

Bioinformatics Analysis of The Expression of ATP Binding Cassette Transporters and The Screening of Regulatory Genes in PTEN/PI3K/Akt/mTOR Signaling Pathway in The Hepatocellular Carcinoma

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Bioinformatics analysis of the Expression of ATP Binding Cassette Transporters and the Screening of Regulatory genes in PTEN/PI3K/Akt/mTOR signaling pathway in the hepatocellular carcinoma

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Abstract: Objective Here we performed the Bioinformatics analysis on the data from The Cancer Genome Atlas (TCGA), in order to find the correlation between the expression of ATP Binding Cassette (ABC) Transporters' genes and hepatocellular carcinoma (HCC) prognosis; **Methods** Transcriptome profiles and clinical data of HCC were obtained from TCGA database. Package edgeR was used to analyze differential gene expression. Patients were divided into low-ABC expression and high-ABC expression groups based on the median expression level of ABC genes in cancer. The overall survival and short-term survival (n= 341) of the two groups was analyzed using the log-rank test and Wilcoxon test; **Results** We found that ABC gene expression was correlated with the expression of PIK3C2B ($p<0.001$, ABCC1: $r=0.27$; ABCC10: $r=0.57$; ABCC4: $r=0.20$; ABCC5: $r=0.28$; ABCB9: $r=0.17$; ABCD1: $r=0.21$). All patients with low-ABC expression showed significantly increased overall survival. Significantly decreased overall survival (Log-rank test: $p<0.05$, Wilcoxon test: $p<0.05$) was found in patients with high expression of ABCC1 (HR=1.58), ABCD1 (HR=1.45), ABCC4 (HR=1.56), and ABCC5 (HR=1.64), while decreased short-term survival (Log-rank test: $p>0.05$, Wilcoxon test: $p<0.05$) was correlated with the increased expression of ABCC10 (HR=1.29), PIK3C2B (HR=1.29) and ABCB9

(HR=1.23); **Conclusions** Our findings indicate that the specific ABC gene expression correlates with the prognosis of HCC. Therefore, ABC expression profile could be a potential indicator for HCC patients.

Keywords: Hepatocellular carcinoma; ABC Transporters; Multidrug Resistance; Bioinformatics

Declarations:

Funding: National Key Research and Development Project, No.2017YFB0403805;

Conflicts of interest: None;

Availability of data and material: TCGA database;

Code availability: GraphPad Prism 8.0 software and custom R and perl code;

Authors' contributions: Major.

1. Introduction

HCC is a potentially fatal cancer, and its mortality rate ranks second among all cancers worldwide, mainly due to its high metastasis and recurrence rate [1]. Radical surgical treatment is applied only to patients with early-stage HCC, but patients treated with radical surgery have a high recurrence rate and unsatisfactory long-term prognosis [2, 3]. The efflux transporters of the ABC superfamily, such as ABCB1 (p-glycoprotein, P-gp), ABCC (multidrug resistance-associated protein, MRP) family, and ABCG2 (breast cancer resistance protein, BCRP), have been identified as major determinants of chemoresistance in tumor cells [4, 5]. ABCC1 is commonly expressed in the human body. In polarized epithelial cells, it is localized to the basolateral membrane [4], which suggests that up-regulated ABCC1 is a protective mechanism to compensate for liver inflammation. Metabolic pathways associated with immunotherapy: The PI3K/AKT/mTOR pathway plays a critical role in cancer, including HCC [6, 7]. The mTOR pathway undergoes aberrant activation in as many as 50% – 60% of HCC cases [8], leading to hepatocarcinogenesis and is associated with poorly differentiated tumors, early recurrence, and poor prognosis [9, 10]. It has been reported that in other tumors, the above ABC genes are regulated by genes related to the PTEN/PI3K/Akt/mTOR pathway. For example, inhibition of GOLPH3 can reverse the resistance of colon cancer cells to oxaliplatin and down-regulate ABCB1, ABCC1, and ABCG2 expression by inhibiting the PI3K/AKT/mTOR pathway [11]. The oncogenic activity of the Akt/mTOR axis is dependent on ABCC4, which marks an aggressive subtype of human HCC [12]. NOTCH1 has been reported to regulate PTEN directly (via CSL activation) [13] and indirectly (via HES1 and cMYC inhibition) [14]. Notch1 affects chemotherapeutic activity by causing up-regulation of MDR1, ABCC5 through potential effects on the downstream PI3K/AKT/mTOR pathway [15]. Earlier functional studies

have shown that NEK2 mediates drug resistance (cisplatin or lipid-doxorubicin) through the expression of the ABCC10 transporter. Nek2-mediated resistance was blocked by specific PI3K or AKT inhibitors. In vitro data indicate that pAKT and NF- κ B signaling pathways are involved in nek2-induced drug resistance in liver cancer [16]. While there is substantial evidence that the PTEN/PI3K/Akt/mTOR pathway influences the role of ABC transporters in tumor multidrug resistance, the precise molecular mechanisms are unknown.

2. Material and methods

2.1. Expression of the crucial genes in Hepatocellular carcinoma

In the TCGA database, the TCGA-LIHC project is searched in humans. Download 'transcriptome profiling' and 'clinical' data. The expression levels of ABC genes and PTEN/PI3K/Akt/mTOR signaling pathway-related genes were analyzed and compared with tumors and corresponding normal tissues. Differential expression analysis was carried out between HCC and control samples to screen differentially expressed genes (DEGs) using the edgeR package in R [17]. Significant DEGs were required with $P < 0.05$ and $|\log \text{fold change (FC)}| > 1$. Meanwhile, the expression levels of significant DEGs in human liver cancer were plotted as a bar graph using GraphPadPrism8.0 software (GraphPad, San Diego, California, USA).

2.2. Validation of protein expression of the crucial genes

Human Protein Atlas (<http://www.proteinatlas.org>) was used to validate protein expression differences of ABC genes and PIK3C2B gene in normal and HCC tissues.

2.3. Survival analysis

Patients were divided into low-ABC expression and high-ABC expression groups based on the median expression level of ABC genes in cancer. The overall survival (OS) and short-term survival ($n=341$) of the two groups was analyzed using the log-rank test and Wilcoxon test. A p value < 0.05 was considered statistically significant ($*p < 0.05$; $**p < 0.01$; and $***p < 0.001$). The results are expressed as the mean SEM unless indicated otherwise. Each time point in the Log-Rank test was given a weight of 1. That is, the effect of the number of survivors at the beginning of each observation point on the statistical model was not considered. Changes in mortality at each time point contributed equally to the entire model. The Breslow test adds weight to the Log-Rank test, setting the weight to the number of survivors at the beginning of each time point. That is, the change in mortality at the beginning stage of a large number of survivors contributed to the model, while the change in

mortality at the beginning stage of a small number of survivors contributed less to the model. So it is easier to detect early differences. If the assumption of hazard ratio holds, then the Log-Rank test is more standard and statistically more reliable.

2.4. Correlation analysis

Using ggstatsplot package [18] in R, correlation analysis between ABC genes and PIK3C2B gene was performed to understand the correlation between the expression of these genes.

3. Results

3.1. Expression of the crucial genes in Hepatocellular carcinoma

A total of 15 ABC DEGs and one PTEN/PI3K/Akt/mTOR signaling pathway-related DEGs, PIK3C2B, were up-regulated in HCC on the data (n=419) from The Cancer Genome Atlas (TCGA). As shown in the following figure (Fig. 1), 6 ABC genes associated with HCC prognosis as well as the PIK3C2B gene were used as histograms with GraphPadPrism8.0 software (GraphPad, San Diego, California, USA).

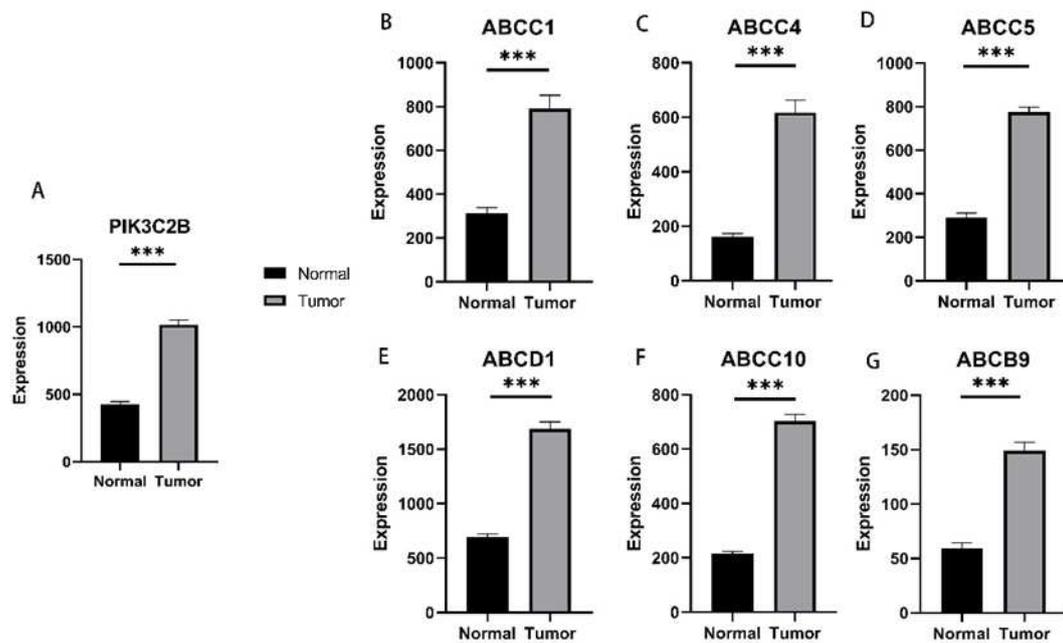


Fig. 1 The mRNA expression of the PIK3C2B (a), ABCC1 (b), ABCC4 (c), ABCC5 (d), ABCD1 (e), ABCC10 (f) and ABCB9 (g) in normal liver tissues and LIHC tissues from the TCGA database. The black bars in boxplots represent normal samples, the gray bars in boxplots represent tumor samples. LIHC liver hepatocellular carcinoma. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

3.2. Validation of protein expression of the crucial genes

In order to identify the expression of DEGs at the protein level, the protein expression levels of

5 ABC DEGs and PIK3C2B gene in hepatocellular carcinoma and healthy tissues were determined using the Human Protein Atlas (HPA) database. According to HPA immunohistochemistry results, the protein expressions of ABCB9, ABCC10 and PIK3C2B were higher in hepatocellular carcinoma tissues than in healthy liver tissues, and the protein expressions of ABCC1, ABCC4 and ABCC5 were not significant in tissues (Fig. 2).

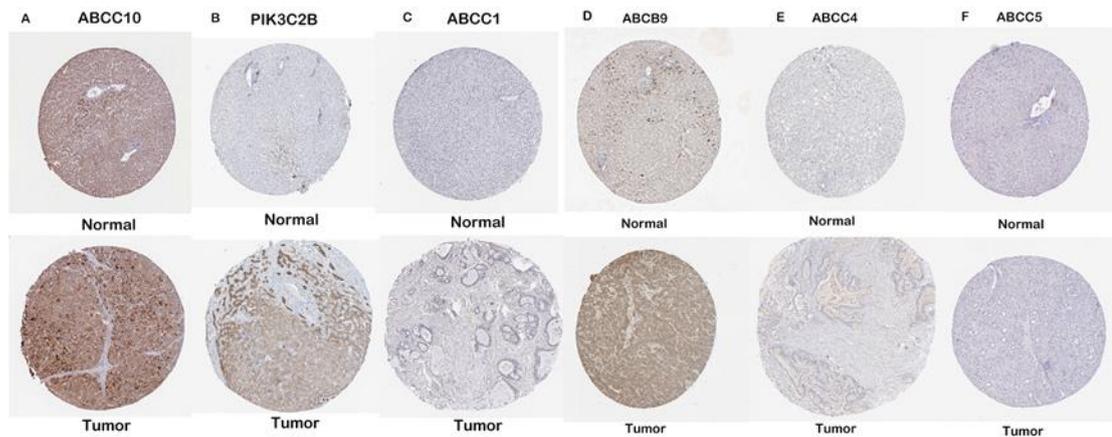


Fig. 2 The immunohistochemistry of the ABCC10 (a), PIK3C2B (b), ABCC1 (c), ABCB9 (d), ABCC4 (e) and ABCC5 (f) in normal liver tissues and LIHC tissues from the HPA database.

3.3. Survival analysis

In HCC patients, among the highly expressed genes, survival analysis of ABCC1 (HR=1.58), ABCD1 (HR=1.45), ABCC4 (HR=1.56), and ABCC5 (HR=1.64) showed statistically significant with Log-rank test and Gehan-Breslow-Wilcoxon test (Log-rank test: $p < 0.05$, Wilcoxon test: $p < 0.05$), survival analysis of ABCC10 (HR=1.29) and ABCB9 (HR=1.23) showed statistically significant only with Gehan-Breslow-Wilcoxon test (Log-rank test: $p > 0.05$, Wilcoxon test: $p < 0.05$). The overall survival of patients with above ABC genes low expression were all significantly higher than those of patients with high expression in HCC (Fig. 3). PIK3C2B, was found to be upregulated, but the effect on prognosis was not statistically significant.

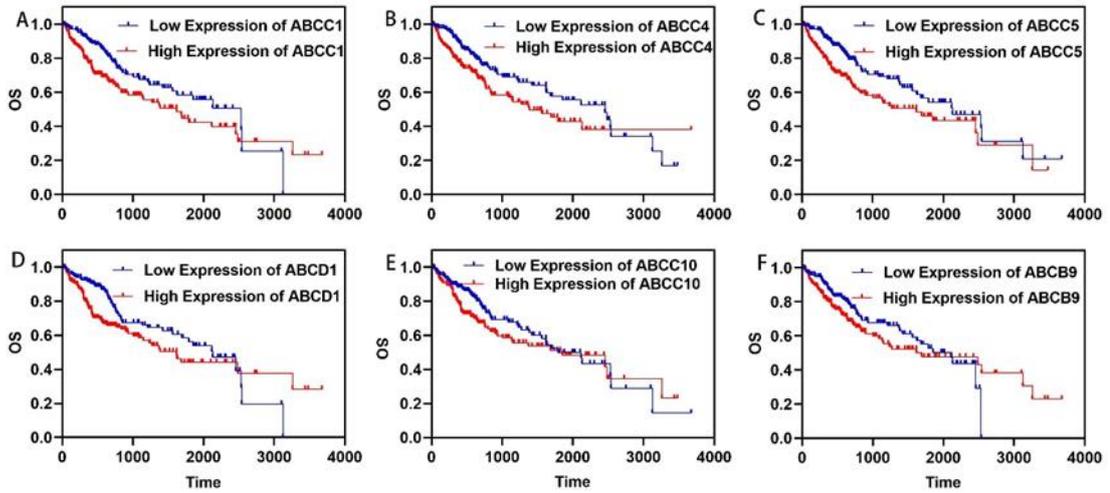


Fig. 3 The survivorship curve of the ABCC1(a), ABCC4 (b), ABCC5 (c), ABCD1 (d), ABCC10 (e) and ABCB9 in LHC tissues from the TCGA database. The blue curves in line charts represent low expression groups, the red curves in line charts represent high expression groups. LHC liver hepatocellular carcinoma.

3.4. Correlation analysis

The results of the correlation analysis of ABC genes and PIK3C2B gene were as follows: $p < 0.001$, ABCC1: $r = 0.27$; ABCC10: $r = 0.57$; ABCC4: $r = 0.20$; ABCC5: $r = 0.28$; ABCB9: $r = 0.17$; ABCD1: $r = 0.21$ (Fig. 4).

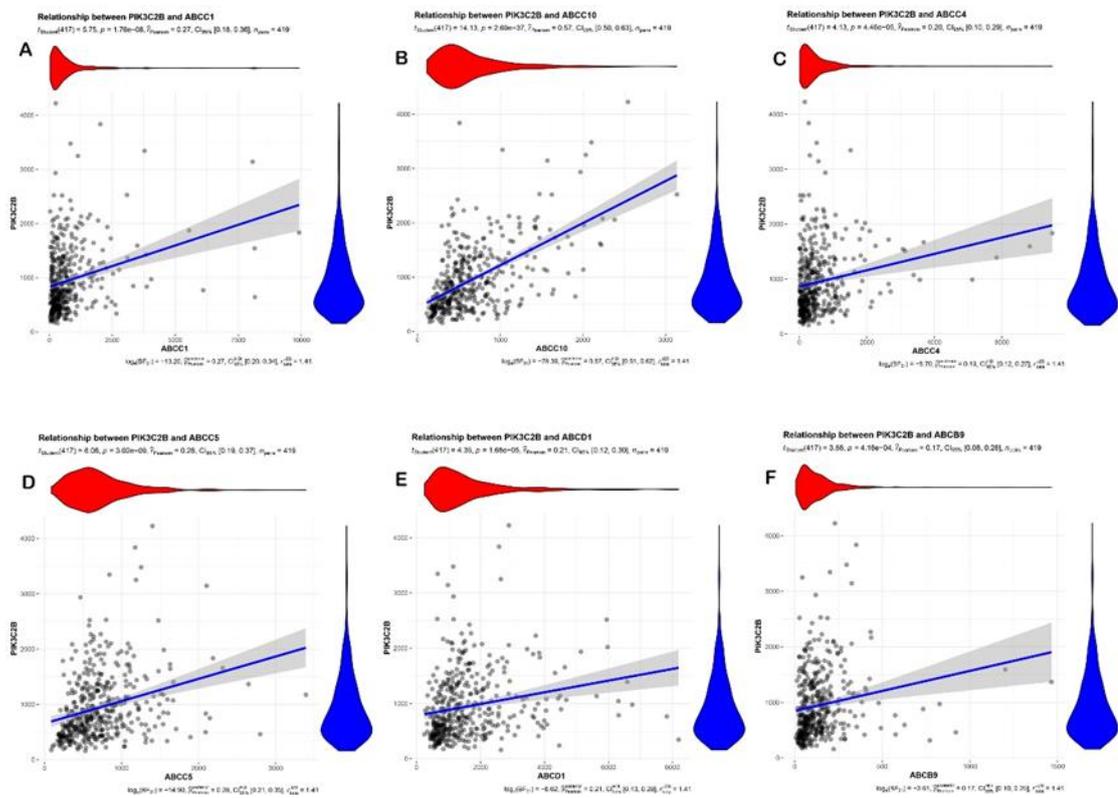


Fig. 4 The correlation between PIK3C2B and ABCC1 (a), ABCC10 (b), ABCC4 (c), ABCC5 (d), ABCD1 (e) and

ABCB9 (f) in LIHC tissues from the TCGA database. LIHC liver hepatocellular carcinoma.

4. Discussion

In HCC, the mRNA expression levels of ABCC1, ABCC4, ABCC5, ABCC10, ACD1, ABCB9, and PIK3C2B genes are elevated. According to the results of immunohistochemistry in HPA, the protein expression levels of ABCB9, ABCC10 and PIK3C2B were significantly increased. The results of survival analysis showed that OS were higher in the low ABC genes expression groups. Among them, the expression of ABCC10 and ABCB9 genes may have a great difference in the early survival of HCC, but not in long-term survival. These findings may suggest that these ABC genes play an important role in the development of HCC and can be used as prognostic biomarkers. The current study demonstrated that phosphatidylinositol 3-kinase-C2 β (PI3K-C2 β) is responsible for cisplatin resistance in esophageal squamous cell carcinoma (ESCC) by activating the Akt pathway. It could increase the level of phosphorylated Akt and significantly inhibited cisplatin-induced apoptosis and cleavage of caspase-3 in ESCC cells. Whether it is via a caspase-dependent apoptosis pathway or other molecular mechanisms is still not clear [19]. Given the role of the PTEN/PI3K/Akt/mTOR axis in inducing multidrug resistance to ABC transporters in tumors [20], we examined the correlation of PIK3C2B with ABC genes associated with HCC prognosis. It is speculated that PIK3C2B is an upstream regulatory gene of ABCC10 gene in HCC, in this study, we revealed for the first time that PI3K-C2 β induces multidrug resistance in tumors through possible regulation of ABCC10 in hepatocellular carcinoma. While the specific role of these genes for HCC needs to be further experimentally validated, their prognostic value needs to be validated in clinical trials.

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