Lipids in Health and Disease

RESEARCH

Open Access

Screening and molecular docking verification of feature genes related to phospholipid metabolism in hepatocarcinoma caused by hepatitis B

Jian Zhang[1†], Fengmei Zhang[2†], Lei Zhang[2*†], Meiling Zhang[3], Shuye Liu[2*] and Ying Ma[3*]

Abstract

Background The progression of tumours is related to abnormal phospholipid metabolism. This study is anticipated to present a fresh perspective for disease therapy targets of hepatocarcinoma caused by hepatitis B virus in the future by screening feature genes related to phospholipid metabolism.

Methods This study analysed GSE121248 to pinpoint differentially expressed genes (DEGs). By examining the overlap between the metabolism-related genes and DEGs, the research focused on the genes involved in phospholipid metabolism. To find feature genes, functional enrichment studies were carried out and a network diagram was proposed. These findings were validated via data base of The Cancer Genome Atlas (TCGA). Further analyses included immune infiltration studies and metabolomics. Finally, the relationships between differentially abundant metabolites and feature genes were confirmed by molecular docking, providing a thorough comprehension of the molecular mechanisms.

Results The seven genes with the highest degree of connection (PTGS2, IGF1, SPP1, BCHE, NR1I2, NAMPT, and FABP1) were identified as feature genes. In the TCGA database, the seven feature genes also had certain diagnostic efficiency. Immune infiltration analysis revealed that feature genes regulate the infiltration of various immune cells. Metabolomics successfully identified the different metabolites of the phospholipid metabolism pathway between patients and normal individuals. The docking study indicated that different metabolites may play essential roles in causing disease by targeting feature genes.

†Jian Zhang, Fengmei Zhang and Lei Zhang contributed equally to this work.

*Correspondence:
Lei Zhang
rikkichang12345@163.com
Shuye Liu
lshye@163.com
Ying Ma
maying@tmu.edu.cn

Full list of author information is available at the end of the article

Conclusions In this study, for the first time, it reveals the possible involvement of genes linked to phospholipid metabolism-related genes using bioinformatics analysis. Identifying genes and probable therapeutic targets could provide clues for the further treatment of disease.

Keywords Metabolomics, Bioinformatics, Hepatocellular carcinoma

Background

Hepatocellular cancer (HCC) has a very high mortality rate, primarily because of its invasive and metastatic nature [1]. Hepatitis B virus (HBV) infection causes liver scarring, which has become the main risk factor for HCC worldwide [2]. Depending mostly on the disease's clinical stage, current treatment options for HCC include biotherapy, radiation and liver transplantation [3, 4].However, studies have shown that regardless of which treatment scheme is adopted, the treatments are primarily suitable for patients with early-stage, and not for the advanced HCC [5, 6]. Hence, prompt identification and treatment serve as effective approaches to increase the survival rate of individuals with HCC.

Phospholipid metabolism means that phospholipids are hydrolysed into glycerol, fatty acids, phosphoric acid and amino alcohols by various phospholipases in organisms and then participate in various metabolic processes in vivo [7]. Studies have shown that tumour cells need to constantly reprogram their metabolism to satisfy their rapid proliferation and meet the needs of the tumour microenvironment (TME), which has relatively strict nutritional requirements [8]. Changes in the TME also strongly affect the biological processes of other cells, which ultimately results in the growth of tumours [9]. Therefore, metabolic disorders are a typical feature of cancer. As an important aspect of lipid metabolism, disordered phospholipid metabolism can change the endoplasmic reticulum and Golgi apparatus amplification of immune cells and ultimately shape the cancer-promoting phenotype [10].

Previous researches have demonstrated that NUSAP1 is an important gene connecting chronic HBV infection and hepatocarcinoma caused by hepatitis B (HBV-HCC) [11]. Most of the researches on HBV-HCC simply focused on screening the potential key genes. Disorders of phospholipid metabolism are very important for maintenance of the TME, and they also have important influences on the efficacy of chemotherapy and immunotherapy in cancer patients. However, there are no reports regarding the possible involvement of genes linked to phospholipid metabolism in HBV-HCC. In this study, genes related to phospholipid metabolism in HBV-HCC were detected for the first time by bioinformatics. Metabolites involved in the phospholipid metabolism pathway that differ between healthy individuals and HBV-HCC patients were identified via metabolomics technology. The feature genes related to phospholipid metabolism were verified by molecular docking, which provides ways for treating HBV-HCC.

Methods

Source of data

To examine alterations in the gene expression profiles of patients with HBV-HCC, GSE121248 was downloaded from the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/gds/) for this study (Kam Hui, 2018). The gene expression data (GSE121248) was produced using the GPL570. This dataset includes 70 tissue samples from HBV-HCC and 37 samples from nearby normal tissues. The differentially expressed genes (DEGs) were searched to fill the |logFC| ≥1.0 and an adjusted *P* value of < 0.05 by GEO2R.

Phospholipid metabolism genes and Venn analysis

The list of genes related to phospholipid metabolism from GeneCards (https://www.Genecards.org/) was downloaded. In this study, Venn diagrams of DEGs and genes associated with phospholipid metabolism were created using ggplot2 (Hadley Wickham, Australia) and Venn Diagram (Chen Jin, United States). The DEGs that intersected with phospholipid metabolism-related genes were incorporated into further analyses.

Enrichment analysis

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses were carried out to find which are involved in the identified DEGs [12]. GO consists of three parts: (1) biological process (BP), (2) cellular component (CC), (3) molecular function (MF) assessment. KEGG is a knowledge base for describing molecular interactions and revealing known metabolic pathways [13]. To clarify the key functional and pathway distinctions among the DEGs, the study used the clusterProfiler (4.4.4) tool in R (4.2.1). The species was classified as *Homo sapiens.*

Protein–protein interaction (PPI) network construction, screening feature genes and identification of corresponding proteins

First, STRING (https://www.string-db.org/) was applied to build the PPI network of DEGs. The cytoHubba plug-in of Cytoscape software was subsequently used to discover the top seven genes. The feature gene to be calculated via the MCC algorithm was selected. Finally,

identification of the corresponding proteins uses the UniProt protein database (https://www.UniProt.org).

Verification of feature gene expression in the cancer genome atla (TCGA)

The study utilized the TCGA database (https://portal.gdc.cancer.gov/) to validate the consistency and stability of feature genes. This validation enhanced the credibility of the research findings and offered a theoretical foundation for future experimental investigations. The expression profiles and clinical data from TCGA can be accessed by downloading them through the R package TCGA biolinks. The clinical data of 104 HBV-HCC patients and 50 healthy patient samples were extracted. R was used to perform all statistical analyses and generate all graphs.

Immune infiltration analysis

R package CIBERSORT was used to confirm the associations between feature genes and the immune microenvironment with the fractions of 22 immune cell types from GSE121248. Correlation analysis and visualization were conducted via the R packages corrplot and linkET. This study used the ggplot2 package to create an expression heatmap. The difference features of immune infiltration in healthy tissue and tumour tissue can be evaluated by using Wilcoxon test.

Screening of phospholipid metabolites in HBV-HCC via metabolomics

The study included thirty-two HBV-HCC patients who were enrolled to the Third Central Hospital's liver surgery department. There were 18 males and 14 females, aged between 45 and 78 years. The 29 healthy volunteers were considered as control group who underwent physical examinations at hospital during the same period. There were 16 males and 13 females, aged between 47 and 79 years. Informed consent and human protocols were approved by the institutional ethics board of Tianjin Third Central Hospital (Approval No: TJWJ2023XK020). Fasting serum samples were obtained from all participants. Serum was extracted from fresh samples through centrifugation at 2500 × g for 5 min and subsequently frozen at -80 °C. This study utilized high-performance liquid chromatography(Thermo Fisher, United States) and mass spectrometry (Thermo Fisher, United States) system. Following thawing, each serum sample (100 µl) was combined with 400 µl of methanol. The chromatographic elution was performed using a binary solvent gradient. The mass spectrometry system used the positive ion scanning mode. The acquired data were imported into MZmine 2.0 software for detection. The SIMCA 14.1 software was used for further analysis. The principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA) models were constructed via pattern recognition. Potential markers were preliminarily screened according to VIP values and confidence intervals, and the selected characteristic metabolites were then tested via SPSS 17.0.

Differentially abundant metabolite docking with screening proteins

The proteins were optimized via the protein preparation module in Schrodinger software, which involves adjusting chemical bonds in the protein structure, addressing metal ions, hydrogenating, and removing impurity atoms and water molecules. OPLS 2005 was employed for protein structure optimization, with a convergence criterion set at a root mean square deviation (RMSD) of 0.5 Å. Through desalting, charging, pH = 7 ± 2 ionization, and optimizing the force field of molecules as OPLS_2005, the small molecules were optimized by using the LigPrep module to form tautomers of molecules.

Molecular docking was performed utilizing the docking module within Schrodinger software, with "receptor box generation" being chosen. The receptor box file was automatically generated, with the original ligand small molecule serving as the central point of the box. Flexible docking using standard precision was employed.

Results

Selection of DEGs

All subjects in GSE121248 were patients with HBV-HCC, corresponding to 107 microarray expression datasets, including 70 cases of HBV-HCC cancer tissues and 37 cases of adjacent tissues. GSE121248 was generated via the GPL570 platform. The online analysis tool GEO2R was used for DEGs. This study identified 953 DEGs in the liver tissues of patients, with 327 genes upregulated and 626 downregulated. The DEGs were visualized via a volcano map (Fig. 1a). In addition, the study assessed the standardization and cross comparability of this dataset (Fig. 1d). The median of the selected samples is basically on the same horizontal line, indicating that this group of data has been neutralized and can be directly applied for analysis.

Screening common genes associated with HBV-HCC and phospholipid metabolism

This study identified 472 target genes related to phospholipid metabolism via GeneCards. Twenty-seven overlapping genes were identified (Fig. 1b). Compared with those in tumours and adjacent normal tissues, 8 genes were upregulated, namely, CDKN3, ACSL4, SPP1, PLCB1, DTNA, ITGA2, SLC44A5 and GDPD1, and 19 were downregulated, namely, ABCB4, LDLR, PRKAR2B, PTGS2, GPD1, BCHE, FABP1, EPHA3, PON1, NR1I2,

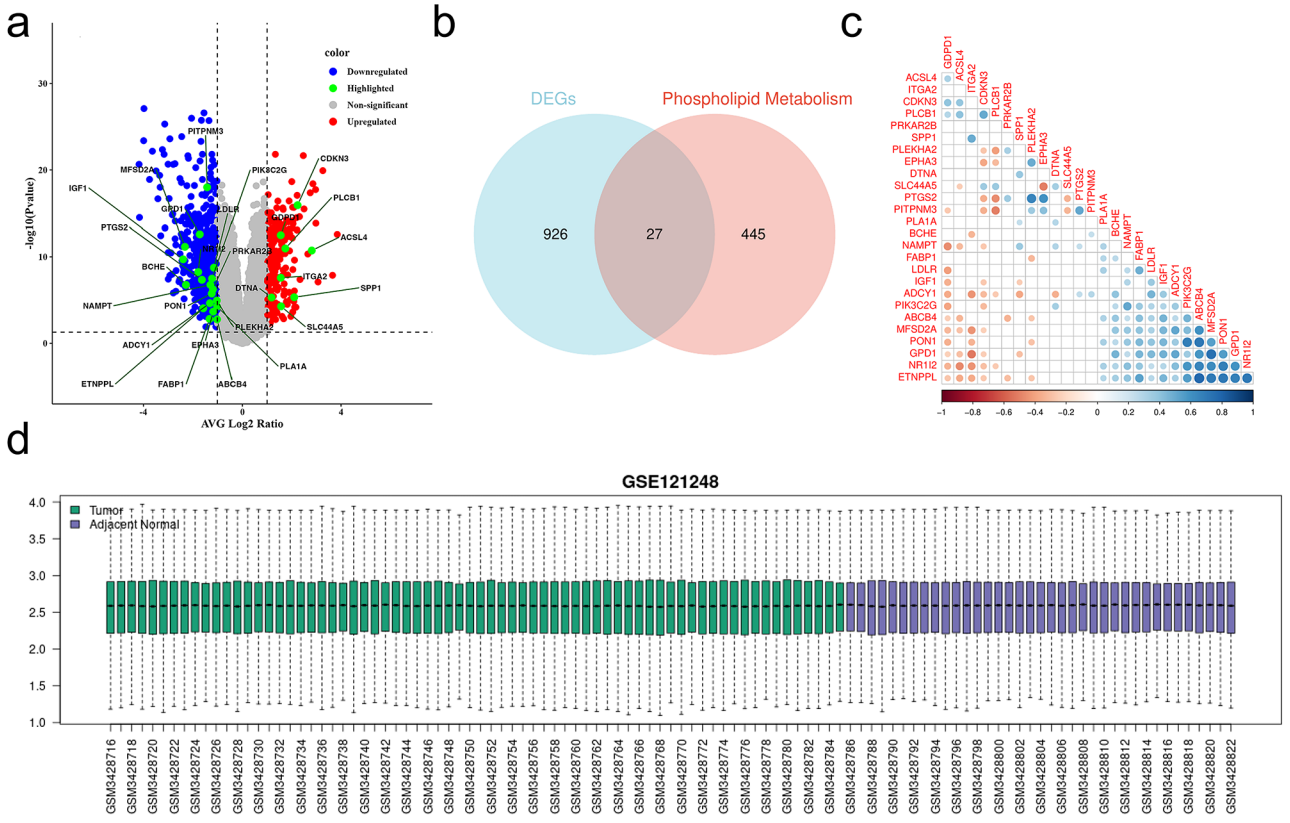

Fig. 1 DEGs analysis of HBV-HCC. (**a**) Volcanic plots in GSE121248. Upregulated DEGs (red), downregulated DEGs (blue), and no differentially expressed (grey). (**b**) Venn diagram of phospholipid metabolism-related DEGs. (**c**) Visualization of the associations between the expression of the 27 identified genes in HBV-HCC tissues. (**d**) Neutralization processing of the microarray data

Table 1 GO and KEGG enrichment analysis for HBV-HCC

Ontology	ID	Description	Count	*P* value
BP	GO:0046486	glycerolipid metabolic process	10	< 0.001
	GO:0006650	glycerophospholipid metabolic process	9	< 0.001
	GO:0006644	phospholipid metabolic process	9	< 0.001
CC	GO:0045121	membrane raft	4	< 0.001
	GO:0098857	membrane microdomain	4	< 0.001
	GO:0034358	plasma lipoprotein particle	2	0.001
MF	GO:0004620	phospholipase activity	4	< 0.001
	GO:0016298	lipase activity	4	< 0.001
	GO:0052689	carboxylic ester hydrolase activity	4	< 0.001
KEGG	hsa04913	ovarian steroidogenesis	4	< 0.001
	hsa04927	cortisol synthesis and secretion	3	< 0.001
	hsa04976	bile secretion	3	0.001

IGF1, ADCY1, PIK3C2G, PLA1A, ETNPPL, MFSD2A, PITPNM3, PLEKHA2 and NAMPT.

The analysis of pearson correlation was conducted on the 27 genes expression values, and a heatmap displaying the significantly correlated gene pairs was produced (Fig. 1c). According to the thermogram, the correlations among 27 genes are very strong; GDPD 1 has a strong negative correlation with other genes, whereas ETNPPL has a positive correlation.

Erichment analysis

The GO and KEGG pathways were analysed to discover the correlation between phospholipid metabolism and HBV-HCC (Table 1; Fig. 2). GO enrichment analysis revealed that BP was enriched mainly in glycerolipid metabolic processes, glycerophospholipid metabolic and phospholipid metabolic processes. The CC were predominantly enriched in membrane rafts, membrane microdomains and plasma lipoprotein particles. MF showed significant enrichment in lipase activity, phospholipase activity and carboxylic ester hydrolase activity. KEGG indicated the DEGs were mainly associated with pathways such as ovarian steroidogenesis, cortisol synthesis and secretion, and bile secretion.

Screening of feature genes via PPI network

This study used PPI data from STRING to plot the PPIs of 27 different genes to further study the role of the

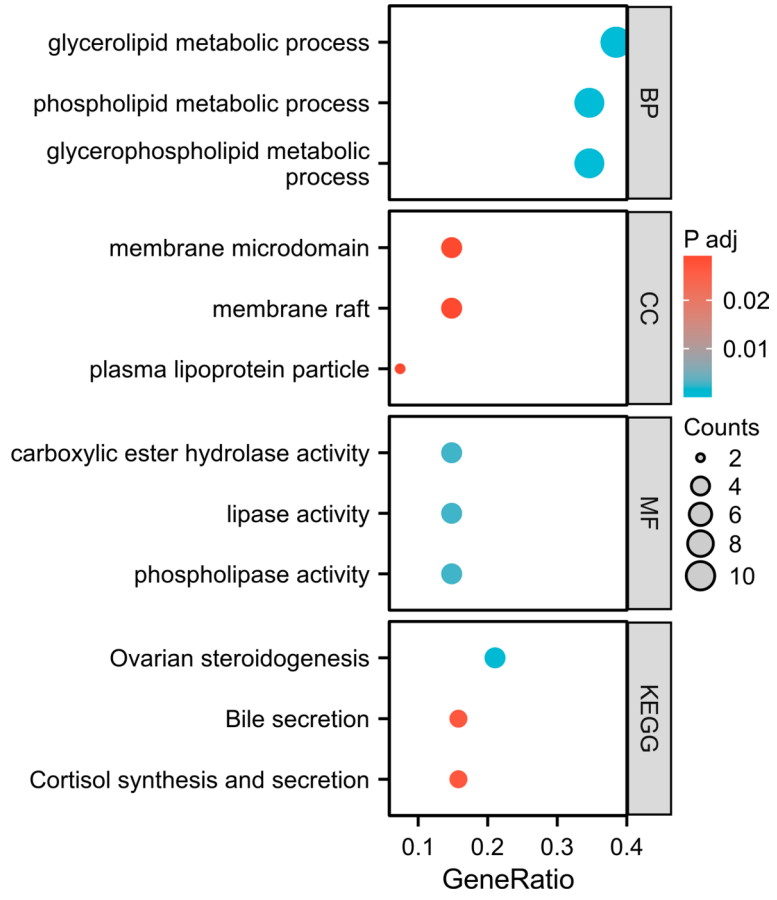

Fig. 2 Enrichment analyses. In the bubble chart, the count represents the number of genes, and the colour represents the adjusted *P* value

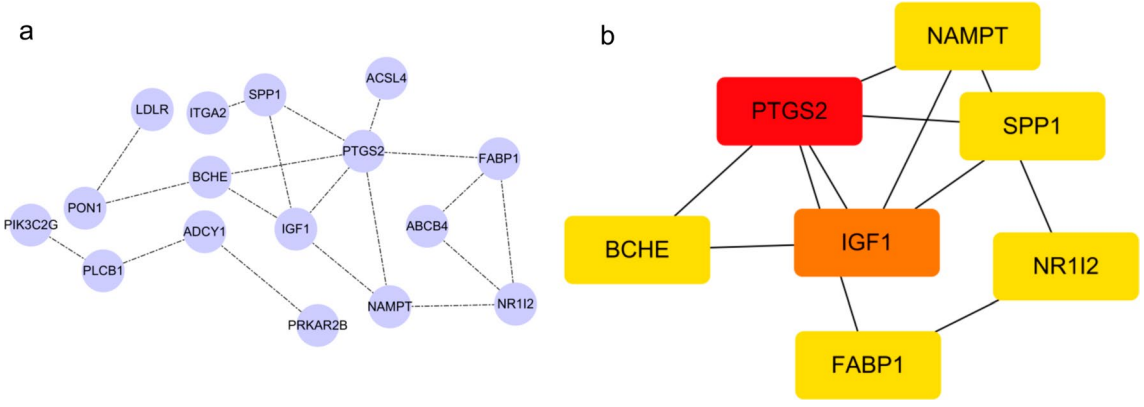

Fig. 3 Network construction diagram. (**a**) PPI network construction. (**b**) Identification of feature genes

Table 2 Protein names corresponding to feature genes

Gene name	Protein name
PTGS2	COX-2
IGF1	Insulin-like growth Factor I
SPP1	Sphingosine-1-phosphate phosphatase 1
BCHE	Cholinesterase
NR1I2	Orphan nuclear receptor PAR1
NAMPT	Nicotinamide phosphoribosyl transferase
FABP1	Fatty acid binding protein

DEGs. The results revealed 16 gene nodes (Fig. 3a). The seven DEGs with the strongest connectivity were identified using Cytoscape cytoHubba plug-in (Fig. 3b). These genes included PTGS2, IGF1, SPP1, BCHE, NR1I2, NAMPT, and FABP1. The protein name corresponding to the feature gene was found in the UniProt protein database (Table 2).

Verification of feature gene

The area under the ROC curve (AUC) of the seven feature genes were all greater than 0.6, which indicated they all had certain diagnostic efficiency (Fig. 4a). The content levels of seven genes showed significant differences ($P<0.01$). Among them, the expression of SPP1 was upregulated, while the expression of the other genes was downregulated (Fig. 4b-h).

The analysis of immune infiltration

The contents of 22 types of immune cells were analysed to determine their influence on feature genes in each GSE121248 sample. As shown in Fig. 5, the feature genes (PTGS2, IGF1, SPP1, BCHE, NR1I2, NAMPT, FABP1) regulate the infiltration of various immune cells, such as elevated SPP1 expression exhibited a positive correlation with neutrophils, elevated NR1I2 expression was inversely correlated with eosinophils and elevated BCHE expression exhibited a positive correlation with the number of plasma cells. The upregulation or downregulation of these feature genes can promote the reprogramming of phospholipid metabolism, activate the glycolysis process, recruit inflammatory cells, inhibit the antitumour immune response, promote angiogenesis and promote tumour drug resistance, which can lead to the occurrence of liver cancer [14].

Differentially abundant metabolite identification

The serum total ion current diagrams were obtained via the metabolomics platform (Fig. 6a). After PCA (Fig. 6b) analysis and OPLS-DA (Fig. 6c) analysis of all the samples, the results indicated a clear distinction between serum samples from the healthy group and the comparison group. This model had good fitting and prediction ability (R2X = 43.8%, R 2Y = 97.2%, and Q2 = 96.3%). The OPLS-DA model was employed to identify distinctive characteristic ions. Nonparametric tests were carried out on the screened metabolites, and the metabolites with $P \geq 0.05$ were excluded. Through the comparison of chromatographic peaks and the search for secondary mass spectrometry data, different metabolites in the phospholipid metabolism pathway in the HBV-HCC group were further identified (Table 3). The diagnostic efficacy of the differentially abundant metabolites was verified by ROC curve analysis (Fig. 7). AUC is commonly utilized to assess diagnostic tests. The findings indicated that the AUCs of the metabolites were all greater than 0.7, suggesting good diagnostic efficiency.

Molecular docking

To explore the potential interactions between metabolites and key targets in greater detail, the study performed molecular docking studies. No corresponding agonists or inhibitors were found for the proteins related to IGF-1 and SPP1, and the binding sites were unclear; thus, only five key targets (PTGS2, BCHE, NR1I 2, NAMPT and FABP 1) could be analysed by molecular docking. The binding strength between metabolites and targets was evaluated. An increased absolute value of the docking score signifies a more stable binding conformation between the metabolite and protein. Table 4 shows the docking scores of metabolites with five types of

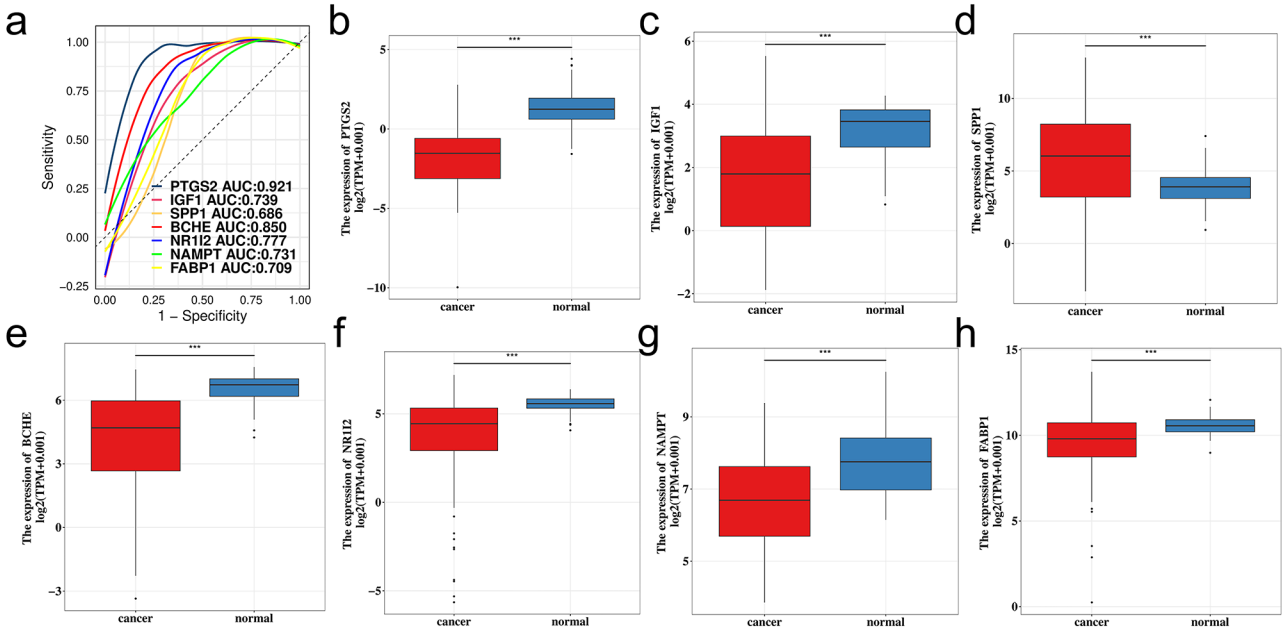

Fig. 4 Validation of feature genes expression levels. (**a**) ROC curve, (**b**) PTGS2, (**c**) IGF1, (**d**) SPP1, (**e**) BCHE, (**f**) NR1I2, (**g**) NAMPT, (**h**) FABP1, $P<0.001$ is shown in ***

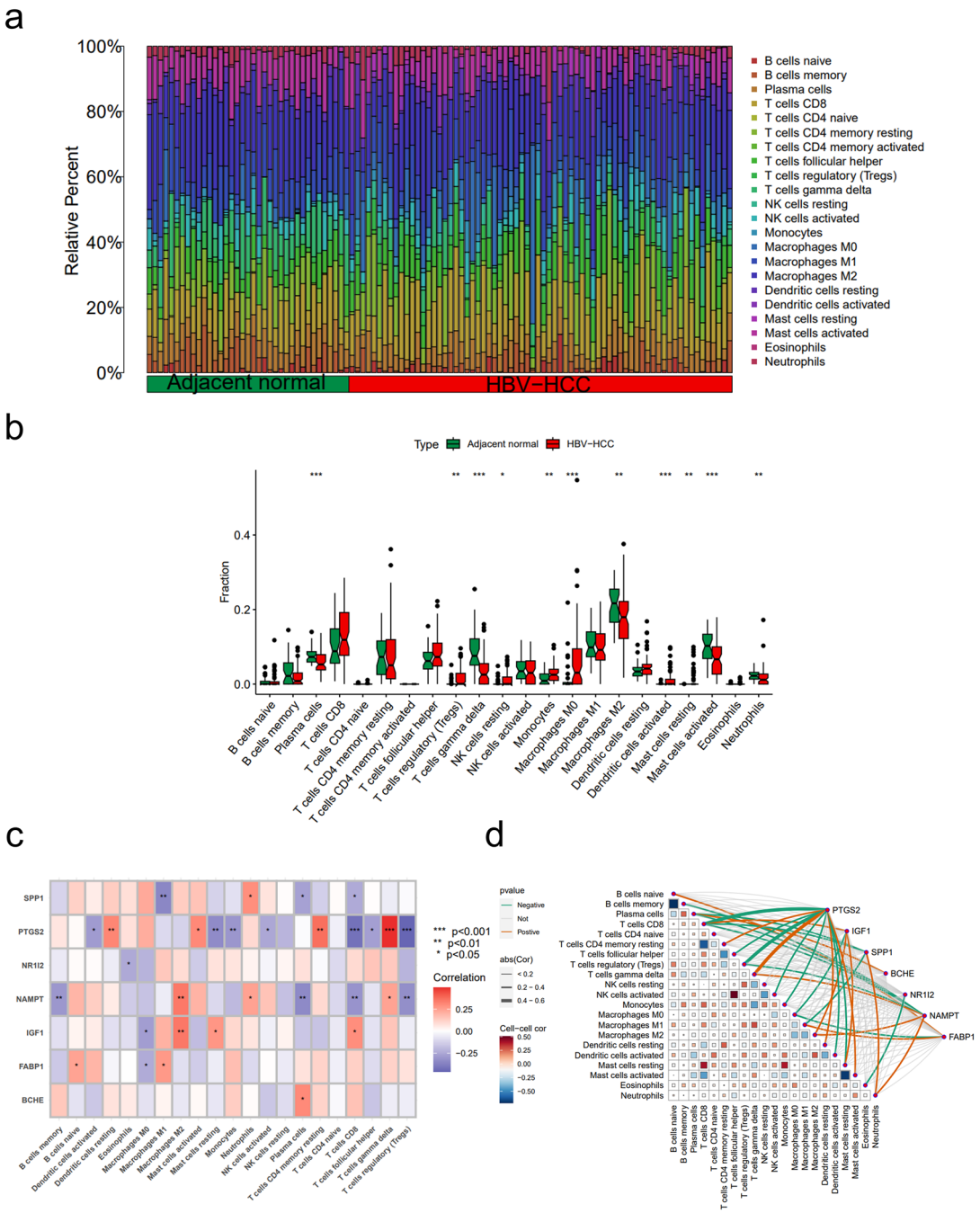

Fig. 5 Immune infiltration analysis. (**a**) Bar plot. (**b**) Box diagram of the immune cell content between HBV-HCC tissue and adjacent healthy tissue. (**c**) Immune and feature gene correlation heatmap. $P < 0.05$ is shown in*, $P < 0.01$ is shown in**, and $P < 0.001$ is shown in ***. Positive correlation (red), negative correlation (blue). (**d**) Visualization of immune and feature gene correlation results. The links represent the correlation between genes and immune cells, positive correlation (orange), negative correlation (green) and insignificant correlation(grey)

proteins, and the metabolites have good docking results with the corresponding proteins PTGS2, NR1I2 and FABP1. The docking score between metabolite 7 and the fatty acid-binding protein was the highest (-14.400 kcal/mol), which indicated that the binding conformation of metabolite 7 and the fatty acid-binding protein was the most stable and that the binding force was the strongest. Metabolites 5 and 7 had no docking score with PTGS2, which showed that these metabolites could not bind with PTGS2. Figures 8, 9, 10, 11 and 12 show the key amino acids that interact with the ligands. The compounds could form stable interactions with these five proteins.

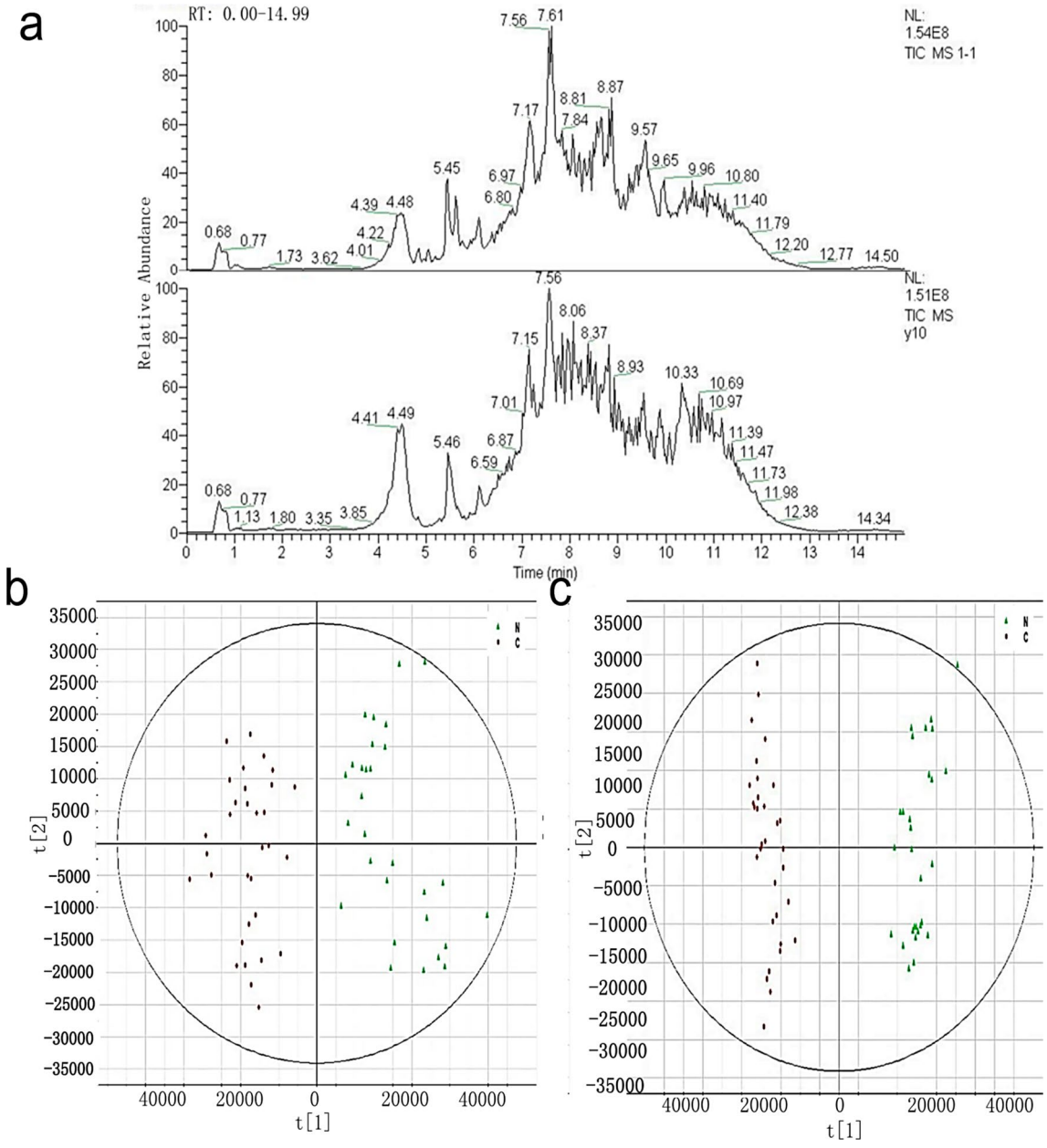

Fig. 6 Experimental results of metabolomics. (**a**) Serum total ion current diagrams. The upper image shows the HBV-HCC group, and the lower image shows the healthy control group. (**b**) PCA (**c**) OPLS-DA. HBV-HCC group (Red), healthy control group (green)

Table 3 Differentially abundant metabolites in the phospholipid metabolic pathway

Serial number	m/z	RT(min)	Metabolite	Content (*N* vs. HCC)
Metabolite 1	544.3426	7.07338	LysoPC(0:0/20:4(5Z,8Z,11Z,14Z))	↓
Metabolite 2	568.3425	7.021236	LysoPC(22:6(4Z,7Z,10Z,13Z,16Z,19Z)/0:0)	↓
Metabolite 3	480.3473	8.097989	LysoPC(P-16:0/0:0)	↓
Metabolite 4	546.3582	7.410748	LysoPC(20:3(8Z,11Z,14Z)/0:0)	↓
Metabolite 5	570.3584	7.250413	LysoPC(22:5(4Z,7Z,10Z,13Z,16Z)/0:0)	↓
Metabolite 6	542.3269	6.730612	LysoPC(20:5(5Z,8Z,11Z,14Z,17Z)/0:0)	↓
Metabolite 7	637.309	8.489258	PA(PGJ2/8:0)	↑

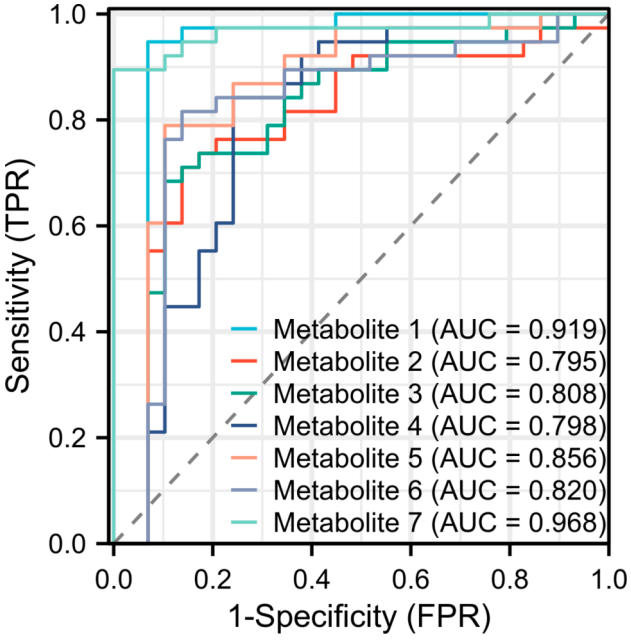

Fig. 7 ROC curve. The AUC is usually used to evaluate diagnostic tests. When the AUC is closer to 1, the variable has a better diagnostic effect in predicting the outcome

Discussion

HCC is caused mainly by HBV infection in Asia [15]. Currently, there is no mature diagnostic strategy with high specificity and sensitivity, and the treatment options are limited (e.g., sorafenib, cis-Pt) [16]. Liver enzyme abnormalities and histopathological changes are more commonly observed in patients with coexisting hepatitis B [17]. Researchers have worked hard to study biomarkers related to HBV-HCC [18, 19]. Wang et al. [20] examined the risk factors, recurrence patterns, and patient long-term outcomes with HBV-HCC recurrence after hepatectomy. Yiming Shao et al. [21] offered the early diagnosis and treatment reference by studing the molecular mechanism in HBV-HCC. Lim et al. [22] revealed the distinct immune microenvironment of HBV-HCC. This research identified 27 DEGs associated with phospholipid metabolism in HBV-HCC, with 8 genes being upregulated and 19 downregulated. Furthermore, this study explored the biological functions of these genes. The results indicated that the inflammatory state of the liver was maintained and developed by stimulating molecules to reach the liver through ovarian steroidogenesis and bile secretion. These metabolic pathways offer the opportunity to investigate potential treatment and

Table 4 Results of molecular docking

Gene name	Protein name	PDB	Metabolite 1	3	5	6	7
BCHE	Cholinesterase	4b0P	-5.656	-6.317	-7.292	-6.986	-8.076
PTGS2	COX-2	3 nt2	-11.303	-10.973	-	-11.136	-
NR1I2	Orphan nuclear receptor PAR1	3R8D	-13.250	-10.111	-13.892	-12.954	-13.574
NAMPT	Nicotinamide phosphoribosyltransferase	6e68	-6.953	-7.316	-9.556	-8.637	-9.595
FABP1	Fatty acid-binding protein	7fyB	-11.302	-10.047	-13.437	-11.748	-14.400

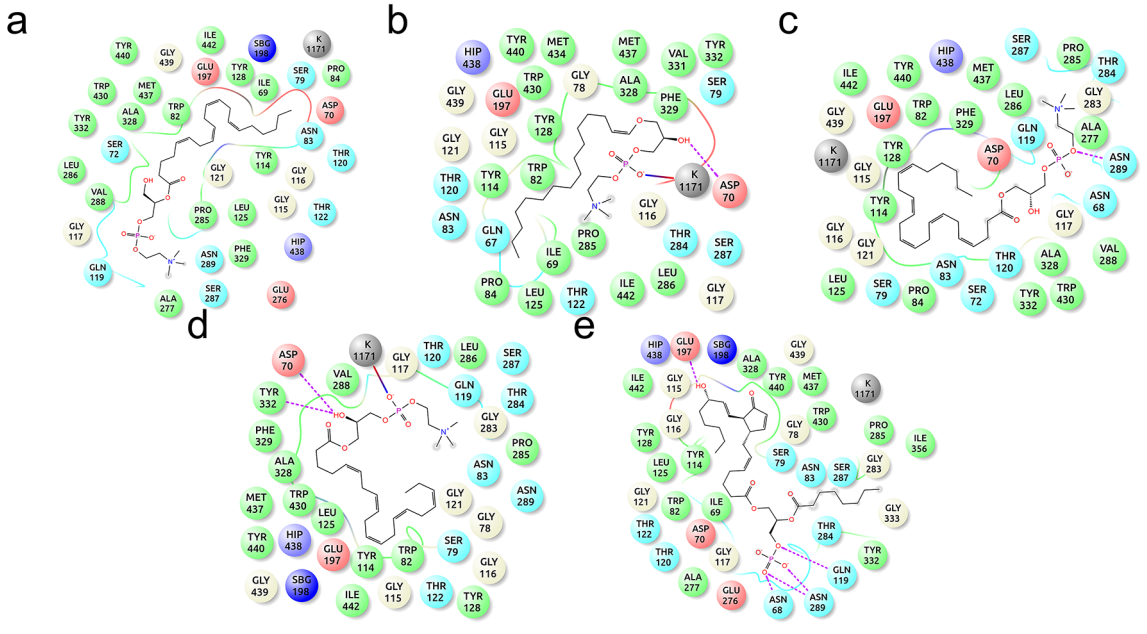

Fig. 8 Docking complex between metabolites and BCHE. (**a**) metabolite1. (**b**) metabolite 3. (**c**) metabolite 5. (**d**) metabolite 6. (**e**) metabolite 7

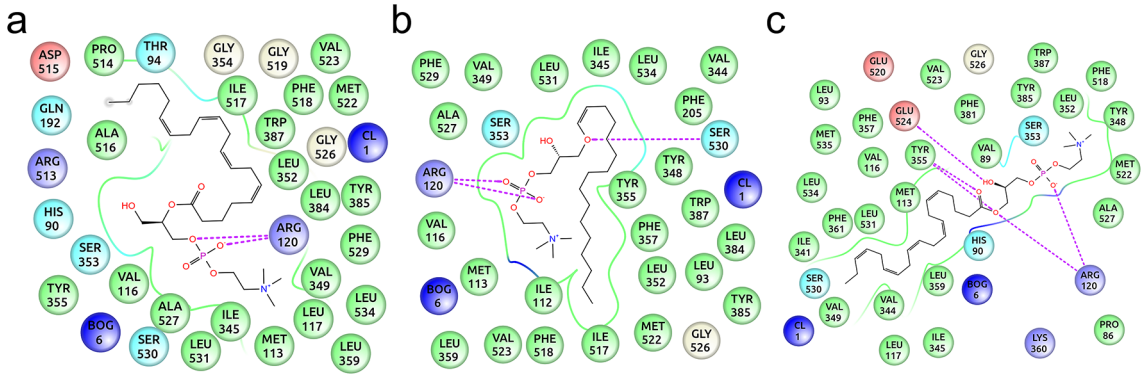

Fig. 9 Docking complex between metabolites and the PTGS2 protein. (**a**) metabolite 1. (**b**) metabolite 3. (**c**) metabolite 6

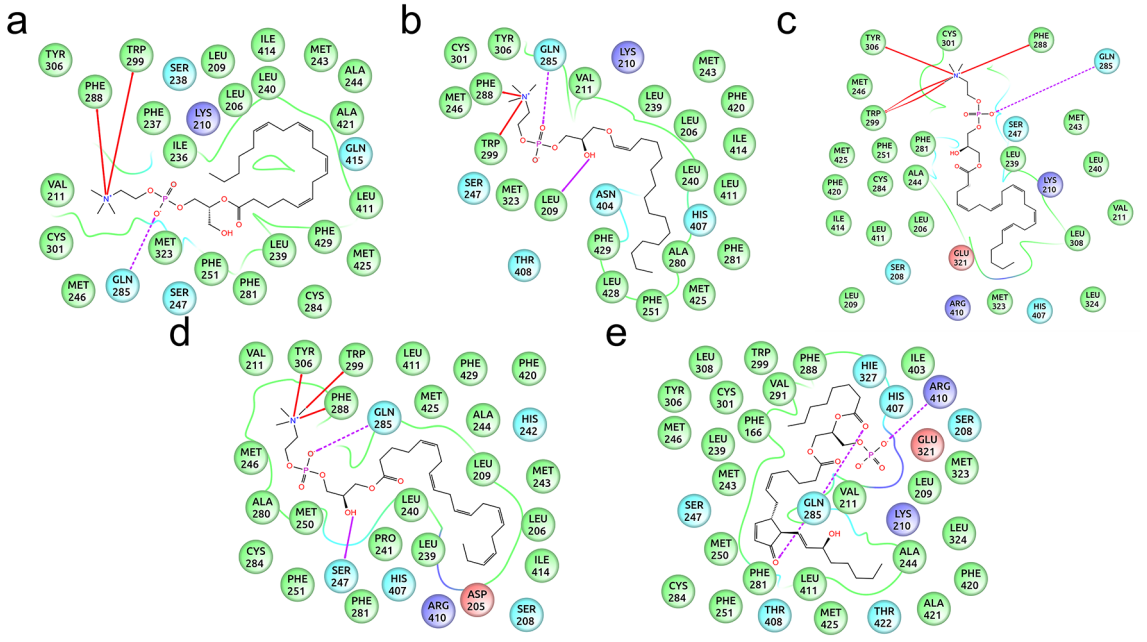

Fig. 10 Docking complex between metabolites and the NR1I2 protein. (**a**) metabolite 1, (**b**) metabolite 3, (**c**) metabolite 5, (**d**) metabolite 6. (**e**) metabolite 7

prevention strategies for diseases. After that, seven feature genes (PTGS2, IGF1, SPP1, BCHE, NR1I2, NAMPT, and FABP1) were identified through Cytoscape. Among them, SPP1 was the sole gene with high expression in HBV-HCC; the other genes had low expression. Finally, the feature genes and potential therapeutic targets of phospholipid metabolism in HBV-HCC were studied via metabolomics and molecular docking technology.

SPP1 is a secreted phosphoprotein that promotes cell adhesion and chemotaxis [23]. It can also promote host defence, bone formation and wound healing by encouraging macrophage migration and promoting Th1 responses, thus preventing viral and bacterial infections. This study revealed that SPP1 is upregulated in HCC, aligning with the findings of this research [24]. SPP1-specific antibodies have been demonstrated to efficiently inhibit HCC cell invasion in vitro and have also been shown to suppress the lung metastasis of HCC cells. Only a few patients are helped by tumour immunotherapy, which severely limits the applicability of immunotherapy in patients with HCC [25]. Ma et al. [26] reported that suppressing SPP1 expression in mice can eliminate the tumour's immunological barrier and limit the infiltration of immune cells into malignant areas, thus demonstrating the potential efficacy of immunotherapy. These results provide a critical step in identifying more effective HCC therapies. Some researchers have also identified SPP1 as an immune-related marker [27, 28]. These findings suggest that SPP1 can work as a prognostic factor in HCC. BCHE is a glycoprotein synthesized and is responsible for drug degradation and detoxification in the body [29]. Recently, a decrease in BCHE activity was reported to be related to

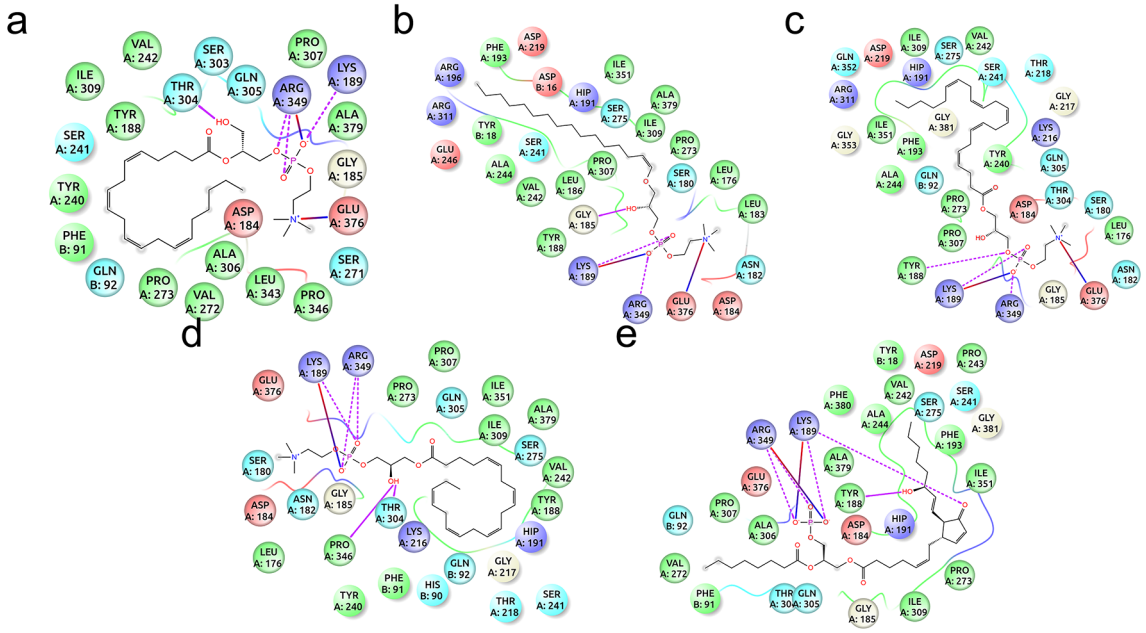

Fig. 11 Docking complex between the metabolite and NAMPT protein. (**a**) metabolite 1. (**b**) metabolite 3. (**c**) metabolite 5. (**d**) metabolite 6. (**e**) metabolite 7

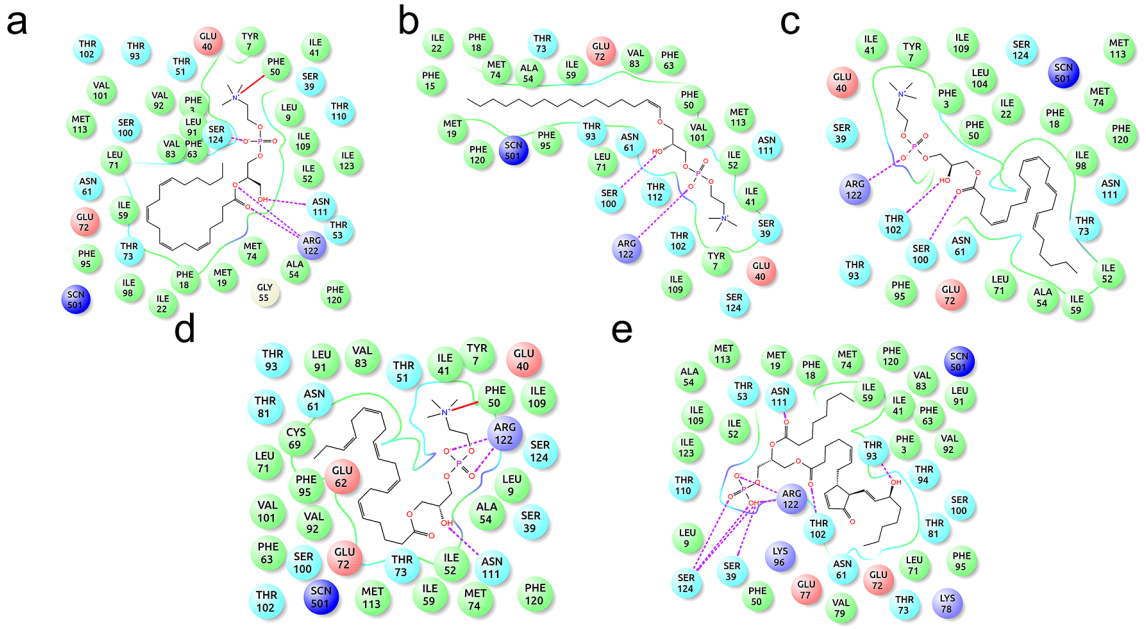

Fig. 12 Docking complex between metabolites and the FABP1 protein. (**a**) metabolite 1. (**b**) metabolite 3. (**c**) metabolite 5. (**d**) metabolite 6. (**e**) metabolite7

liver function injury, which shows that BCHE is a marker of substantial damage to liver organs [30].

NR1I2 belongs to the nuclear receptor family and can control metabolism and regulate the expression of detoxification enzymes [31]. Nuclear receptors are key regulators of lipid metabolism in HCC, contributing to both its pathogenesis and treatment [32]. Reports indicate that NR1I2 expression is commonly associated with chemotherapy resistance in hepatocellular carcinoma cells [33]. It also serves as the primary regulator of CYP3A4 and is involved in controlling T-cell differentiation, playing a crucial role in maintaining immune homeostasis [34]. Furthermore, NR1I2 can significantly inhibit HCC progression by negatively regulating the JNK pathway, which aligns with the findings of this study [35].

IGF-1 is a significant growth hormone that is primarily generated in the liver [36]. IGF-1 in the PI3K/AKT signalling pathway is crucial for regulating proliferation

and death [37]. The bioavailability of IGF-1 in the blood is regulated by IGFBP1, and changes in IGF signal transduction are related to HCC [38]. HBV inhibits the expression of host cells and the secretion of IGFBP1 through HBx, thus increasing the survival and antiapoptotic effects of IGF-1, which may lead to HBV-HCC disease [39]. The serum IGF-1 level in HCC patients were less than that in the healthy controls confirmed by Xu et al. [40]. This finding aligns with the findings presented in this paper. The reason is that IGF-1 is synthesized mainly by the liver, so advanced liver tumours can inhibit normal liver function by replacing normal liver cells. It has also been reported that reduced IGF-1 levels in HCC patients are associated lower survival rates [41]. Additionally, IGF-1 is crucial in HCC, as its gene expression declines with the worsening severity of HCC [42]. Another study found that some patients with advanced liver disease have sarcopenia, which is closely related to IGF-1 [43]. However, lipidomic analyses showed that the levels of major phospholipids (phosphatidylcholine, phosphatidylethanolamine, and phosphatidylglycerol) were increased in sarcopenic muscle and inversely correlated with muscle volume and peak power [44].

NAMPT is an essential enzyme in the NAD+-dependent activation of the SIRT1 pathway. SIRT1 is closely associated with HCC [45, 46]. The demand of cancer cells for NAD increases due to high proliferation rates and high DNA repair rates [47]. Therefore, NAMPT is considered a target of anticancer therapy. The downregulation of NAMPT expression markedly accelerated HCC growth and was inversely correlated with survival rates, particularly in human HCC. Lactic acid accumulation in the tumour microenvironment inhibits NAMPT transcriptional activity in NK cells [48]. PTGS2, also known as a proinflammatory enzyme, is triggered by prostaglandins and contributes to cell proliferation, as well as tumour onset, development and spread [39]. Research on PTGS2, also called cyclooxygenase-2 (COX-2), have shown that COX-2 levels are typically higher in well-differentiated hepatocellular carcinoma compared to poorly differentiated HCC. These findings indicate that COX-2 may contributes to the early stages of HCC development [37]. Furthermore, a notable association exists between COX-2 expression levels and active inflammation in adjacent normal liver tissue. Moreover, elevated COX-2 expression in adjacent normal tissue is significantly linked to a reduction in disease-free survival among patients with HCC [41]. It has been suggested that the expression of COX-2 could be a significant factor in the postoperative recurrence of HCC.

FABP1 is primarily expressed in the liver, which shows low expression in HCC tissues at various clinical stages [49–51]. Patients with elevated FABP1 expression in adjacent tissues generally experience longer lifespans. Another study demonstrated that FABP1 within the microenvironment influenced fatty acid distribution and tumour progression [52]. In addition, FABP1 can also exert antioxidant stress to protect the liver by capturing and removing reactive oxygen species (ROS) [53]. Therefore, people believe that FABP1 can be considered a tumour suppressor gene and that it works as a regulatory gene in the development and pathogenesis of tumours.

Recently, emerging evidence has highlighted the crucial regulatory function of the tumour TME in the initiation of tumours [54]. Immune infiltration suggest that the activity of feature genes is associated with various immune cells, potentially influencing the progression of HBV-HCC through the regulation of diverse immune-related signalling pathways. These feature genes may be new potential prognostic and immune-related biomarkers for HBV-HCC patients.

Metabolomics effectively identified distinct metabolites in the phospholipid metabolism pathway between individuals with and without HBV-HCC, providing further validation of significant discrepancies in this pathway between the two groups. The docking study indicated that the different metabolites may play essential roles in causing HBV-HCC by targeting key genes, such as PTGS2, BCHE, NR1I2, NAMPT and FABP1. Among them, PA (PGJ2/8:0) had the highest docking score with FABP1, suggesting a strong interaction. PA (PGJ2/8:0) is an oxidized phosphatidic acid (PA). PA can promote steatosis, cell damage, fibrosis and inflammation in liver cells. FABP1 can stably bind to PA (PGJ2/8:0) and contribute to mitochondrial and microsomal phospholipid metabolism by controlling the PA synthesis and utilization [55]. An abnormal increase in the PA (PGJ2/8:0) level is an important biochemical reaction event that promotes the progression of HBV-HCC. FABP1, a key enzyme for the transformation of PA (PGJ2/8:0) into phosphatidic acid microsomes, could considered as a potential protein for the treatment of HBV-HCC.

Study strengths and limitations

For the first time, the possible involvement of genes linked to phospholipid metabolism in HBV-HCC were revealed. Disease classification and metabolic pathways are more detailed than those in previous studies [56, 57]. Metabolomics and molecular docking were added to make the article more complete. However, there is a lack of functional experiments on the biological effects of these compounds in HCC cells. These experiments should be performed in the future, which will enable us to better confirm the effect of identified genes in HBV-HCC and provide latent targets and a theoretical basis for developing new strategies for tumour intervention and treatment in the future.

Conclusions

Above all, this finding uncovered the possible role of phospholipid metabolism in HBV-HCC and revealed related feature genes and potential therapeutic targets via bioinformatics methods. This study, which connects phospholipid metabolism with tumour development, deepens people understanding of the molecular regulatory mechanisms underlying abnormal metabolism in tumour cells. It also offers potential targets and a theoretical foundation for future intervention strategies in HBV-HCC patients.

Acknowledgements
Not applicable.

Author contributions
Jian Zhang and Fengmei Zhang wrote the main manuscript. Lei Zhang completed metabonomics. Meiling Zhang assisted Ying Ma in molecular docking. Shuye Liu provided guidance for experimental design. Ying Ma completed molecular docking.

Funding
Tianjin Health Science and Technology Project (Grant No. TJWJ2023XK020) and Tianjin Key Medical Discipline (Specialty) Construction Project (Grant No. TJYXZDXK-047 A) provided funding for this work.

Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethical approval
The ethics committee of Tianjin Third Central Hospital approved the study (Approval No: TJWJ2023XK020).

Competing interests
The authors declare no competing interests.

Author details
[1]Department of Clinical Laboratory, The Second Hospital of Tianjin Medical University, Tianjin 300211, China
[2]Department of Clinical Laboratory, Tianjin Key Laboratory of Extracorporeal Life Support for Critical Diseases, Artificial Cell Engineering Technology Research Center, The Third Central Hospital of Tianjin, Tianjin Institute of Hepatobiliary Disease, Tianjin 300170, China
[3]Tianjin Key Laboratory on Technologies Enabling Development of Clinical Therapeutics and Diagnostics (Theranostics), School of Pharmacy, Tianjin Medical University, Tianjin 300070, China

Received: 25 June 2024 / Accepted: 13 August 2024
Published online: 24 August 2024

References

1. Lee JH, Suh JH, Kang HJ, Choi SY, Jung SW, Lee-Kwon W, et al. Tonicity-responsive enhancer-binding protein promotes stemness of liver cancer and cisplatin resistance. EBioMedicine. 2020;58:102926.
2. Eller C, Heydmann L, Colpitts CC, El Saghire H, Piccioni F, Jühling F, et al. A genome-wide gain-of-function screen identifies CDKN2C as a HBV host factor. Nat Commun. 2020;11:2707.
3. Xie Y, Wu H, He Y, Liu L, Huang IB, Zhou L, et al. Targeting AXL induces tumor-intrinsic immunogenic response in tyrosine kinase inhibitor-resistant liver cancer. Cell Death Dis. 2024;15:110.
4. Yang Q, Zhuo Z, Qiu X, Luo R, Guo K, Wu H, et al. Adverse clinical outcomes and immunosuppressive microenvironment of RHO-GTPase activation pattern in hepatocellular carcinoma. J Transl Med. 2024;22:122.
5. Xu W, Yang M, Zhang W, Jia W, Zhang H, Zhang Y. Tumor microenvironment responsive nano-platform for overcoming sorafenib resistance of hepatocellular carcinoma. Mater Today Bio. 2024;24:100902.
6. Song Y, Lau HC, Zhang X, Yu J. Bile acids, gut microbiota, and therapeutic insights in hepatocellular carcinoma. Cancer Biol Med. 2023;21:144–62.
7. Liang D, Minikes AM, Jiang X. Ferroptosis at the intersection of lipid metabolism and cellular signaling. Mol Cell. 2022;82:2215–27.
8. Dang Q, Sun Z, Wang Y, Wang L, Liu Z, Han X. Ferroptosis: a double-edged sword mediating immune tolerance of cancer. Cell Death Dis. 2022;13:925.
9. Schneider G. S1P signaling in the tumor microenvironment. Adv Exp Med Biol. 2020;1223:129–53.
10. Yin Y, Sichler A, Ecker J, Laschinger M, Liebisch G, Höring M, et al. Gut microbiota promote liver regeneration through hepatic membrane phospholipid biosynthesis. J Hepatol. 2023;78:820–35.
11. Meng J, Yang Z, Jiang X, Zou J. Unveiling NUSAP1 as a common gene signature linking chronic HBV infection and HBV-related HCC. Discov Oncol. 2024;15:61.
12. Guo S, Xing N, Du Q, Luo B, Wang S. Deciphering hepatocellular carcinoma pathogenesis and therapeutics: a study on anoikis, ceRNA regulatory network and traditional Chinese medicine. Front Pharmacol. 2023;14:1325992.
13. Chen QL, Qiao F, Lu WT, Shi HL, Zhou CX. Bioinformatics analysis of primary biliary cholangitis key genes and molecular mechanisms. Zhonghua Gan Zang Bing Za Zhi. 2023;31:1209–16.
14. Xu Z, Yuan KF. Lipid metabolic reprogramming and metabolic stress in liver cancer. J Sichuan Univ (Med Sci). 2021;52:561–5.
15. Devarbhavi H, Asrani SK, Arab JP, Nartey YA, Pose E, Kamath PS. Global burden of liver disease: 2023 update. J Hepatol. 2023;79:516–37.
16. Chuang YC, Tsai KN, Ou JJ. Pathogenicity and virulence of hepatitis B virus. Virulence. 2022;13:258–96.
17. Estakhri A. The effect of NAFLD (non-alcoholic fatty liver disease) on long-term outcome of chronic hepatitis B in Iranian patients. Open J Gastroenterol. 2012;2:18–21.
18. Cacoub P, Asselah T. Hepatitis B virus infection and extra-hepatic manifestations: a systemic disease. Am J Gastroenterol. 2022;117:253–63.
19. Péneau C, Imbeaud S, La Bella T, Hirsch TZ, Caruso S, Calderaro J, et al. Hepatitis B virus integrations promote local and distant oncogenic driver alterations in hepatocellular carcinoma. Gut. 2022;71:616–26.
20. Wang MD, Li C, Liang L, Xing H, Sun LY, Quan B, et al. Early and late recurrence of hepatitis B virus-associated hepatocellular carcinoma. Oncologist. 2020;25:e1541–51.
21. Shao Y, Su L, Hao R, Wang Q, Naranmandura H. Advances on molecular mechanism of hepatitis B virus-induced hepatocellular carcinoma. Zhejiang Da Xue Xue Bao Yi Xue Ban. 2021;50:113–22.
22. Lim CJ, Lee YH, Pan L, Lai L, Chua C, Wasser M, et al. Multidimensional analyses reveal distinct immune microenvironment in hepatitis B virus-related hepatocellular carcinoma. Gut. 2019;68:916–27.
23. Liu L, Zhang R, Deng J, Dai X, Zhu X, Fu Q, et al. Construction of TME and identification of crosstalk between malignant cells and macrophages by SPP1 in hepatocellular carcinoma. Cancer Immunol Immunother. 2022;71:121–36.
24. Eun JW, Yoon JH, Ahn HR, Kim S, Kim YB, Lim SB, et al. Cancer-associated fibroblast-derived secreted phosphoprotein 1 contributes to resistance of hepatocellular carcinoma to sorafenib and lenvatinib. Cancer Commun (Lond). 2023;43:455–79.
25. He H, Chen S, Fan Z, Dong Y, Wang Y, Li S, et al. Multi-dimensional single-cell characterization revealed suppressive immune microenvironment in AFP-positive hepatocellular carcinoma. Cell Discov. 2023;9:60.
26. Ma L, Wang L, Khatib SA, Chang CW, Heinrich S, Dominguez DA, et al. Single-cell atlas of tumor cell evolution in response to therapy in hepatocellular carcinoma and intrahepatic cholangiocarcinoma. J Hepatol. 2021;75:1397–408.
27. Xiang T, Cheng N, Huang B, Zhang X, Zeng P. Important oncogenic and immunogenic roles of SPP1 and CSF1 in hepatocellular carcinoma. Med Oncol. 2023;40:158.
28. Chen S, Tang L, Guillot A, Liu H. Bariatric surgery associates with nonalcoholic steatohepatitis/hepatocellular carcinoma amelioration via SPP1 suppression. Metabolites. 2022;13:11.
29. Lu S, Meng Z, Tan Y, Wu C, Huang Z, Huang J, et al. An advanced network pharmacology study to explore the novel molecular mechanism of compound kushen injection for treating hepatocellular carcinoma by bioinformatics and experimental verification. BMC Complement Med Ther. 2022;22:54.
30. Gok M, Cicek C, Bodur E. Butyrylcholinesterase in lipid metabolism: a new outlook. J Neurochem. 2024;168:381–5.

31. Zhang R, Huang M, Wang H, Wu S, Yao J, Ge Y, et al. Identification of potential biomarkers from hepatocellular carcinoma with MT1 deletion. Pathol Oncol Res. 2021;27:597527.
32. Yang Z, Danzeng A, Liu Q, Zeng C, Xu L, Mo J, et al. The role of nuclear receptors in the pathogenesis and treatment of non-alcoholic fatty liver disease. Int J Biol Sci. 2024;20:113–26.
33. Zhu D, Zhu Y, Liu L, He X, Fu S. Metabolomic analysis of vascular cognitive impairment due to hepatocellular carcinoma. Front Neurol. 2022;13:1109019.
34. Sun G, Sun K, Shen C. Human nuclear receptors (NRs) genes have prognostic significance in hepatocellular carcinoma patients. World J Surg Oncol. 2021;19:137.
35. Tanaka Y, Uchi H, Ito T, Furue M. Indirubin-pregnane X receptor-JNK axis accelerates skin wound healing. Sci Rep. 2019;9:18174.
36. Li Y, Li K, Pan T, Xie Q, Cheng Y, Wu X, et al. Translocation of IGF-1R in endoplasmic reticulum enhances SERCA2 activity to trigger Ca^{2+} (ER) perturbation in hepatocellular carcinoma. Acta Pharm Sin B. 2023;13:3744–55.
37. Wu D, Zhang L, Ma S, Zhao Y, Chen R, Zhang F, et al. Low growth hormone levels predict poor outcome of hepatitis B virus-related acute-on-chronic liver failure. Front Med (Lausanne). 2021;8:655863.
38. Ngo MT, Jeng HY, Kuo YC, Nanda JD, Brahmadhi A, Ling TY, et al. The role of IGF/IGF-1R signaling in hepatocellular carcinomas: stemness-related properties and drug resistance. Int J Mol Sci. 2021;22:1931.
39. Nielsen KO, Mirza AH, Kaur S, Jacobsen KS, Winther TN, Glebe D, et al. Hepatitis B virus suppresses the secretion of insulin-like growth factor binding protein 1 to facilitate anti-apoptotic IGF-1 effects in HepG2 cells. Exp Cell Res. 2018;370:399–408.
40. Xu G, Chu J, Shi Y, Huang L, Fu J. The regulation of proliferation and apoptosis in hepatocellular carcinoma via insulin-like growth factor 1 receptor. Growth Horm IGF Res. 2022;66:101499.
41. Lacin S, Yalcin S, Karakas Y, Hassan MM, Amin H, Mohamed YI, et al. Prognostic significance of serum insulin-like growth factor-1 in hepatocellular cancer patients: a validation study. J Hepatocell Carcinoma. 2020;7:143–53.
42. Osganian SA, Subudhi S, Masia R, Drescher HK, Bartsch LM, Chicote ML, et al. Expression of IGF-1 receptor and GH receptor in hepatic tissue of patients with nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. Growth Horm IGF Res. 2022;65:101482.
43. Tarantino G, Sinatti G, Citro V, Santini SJ, Balsano C. Sarcopenia, a condition shared by various diseases: can we alleviate or delay the progression? Intern Emerg Med. 2023;18:1887–95.
44. Hinkley JM, Cornnell HH, Standley RA, Chen EY, Narain NR, Greenwood BP, et al. Older adults with Sarcopenia have distinct skeletal muscle phosphodiester, phosphocreatine, and phospholipid profiles. Aging Cell. 2020;19:e13135.
45. Tian C, Huang R, Xiang M. SIRT1: harnessing multiple pathways to hinder NAFLD. Pharmacol Res. 2024;203:107155.
46. Lin YC, Wu HC, Liao CC, Chou YC, Pan SF, Chiu CM. Secretion of one adipokine Nampt/Visfatin suppresses the inflammatory stress-induced NF-κB activity and affects nampt-dependent cell viability in Huh-7 cells. Mediators Inflamm. 2015;2015:392471.
47. Lin CH, Kuo JC, Li D, Koenig AB, Pan A, Yan P, et al. AZD5153, a bivalent BRD4 inhibitor, suppresses hepatocarcinogenesis by altering BRD4 chromosomal landscape and modulating the transcriptome of HCC cells. Front Cell Dev Biol. 2022;10:853652.
48. Guo X, Tan S, Wang T, Sun R, Li S, Tian P, et al. NAD^+ salvage governs mitochondrial metabolism, invigorating natural killer cell antitumor immunity. Hepatology. 2023;78:468–85.
49. Lin YX, Chen K, An FM, Wang YF, Wu XB, Zhan Q, et al. Study of abnormal lipid metabolism analysis and significance of fatty acid binding protein expression in patients with hepatocellular carcinoma. Zhonghua Gan Zang Bing Za Zhi. 2021;29:1006–13.
50. Eguchi A, Iwasa M. The role of elevated liver-type fatty acid-binding proteins in liver diseases. Pharm Res. 2021;38:89–95.
51. Dum D, Ocokoljic A, Lennartz M, Hube-Magg C, Reiswich V, Höflmayer D, et al. FABP1 expression in human tumors: a tissue microarray study on 17,071 tumors. Virchows Arch. 2022;481:945–61.
52. Tang W, Sun G, Ji GW, Feng T, Zhang Q, Cao H, et al. Single-cell RNA-sequencing atlas reveals an FABP1-dependent immunosuppressive environment in hepatocellular carcinoma. J Immunother Cancer. 2023;11:e007030.
53. Lin YX, Wu XB, Zheng CW, Zhang QL, Zhang GQ, Chen K, et al. Mechanistic investigation on the regulation of FABP1 by the IL-6/miR-603 signaling in the pathogenesis of hepatocellular carcinoma. Biomed Res Int. 2021;2021:8579658.
54. Barkley D, Moncada R, Pour M, Liberman DA, Dryg I, Werba G, et al. Cancer cell states recur across tumor types and form specific interactions with the tumor microenvironment. Nat Genet. 2022;54:1192–201.
55. Vancura A, Haldar D. Regulation of mitochondrial and microsomal phospholipid synthesis by liver fatty acid-binding protein. J Biol Chem. 1992;267:14353–9.
56. Li L, Lei Q, Zhang S, Kong L, Qin B. Screening and identification of key biomarkers in hepatocellular carcinoma: evidence from bioinformatic analysis. Oncol Rep. 2017;38:2607–18.
57. Song X, Du R, Gui H, Zhou M, Zhong W, Mao C, et al. Identification of potential hub genes related to the progression and prognosis of hepatocellular carcinoma through integrated bioinformatics analysis. Oncol Rep. 2020;43:133–46.

Publisher's Note