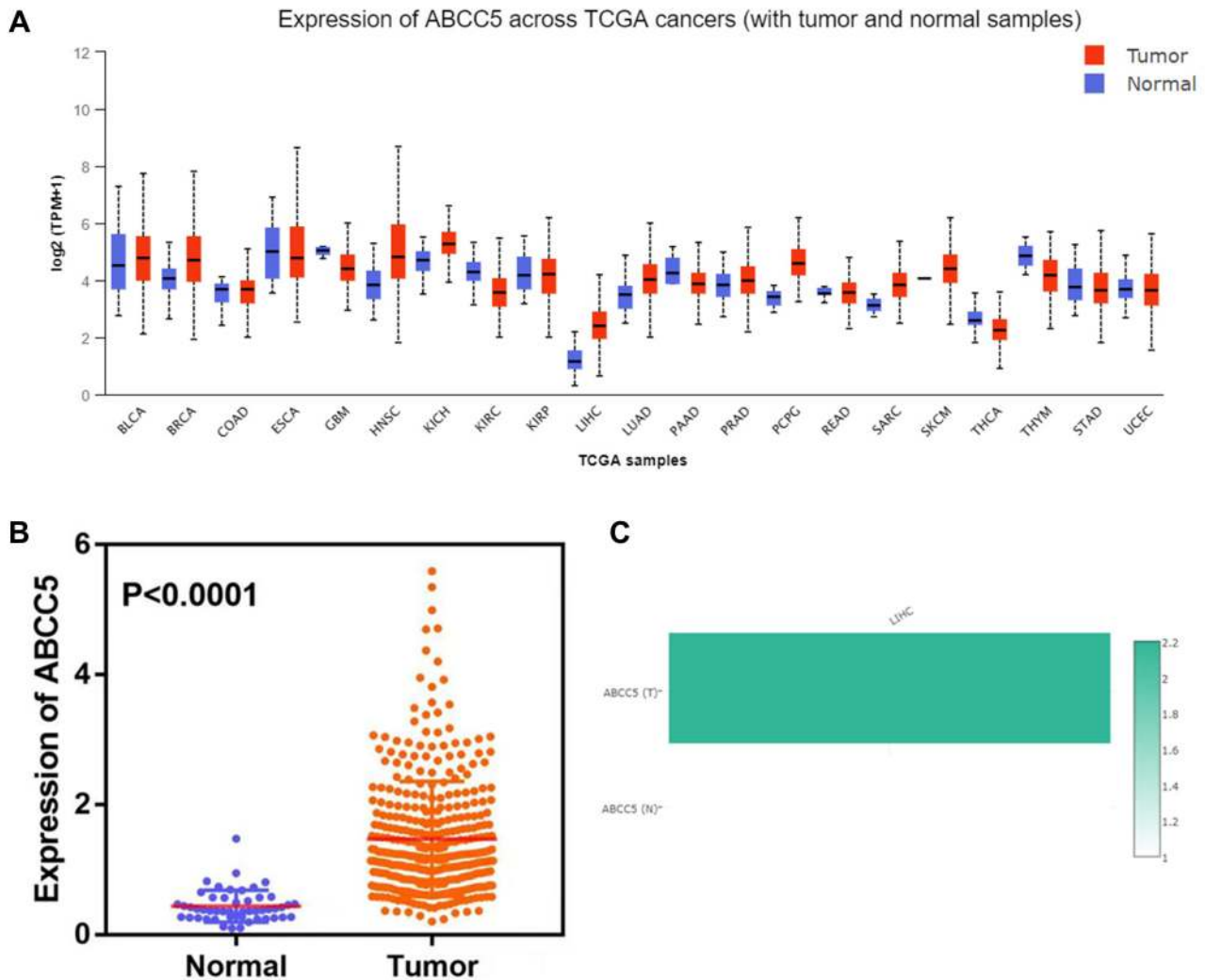
### GO and KEGG Enrichment Analyses of Co-Expressed Genes

We performed GO and KEGG enrichment analyses to identify the potential functions that involve genes in ABC transporter family. The genes were closely associated with transmembrane transport (especially drugs'), antigen processing and presentation in biological process (BP). While in cellular component (CC), they were majorly enriched in membrane, ABC transporter complex, integral component of membrane, integral component of plasma membrane, and TAP complex. Furthermore, in molecular function (MF), these genes were significantly linked with ATPase activity and transporter activity. KEGG analyses were also conducted, and the results showed that the ABC transporters were the most highly enriched pathway as expected, followed by bile secretion (Figure 4B). The results of functional analyses were consistent with ABCC5 as a member of ABC transporter family, and pointed to its function in transmembrane transporting, especially in drug transmembrane transporting, in LIHC progression.





**Figure 1** (A) mRNA levels of the ATP-binding Cassette transporter family in LHC (ONCOMINE). The figure shows the number of datasets with statistically significant mRNA overexpression (red) or downregulated expression (blue) of the ABC transporter family. (B) Transcription of the ABC transporter family in LHC (UALCAN). (C) Stage plot showing the correlation between the ABC transporter family expression and tumor stage in LHC (GEPIA).



**Figure 2** (A) Expression of ABCC5 across cancers identified by the TCGA: ABCC5 was significantly differently expressed in LIHC compared to normal tissue. (B) Analysis of 369 LIHC and 50 normal tissues showed that ABCC5 was significantly overexpressed in LIHC ( $P < 0.0001$ ). (C) Expression of ABCC5 in LIHC as found in the GEPIA database: the expression of ABCC5 in LIHC was approximately 2.2 times higher than that in normal tissue.

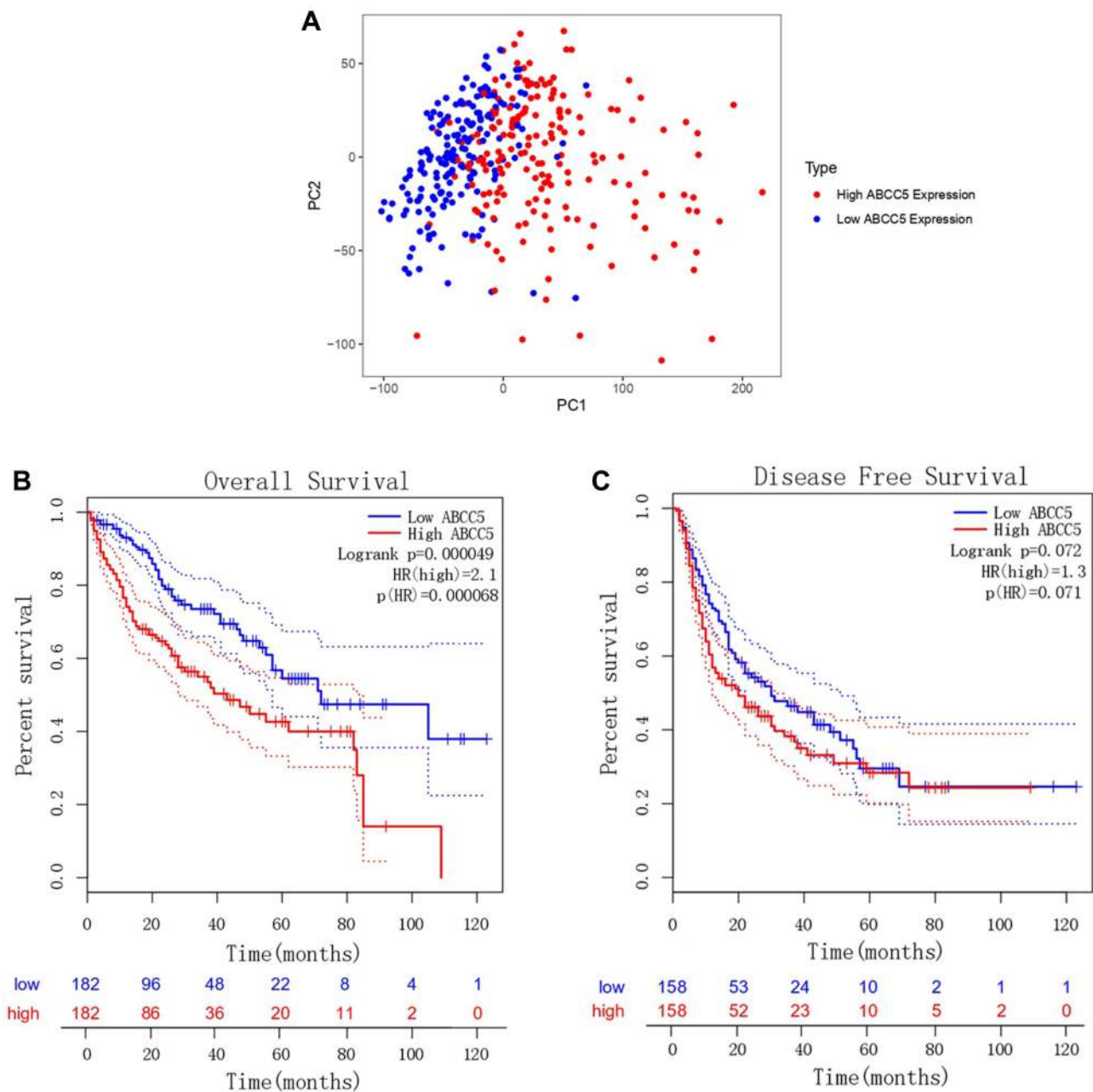
## Correlation Between Immune Infiltration and Expression of ABCC5 in LIHC

We conducted analyses with TIMER to evaluate the potential correlation between the expression of ABCC5 and immune infiltration in LIHC. Firstly, the “SCNA” module analysis indicated that the immune infiltration levels of some cells were related to altered copy numbers of ABCC5, such as neutrophils and macrophages (Figure 5A). Secondly, we took data from TCGA as a training group and those from GSE112790 as a validating group. ABCC5 expression appeared to have no apparent relationship with tumor purity, while it was significantly positively correlated with the infiltration levels of macrophages, neutrophils, and dendritic cells in LIHC in both training and validating groups ( $p < 0.05$ ,

Figure 5B). The results implied that ABCC5 may influence the progression and prognosis of LIHC in the way of regulating the level of immune cell infiltration.

## Genetic Alteration Frequency and Drug Sensitivity of ABCC5 in LIHC

We investigated the alteration frequency of the ABCC5 gene in LIHC with data from PanCancer Atlas and Firehose legacy. The results showed that the alteration frequency of ABCC5 in LIHC was 7% and 8%, respectively (Figure 6A, Supplementary Figure 1D). There is a positive correlation between the copy number of ABCC5 and mRNA expression in LIHC tissues (Figure 6B). In addition, we found LIHC samples with deletions showed lower mRNA expression than those with



**Figure 3** (A) Principal component analysis of ABCC5 expression in LIHC. (B) Overall survival analysis of ABCC5 expression in LIHC (GEPIA). (C) Disease-free survival analysis of ABCC5 expression in LIHC (GEPIA).

diploids (Figure 6C). In the aspect of drug sensitivity, we applied GDSA and CTPR database to depict the relationships between ABCC5 expression and drug responses. The bubble heat map showed that high expression levels of ABCC5 had significant correlations with lower half inhibitory concentration (IC50) data in GDSC and CTRP database, respectively, indicating that ABCC5 exhibited drug sensitivity, including Afatinib, Erlotinib, Gefitinib and Lapatinib (Figure 6D).

### Distribution of ABCC5 Expressed Cells in LIHC Tissues

Finally, we tried to explore the exact distribution of ABCC5 expression in different kinds of cells in LIHC tissues. The graphs obtained by t-SNE algorithm revealed that expression of ABCC5 was most concentrated in macrophages, followed by hepatocytes (Figure 7). The gathering expression of ABCC5 in macrophages was consistent with the result of our immune infiltration analysis,

**Table 1** Univariate and Multivariate Cox Analyses of ABCC5 Expression in LIHC

Parameter	Univariate Analysis			Multivariate Analysis		
	HR	95% CI	P	HR	95% CI	P
Age	1.01	0.99–1.02	0.566	–	–	–
Gender	0.79	0.49–1.26	0.324	–	–	–
Grade	1.03	0.75–1.40	0.865	–	–	–
Stage	1.85	1.45–2.37	0.000	1.06	0.39–2.89	0.914
T classification	1.79	1.43–2.26	0.000	1.69	0.68–4.17	0.256
M classification	3.83	1.20–12.20	0.023	1.75	0.46–6.72	0.414
N classification	2.01	0.49–8.22	0.332	–	–	–
ABCC5	1.47	1.20–1.81	0.000	1.47	1.17–1.84	0.001

**Notes:** “–” represents that there is no data.  
**Abbreviations:** HR, hazard ratio; CI, confidence interval.

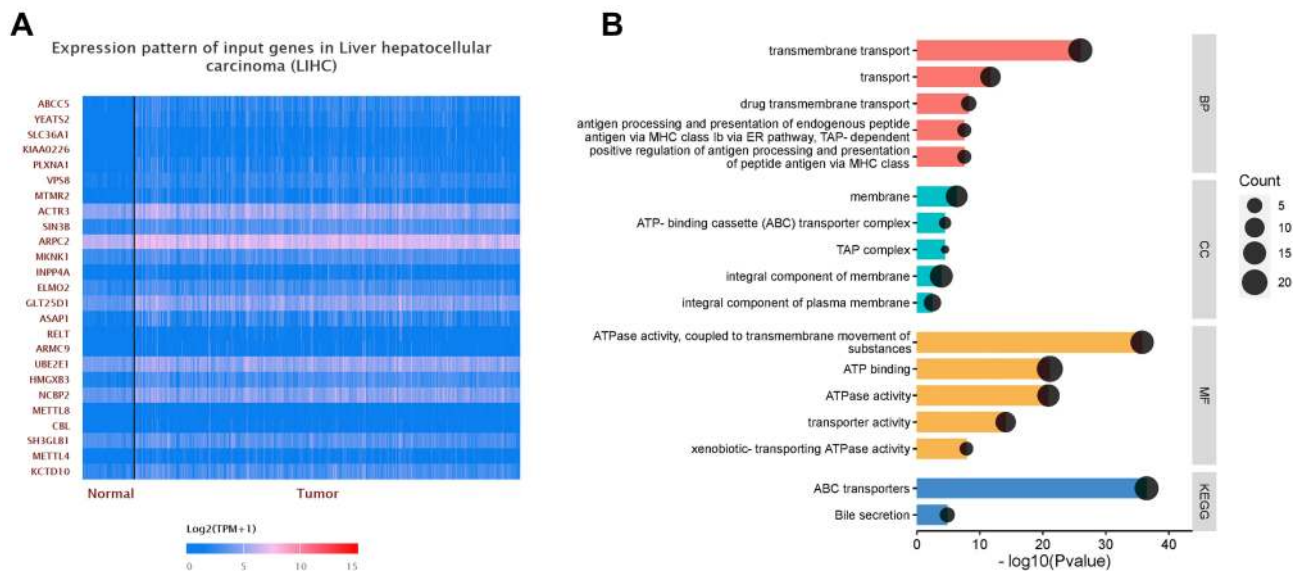
and the high expression of ABCC5 in hepatocytes hinted its potential role in LIHC.

### Discussion

It has been widely accepted that members of the ABC transporter family are related to various diseases, including cancers, and were suspected to influence proliferation, differentiation, migration, and invasion of tumor cells.<sup>13</sup> For example, ABCG2, also known as the breast cancer resistance protein (BCRP), has functions in the development of breast cancer.<sup>22</sup> Furthermore, this family is confirmed to be involved in multidrug resistance, which hampers the effects of anti-cancer pharmacotherapy.<sup>23</sup> Liver cancer, of which LIHC is the most common type,

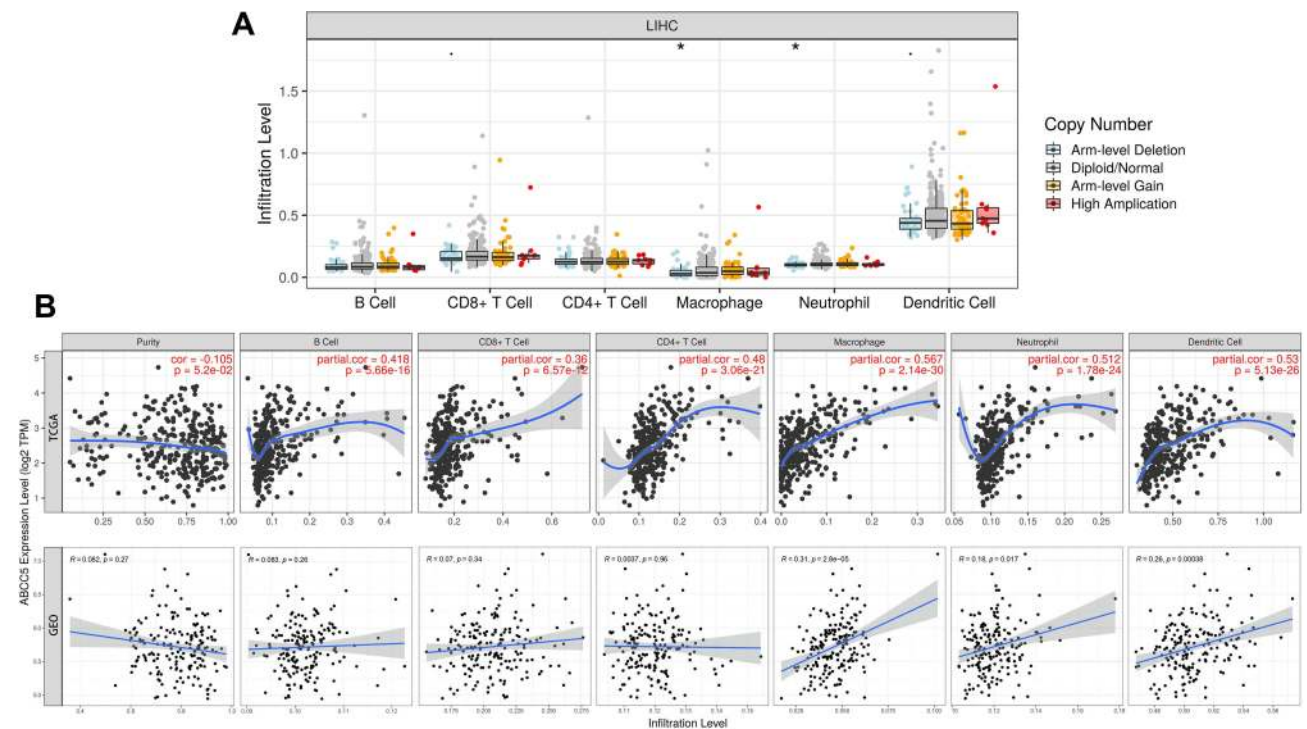
is a considerable threat to public health due to its high mortality. In a study by Siegel et al, survival ranked second lowest for liver cancer (20%), just behind pancreas cancer (10%).<sup>24</sup> Several studies have depicted the relationship between ABC transporters and LIHC. For example, Zhang et al found that low expression of ABCA8 and ABCA9 shortens the survival time of patients, while over-expression of ABCB6 in LIHC was associated with the acceleration of tumor progression by inhibiting ferroptosis.<sup>12</sup>

We researched the aberrant expression of the ABC transporter family in LIHC samples from the ONCOMINE and UALCAN databases. The differently expressed genes between LIHC and normal tissues in both databases were

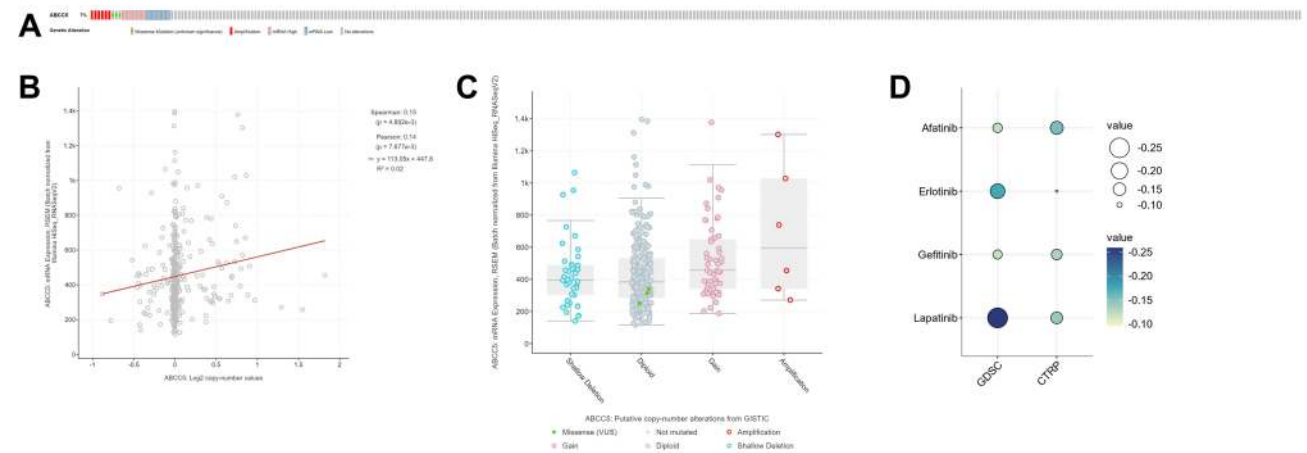


**Figure 4** (A) The heat map of the top 25 co-expressed genes with ABCC5. (B) Functional enrichment analyses of ABCC5 and the co-expression genes in LIHC. GO enrichment analysis showed that the co-expressed genes were significantly enriched in BP, CC, and MF of LIHC. KEGG analysis showed that co-expression genes were mainly enriched in ABC transporters and bile secretion.





**Figure 5 (A)** Altered ABCC5 gene copy numbers was significantly correlated with the immune infiltration levels of several types of immune cell in LIHC (PanCancer Atlas) ( $p < 0.05$ ). **(B)** Immune infiltration linked to ABCC5 in LIHC (TCGA) as training: the expression of ABCC5 was significantly correlated with the infiltration of B cells ( $p = 5.66e-16$ ), CD8 + T cells ( $p = 6.57e-12$ ), CD4 + T cells ( $p = 3.06e-21$ ), macrophages ( $p = 2.14e-30$ ), neutrophils ( $p = 1.78e-24$ ), and dendritic cells ( $p = 5.13e-26$ ). Immune infiltration linked to ABCC5 in LIHC (GSE112790) as validation: the expression of ABCC5 was significantly correlated with the infiltration of macrophages ( $p = 2.8e-5$ ), neutrophils ( $p = 0.017$ ), and dendritic cells ( $p = 0.00038$ ).

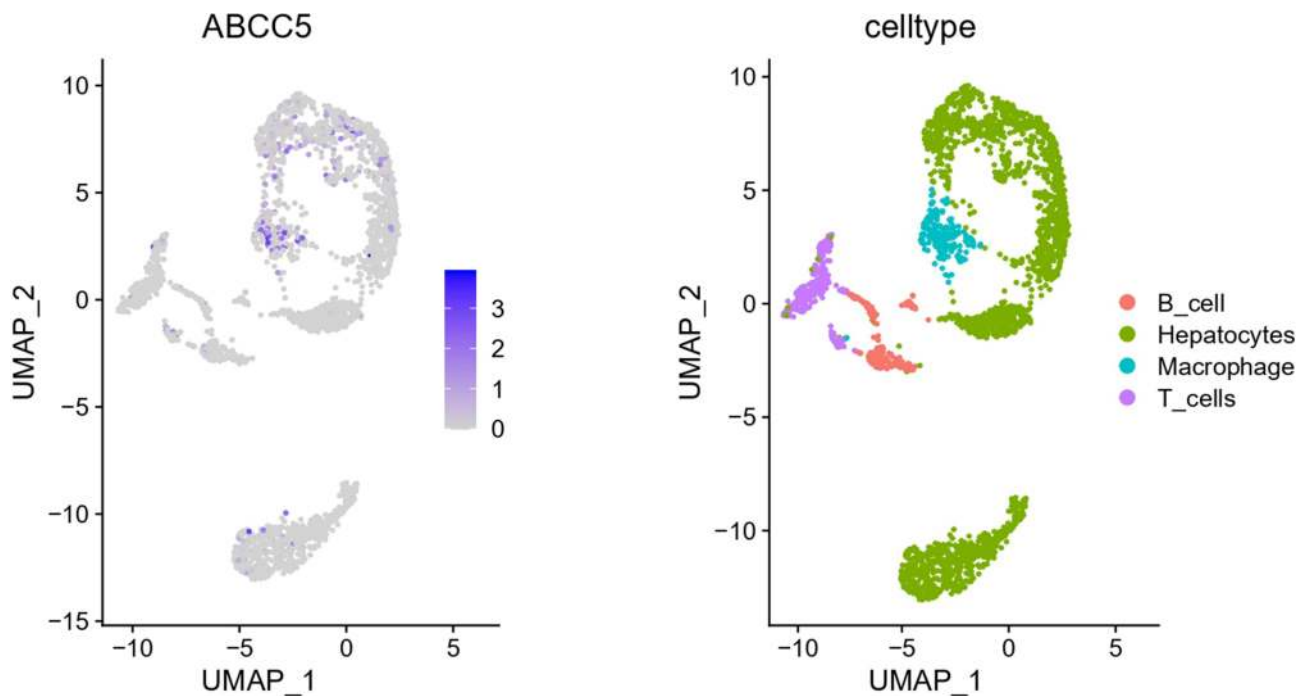


**Figure 6 (A)** ABCC5 alterations in the LIHC cohort. The different types of genetic alterations are highlighted in different colors. **(B)** Correlation between ABCC5 expression and the copy number of ABCC5 in LIHC. **(C)** LIHC samples with deletions showed lower mRNA expression than those with diploids. **(D)** Prediction of drug sensitivity associated with ABCC5 in LIHC, including Afatinib, Erlotinib, Gefitinib, and Lapatinib.

selected. Simultaneously, we evaluated the correlation between the expression of ABC transporter genes and the pathological stages of LIHC patients. The results showed that the expression of ABCC1, ABCC5, ABCE1, and ABCF2 increased as LIHC progressed. Combined with the statistical analyses above, ABCC5 is expressed differently in

LIHC tissues and is strongly related to the pathological stage. Therefore, we selected this gene to further explore its diagnostic, prognostic, and therapeutic value as well as mechanisms in LIHC.

In the human liver, ABCC transporter proteins are differentially expressed and regulated and involved in



**Figure 7** Single-cell analysis revealed the distribution of ABCC5 expressed cells in LIHC tissues.

eliminating toxic metabolites.<sup>14</sup> In the ABCC transporter subfamily, ABCC8 gene is related to congenital hyperinsulinism of infancy.<sup>25</sup> It has been identified that ABCC5 is a member of the MRP subfamily and is associated with resistance to several nucleosides. It is involved in cGMP secretion as well.<sup>6</sup> A previous study has also confirmed that dramatically increased levels of ABCC5 existed in LIHC.<sup>26</sup>

The PCA divided patients of LIHC into high- and low-expression subgroups. Our KM analysis demonstrated that LIHC patients with low transcriptional levels of ABCC5 tend to have a more prolonged OS, and both Cox analyses identified ABCC5 expression as an independent risk factor of OS in LIHC. Subsequently, the construction of a co-expression pattern identified the genes co-expressed with ABCC5 for functional analyses. Both GO and KEGG analyses showed that genes were enriched in pathways related to transmembrane transport, including drug transmembrane transport. Many anti-cancer drugs require transmembrane transporters to access the cancer cells and act on intracellular targets, for which ABC transporters are responsible.<sup>27</sup> In addition to directly affecting drug efficacy by regulating the uptake of endogenous compounds and xenobiotics, transmembrane transporters can also influence cellular metabolic processes like glycolysis and oxidative phosphorylation.<sup>28–30</sup> Therefore, it is certain that an abnormal expression of the ABC transporter family could

interfere with drug absorption and metabolism. ABCC5 was reported to be associated with the tumor response to gemcitabine-based chemoradiotherapy, which ultimately affects patients' survival.<sup>31</sup> Other enriched pathways included ATPase activity and bile secretion. ABC proteins contain nucleotide-binding domains (NBDs), which are ATPase subunits.<sup>32</sup> Moreover, a defect in the bile acid secreted by the liver may also contribute to LIHC oncogenesis.<sup>33</sup>

Analyses of correlation between ABCC5 expression in LIHC and immune infiltration illustrated that the ABCC5 expression level was statistically significant related to cell infiltration of several types of immune cells, including macrophages, neutrophils, and dendritic cells (DCs). It has been reported that a few ABC transporters are involved in cellular processes such as antigen processing and immunity.<sup>15</sup> Macrophages are equally important in LIHC. Macrophages have an essential part in chronic liver inflammation, which is a crucial step in the initiation and progression of LIHC. Tumor-associated macrophages (TAMs) are a well-known component of the tumor microenvironment, the majority of which are M2-polarized macrophages. Yao et al found that M2-polarized macrophages promoted the migration and epithelial-mesenchymal transition of LIHC cells by regulating the TLR4/STAT3 signaling pathway.<sup>34,35</sup> In addition, neutrophils are also accumulated in and become a major component of the tumor microenvironment of LIHC. It has been demonstrated

that they promote LIHC development by inducing the production of the pro-metastatic factor OSM.<sup>36</sup> DCs are a type of antigen-presenting cells. The lack of crosstalk between DCs is one of the causes of tumor tolerance in LIHC.<sup>37</sup> Hence, the elevation of ABCC5 expression levels in LIHC patients may suggest a potential target in immunotherapy, which could benefit the patients' prognosis.<sup>38</sup>

The high frequency of genetic alterations in the ABCC5 gene and the significant difference in ABCC5 protein levels between tumor and normal samples both confirm the sensitivity of ABCC5 as a biomarker for LIHC at the gene and protein levels. There have been reports of numerous new biomarkers for the diagnosis and prognosis of LIHC, such as lncRNA-D16366, deoxycytidine kinase, and circ\_0067934.<sup>39–41</sup> Though all these new biomarkers lack sufficient clinical testing and validation, they may have the potential to become diagnostic, prognostic, and therapeutic targets in LIHC and provide insights into the mechanisms of initiation and progression of LIHC.

At present, cancer treatment has entered the era of precision medicine. As mentioned previously, ABCC5 may play an important role in LIHC, so it is crucial to explore drugs related to ABCC5. We identified 4 different types of drugs that were significantly sensitive to ABCC5 in LIHC. It is beneficial to improve the prognosis of LIHC patients and explore the mechanism of ABCC5.

This study has several limitations that should be addressed. Firstly, the data we applied for statistical analyses were obtained from public databases and did not reflect authentic clinical conditions. Secondly, we moderately investigated the prognostic value of ABCC5 in LIHC and more in-depth research is required to validate its clinical application value. Thirdly, prospective studies should be performed since our study has been retrospective. Fourthly, clinical tissues for validation were not included in this study and further experiments with animal and human samples are necessary to clarify the actual underlying mechanisms and relationships between ABCC5 and LIHC.

## Conclusion

In the bioinformatic analysis, ABCC5 is the only member in ABC transporter family that is both differentially expressed between LIHC and normal liver tissues, and strongly related to pathological stage of LIHC. ABCC5 is confirmed as an independent risk factor for LIHC patients. It may facilitate LIHC progression through different mechanisms, including transmembrane transport and immune infiltration. ABCC5 may be

a potential biomarker and target for diagnosis, prognosis, and therapy of LIHC.

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## Disclosure

The authors report no conflicts of interest in this work.

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