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Clinical pathological significance and biological function of PLIN1 in hepatocellular carcinoma: bioinformatics analysis and in vitro experiments

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Abstract

Background & aims Perilipin 1 (PLIN1) is an essential lipid droplet surface protein that participates in cell life activities by regulating energy balance and lipid metabolism. PLIN1 has been shown to be closely related to the development of numerous tumor types. The purpose of this work was to elucidate the clinicopathologic significance of PLIN1 in hepatocellular carcinoma (HCC), as well as its impact on the biological functions of HCC cells, and to investigate the underlying mechanisms involved.

Methods Public high-throughput RNA microarray and RNA sequencing data were collected to examine PLIN1 levels and clinical significance in patients with HCC. Immunohistochemistry (IHC) and real-time quantitative reverse transcription polymerase chain reaction (RT–qPCR) were conducted to assess the expression levels and the clinicopathological relevance of PLIN1 in HCC. Then, SK and Huh7 cells were transfected with a lentivirus overexpressing PLIN1. CCK8 assay, wound healing assay, transwell assay, and flow cytometric analysis were conducted to explore the effects of PLIN1 overexpression on HCC cell proliferation, migration, invasion, and cell cycle distribution. Ultimately, Gene Ontology (GO) functional annotation and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis were performed to investigate the underlying mechanisms of PLIN1 in HCC progression based on HCC differentially expressed genes and PLIN1 co-expressed genes.

Results PLIN1 was markedly downregulated in HCC tissues, which correlated with a noticeably worse prognosis for HCC patients. Additionally, PLIN1 overexpression inhibited the proliferation, migration, and invasion of SK and Huh7 cells in vitro, as well as arresting the HCC cell cycle at the G0/G1 phase. More significantly, energy conversion-related biological processes, lipid metabolism, and cell cycle signalling pathways were the three most enriched molecular mechanisms.

Conclusion The present study revealed that PLIN1 downregulation is associated with poor prognosis in HCC patients and accelerated HCC progression by promoting cellular proliferation, migration, and metastasis, as well as the mechanisms underlying the regulation of lipid metabolism-related pathways in HCC.

Keywords Hepatocellular carcinoma, PLIN1, Diagnostic marker, Prognosis, Biological functions

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Background

Primary liver cancer is a malignant tumor with high incidence and lethality worldwide. HCC, the most common type of liver cancer, has become the sixth highest incidence cancer and the third leading cause of cancer-related death, according to the global cancer statistics 2022 report [1]. Chronic viral hepatitis is the leading cause of HCC, accounting for approximately 80%-90% of cases. In addition, metabolic dysregulation of the liver can increase the risk of HCC, such as diabetes, obesity, and other metabolic diseases [2]. Hepatitis B virus (HBV) or hepatitis C virus (HCV) carriers with diabetes or obesity are at significantly increased risk, suggesting a synergistic effect of metabolic factors and hepatitis [3]. To date, surgery remains the primary treatment for prolonging the survival of HCC patients [4]. However, the early signs and symptoms of HCC are not obvious or lack specificity, and most patients are diagnosed at an advanced stage, which limits the best treatment period. Even after systemic and translational therapy to gain access to surgery, patients still face a high recurrence rate and mortality risk due to the heterogeneity of the tumor [5]. Therefore, exploring key genes related to the onset and progression of HCC provides an opportunity to improve the quality of life and prolong the survival of patients.

Perilipin-1 (PLIN1), a gene on human chromosome 15q26.1, encodes a protein from the perilipin family. It is involved in regulating lipolysis in adipocytes, cell metabolism, and mitochondrial function [6]. The function of PLIN1 in maintaining lipid homeostasis depends on the regulation of its expression. Numerous investigations revealed a strong correlation between PLIN1 expression and metabolism-related diseases, including obesity [7], atherosclerosis [8], and insulin resistance [9]. Additionally, several human malignant tumors, such as liposarcoma [10], breast cancer [11], and lung squamous cell carcinoma [12], exhibit abnormal expression of PLIN1, indicating a potential role for PLIN1 in the development and metastasis of various tumors. Zhang et al. [13] demonstrated that PLIN1 can be used to distinguish liposarcoma from other soft tissue sarcomas. PLIN1 expression was notably downregulated in liposarcoma subtypes during adipocyte development, leading to the hypothesis that PLIN1 could function as a particular marker for liposarcoma subtype differentiation [14]. According to reports, a poor prognosis for patients with breast cancer is associated with the downregulation of PLIN1 expression [15]. Furthermore, PLIN1 is aberrantly expressed in a number of liver disorders, including hepatitis C virus infection [16], hepatocellular steatosis [17], and non-alcoholic fatty liver disease [18, 19]. There is insufficient information to determine whether PLIN1 can serve as a helpful predictive biomarker or has a role in the development of HCC.

Here, public high-throughput RNA microarray and RNA sequencing data were downloaded to analyze the PLIN1 expression levels. In-house IHC and RT-qPCR were performed to further confirm the result and explore the clinicopathologic significance of PLIN1 in HCC. Furthermore, the effects of PLIN1 on SK and Huh7 cell proliferation, migration, invasion, and cell cycle progression were examined in vitro. Ultimately, the underlying molecular mechanism was determined based on PLIN1-related differentially co-expressed genes (DCGs), which is very beneficial for identifying more aspects of HCC development and treatment.

Materials and methods

Data acquisition and preprocessing

Public high-throughput RNA microarray and RNA sequencing data from HCC and non-HCC liver tissues were collected from public databases, including the Genotype-Tissue Expression (GTEx, https://gtexportal.org), the Cancer Genome Atlas (TCGA, https://portal.gdc.cancer.gov/) and Gene Expression Omnibus (GEO, https://www.ncbi.nlm.nih) databases. The retrieval formula was as follows: ((hepatocellular OR liver) AND (Neoplas* OR Tumo* OR Cancer OR Malignan*)) OR ((HCC) OR (Hepatocellular Carcinoma)). The inclusion criteria were as follows: (1) All the subjects are *Homo sapiens*; (2) The collected samples contain HCC and non-HCC samples; (3) At least 3 cases of tumor and non-tumor samples; (4) PLIN1 expression levels were observed in the dataset. All extracted data were normalized and merged by log2 transformation. Furthermore, the remove BatchEffect() function from the "Limma" package was utilized for removing the batch effects among different platforms.

Clinical application potential of PLIN1

The standardized mean difference (SMD), receiver operating characteristic (ROC) curve, summarized receiver operating characteristic (SROC) curve, and the pooled sensitivity and specificity were used to assess the clinical potential of PLIN1. Stata Version 12.0 was used to conduct the aforementioned analysis. The Kaplan–Meier survival curve was generated via the Gene Expression Profiling Interactive Analysis (GEPIA2, http://gepia2.cancer-pku.cn/#analysis) to provide insight into the prognostic potential of PLIN1 in HCC.

Sample and clinical pathological data collection

Eighty paraffin-embedded HCC tissues and 80 non-tumour liver tissues were collected from the Department of Pathology, the First Affiliated Hospital of Guangxi

Medical University for use in immunohistochemical staining. Furthermore, 84 fresh HCC tissues specimens and 84 matched non-tumour liver tissues specimens were obtained from the sample bank of the First Affiliated Hospital of Guangxi Medical University. The associated clinicopathological characteristics, such as tissue type, sex, age, microsatellite focus, alpha-fetoprotein content (AFP), tumor thrombus, Edmenson grade, vascular invasion, tumor number, and extrahepatic metastasis, were also gathered from the medical records. All the subjects provided appropriate informed consent. The First Affiliated Hospital Ethics Committee of Guangxi Medical University approved the project.

Cell culture

Human HCC cell lines (SK and Huh7) were acquired from the Institute of Biological Sciences, Chinese Academy of Sciences (Shanghai). SK and Huh7 cells were cultivated in high-glucose Dulbecco's modified Eagle's medium (DMEM) (Gibco, USA, Cat. No. C11995500BT) supplemented with 10% fetal bovine serum (FBS) (Gibco, USA, Cat. No. 10099141C) and 1% penicillin–streptomycin (Solarbio, China, Cat. No. P1400-100) in a humidified incubator containing 5% CO2 at 37 °C, and the cells were subcultured when they reached approximately 80% confluence. As soon as the cells entered the exponential growth phase, experiments were carried out.

Immunohistochemical staining

Following surgical resection, tissue samples were preserved for 24 h in 10% neutral formalin, and then dehydrated and embedded in paraffin. The paraffin-embedded tissues were then cut into 4 μm sections for IHC staining. PLIN1 polyclonal antibody was purchased from Abcam (Cambridge, UK, Cat. No. ab213524). IHC staining was performed as described in previous studies [20, 21]. Under a microscope, 10 randomly chosen high-magnification fields were evaluated for staining intensity and percentage of positive cells. Based on the staining intensity, 0 indicates no staining, 1 indicates weak staining, 2 indicates moderate staining, and 3 indicates strong staining. According to the average percentage of positive cells, the scores were as follows: 0 (< 5%), 1 (5% ~ 25%), 2 (26% ~ 50%), 3 (51% ~ 75%), and 4 (76% ~ 100%). The total immunohistochemical staining score was calculated as follows: staining intensity * percentage of positive cells, ≥ 6 was considered positive, and < 6 was considered negative. Immunohistochemical results were independently determined by three senior pathologists.

RT–qPCR

An RNAeasy™ Plus Animal RNA Isolation Kit with Spin Column (Beyotime Biotechnology, China, Cat. No. R0027) was used to extract total RNA in accordance with the manufacturer's instructions. The RNA was reverse-transcribed following the instructions of the PrimeScript RT Master Mix (TaKaRa, Japan, Cat. No. RR036A). TB Green Premix Ex Taq II (Tli RNaseH Plus) (TaKaRa, Japan, Cat. No. RR820A) was utilised for PCR amplification (Thermo Fisher, USA). ACTB was used as the internal control. The relative expression of PLIN1 mRNA in each sample was calculated via the $2^{-\Delta\Delta CT}$ method. Primer Premier created the sequences of the specific primers utilized in this investigation, the sequences of which are listed below:

H- ACTB-F: 5'- CCTGGCACCCAGCACAAT-3'
H- ACTB-R: 5'- GGGCCGGACTCGTCATAC-3'
H-PLIN1-F: 5'-GCA GCA TTG AGA AGG TGG TGG AG-3'
H-PLIN1-R: 5'-ATC GAG AGA GGG TGT TGG TCA GAG-3'

Lentivirus transfection

Lentiviral vectors (LV-PLIN1(80904–1)) were purchased from Genechem Co.,Ltd. (Shanghai, China). HCC cells were inoculated in 6-well plates and pre-cultured for 24 h. Following the experimental guidelines, lentivirus was used to transfect SK and Huh7 cells when the cell confluence reached approximately 30%. The cells transfected with the PLIN1-overexpressing lentiviral vector were designated the experimental group (PLIN1-OV), while the cells transfected with the empty vector were designated the control group (EV). Puromycin was used to screen for transfected cells.

Transwell cell migration and invasion detection

HCC cells in the logarithmic growth phase, including cells stably transfected with PLIN1-overexpressing lentiviral vector and corresponding empty vector cells, were collected. To conduct the migration test, the 24-well plate (Corning, Cat. No. Costar 3422) was filled with 500 μl of 10% FBS-containing medium, and then the chamber was inserted into the plate. Next, each well was infected with 100 μl of serum-free cell suspension containing 3×10^4 cells. For the invasion assay, the transwell chamber was preincubated with Matrigel for 2 h. Next, 100 μl of serum-free cell suspension with a cell concentration of 4×10^4 was inoculated in each well, and 500 μl of 10% FBS-containing medium was poured into each well of 24-well plates in the lower chamber. Afterwards, the chamber and 24-well plates were incubated in a humidified incubator at 37 °C with 5% CO2 for 24 h. The following day, the chamber was removed, and any non-migrating or non-invading cells on the upper

surface were carefully removed with a cotton swab. The cells that were migrating or invading were fixed for 30 min with methanol and stained for 30 min at room temperature using 0.3% crystal violet; the staining time could be extended based on the staining of the cells. Five visual fields were randomly selected under the microscope to observe and count the number of invading and migrating cells.

Wound-healing assay

Each group of logarithmic growth HCC cells was seeded in 6-well plates at a density of 5×10^5/well for SK and 6×10^5/well for Huh7 cells. Once the cells reached 90% confluence, a sterile 200 μl pipette tip was used to make a scratch on the bottom of the plate, which was then replaced with fresh serum-free medium for culture. The ability to migrate was evaluated by examining the migration of cells to the scratch location, which was captured on a microscope at various times following the scratch. ImageJ software was used to determine the healing rate of each group.

Flow cytometric analysis of the cell cycle

The cell cycle distribution of each group was examined using a cell cycle staining kit (Multi Sciences, China, Cat. No. CCS012). For each group, 2×10^5–1×10^6 cells were collected, and 1 ml of PBS was added for resuspension. Then, the cells were gradually added to 3 ml of chilled 75% ethanol and fixed at -20 °C overnight. On the day of detection, the fixed cells were centrifuged, ethanol was added, and the tube wall was gently bounced to release the precipitate. After adding 5 ml of PBS, the cells were left for 15 min to hydrate again. After adding 1 ml of the DNA staining solution, the samples were vortexed for 5–10 s. The inoculum was kept in the dark at room temperature for 30 min. Using a flow cytometer, the test with the lowest loading rate was selected.

CCK-8 cell proliferation assay

A CCK-8 kit (meilunbio, China, Cat. No. MA0218) was used to assess the viability of the cells. HCC cells in the logarithmic growth phase were collected from each group and inoculated into 96-well plates with 100 μl of serum-free cell suspension containing 4×10^4 cells. A set of cell-free wells with only culture media was used as a blank group, with 4 replicates per group. The 96-well culture plate was cultivated in a constant temperature incubator with 5% CO2 at 37 °C until the cells attached to the wall. The wells were filled with 10 μl of CCK-8 solution and then incubated for 1 h at 37 °C in an incubator. The absorbance of each well was then measured using a Multiskan FC microplate spectrophotometer at 450 nm.

Functional enrichment analysis of DCGs

We used R software to screen for PLIN1 co-expressed genes using Pearson's correlation approach based on previously combined datasets from various platforms. The screening criteria were a correlation coefficient (r) > 0.7 and a P value < 0.05. Simultaneously, the "Limma" package of R was utilized to determine the differentially expressed genes (DEGs) in HCC. Genes with a |log2 (fold change) |> 1 and adjusted-P < 0.05 were recognized as DEGs of HCC. By intersecting the two genes, a set of overlapping DCGs can be obtained. The underlying functional pathways regulated by DCGs were identified using GO functional annotation and KEGG pathway analysis, which were carried out using the "clusterProfiler" package with a significance threshold set at a P value < 0.05.

Statistical analysis

Under appropriate conditions, the experiment was independently conducted 3 times. The data are displayed as the mean ± standard deviation (M ± SD). Student's t-test was used to compare the means between two groups, and one-way analysis of variance was used to compare the means between multiple groups. The Kaplan–Meier method was used for survival analysis, and the log-rank test was used for verification. The ROC curve was generated to evaluate the utility of PLIN1 as a biomarker for HCC, and the area under the curve (AUC) was utilized to evaluate its accuracy. SPSS 22.0, Stata Version 12.0, and R Version 4.1.1 were used for all statistical analyses, and GraphPad Prism version 8.0 was used for plotting. All statistical P values were based on two-tailed statistical tests, and a P value < 0.05 indicated statistical significance.

Results

Analysis of PLIN1 expression in HCC based on public databases

After screening, 45 expression profiling microarrays related to PLIN1 in HCC were included (Supplementary Table 1), including an expression matrix after merging TCGA-GTEx and 44 expression matrices from the GEO database, which were obtained from the Affymetrix platform, Agilent platform, Arraystar platform, HiSeq X Ten platform, Illumina platform, and Gene Chip platform. From the above expression matrix, we obtained 1623 HCC and 1105 non-HCC tissue samples. Next, we categorized the expression of PLIN1 in each platform and created a scatter diagram to more clearly illustrate the differences in PLIN1 expression between HCC tissue and

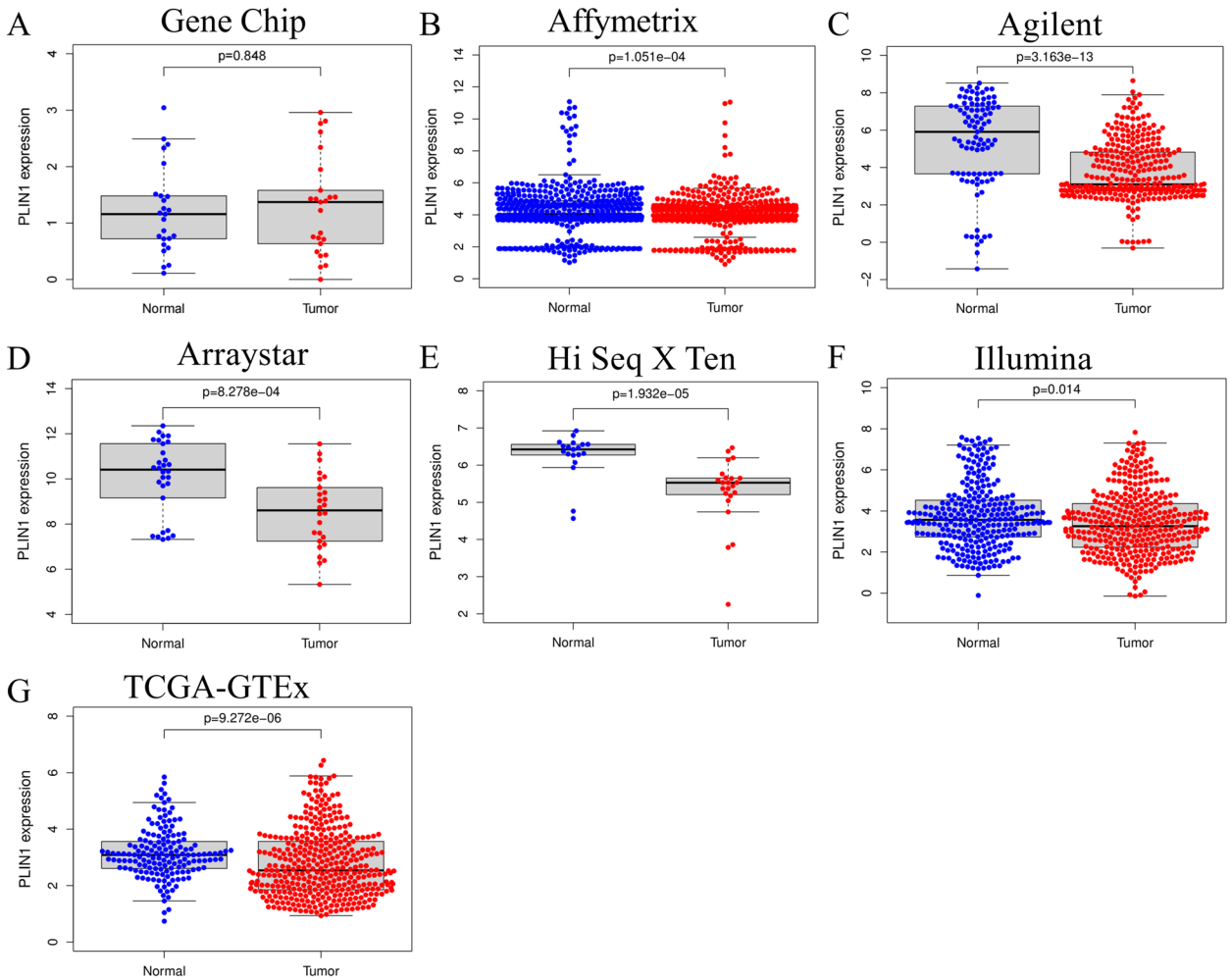

Fig. 1 Expression of PLIN1 on different platforms. **A** Gene Chip platform; **B** Affymetrix platform; **C** Agilent platform; **D** ArrayStar platform; **E** HiSeq X Ten platform; **F** Illumina platform; **G** TCGA-GTEx platform

non-HCC liver tissue (Fig. 1). It was confirmed that six platforms—Affymetrix, Agilent, Arraystar, HiSeq X Ten, Illumina, and TCGA-GTEx—had significantly decreased PLIN1 in HCC ($P < 0.05$).

For a further comprehensive analysis of PLIN1 levels and its potential clinical significance in HCC, a meta-analysis was performed. The results also suggested that PLIN1 was downregulated in HCC tissues (SMD = -0.46 [95% CI: -0.70, -0.22], Fig. 2A), and the area under the SROC was 0.75 [95% CI: 0.71–0.79] (Fig. 2B). The pooled sensitivity and specificity were 0.72 [95% CI: 0.61–0.80] and 0.67 [95% CI: 0.54–0.78], respectively (Fig. 2C, D), indicating that PLIN1 had a certain ability to distinguish between HCC and non-HCC tissues. Linear regression test ($P = 0.261$) and the symmetrical distribution of small circles in the Deeks funnel plot showed that the expression of PLIN1 had no publication bias in the meta-analysis (Fig. 3A).

Analysis of PLIN1 expression in HCC based on internal tissue samples and its relationship with clinicopathological features

To confirm the expression of PLIN1 in HCC. Using IHC staining, we first measured the protein expression of PLIN1. PLIN1 protein was expressed in the cytoplasm. Compared with that in non-tumour liver tissues, PLIN1 expression was significantly lower in HCC tissues (Fig. 4A, B). The downregulation of PLIN1 protein expression in HCC tissues was further validated by immunohistochemical assays from the HPA database (https://www.proteinatlas.org/), which revealed cytoplasmic staining (Fig. 4C, D).

At the mRNA level, PLIN1 expression in 84 pairs of HCC and non-tumour liver tissues was assessed using RT–qPCR. We discovered that PLIN1 mRNA levels were lower in HCC tissues than in non-tumour liver tissues, and the significant ability in distinguishing HCC

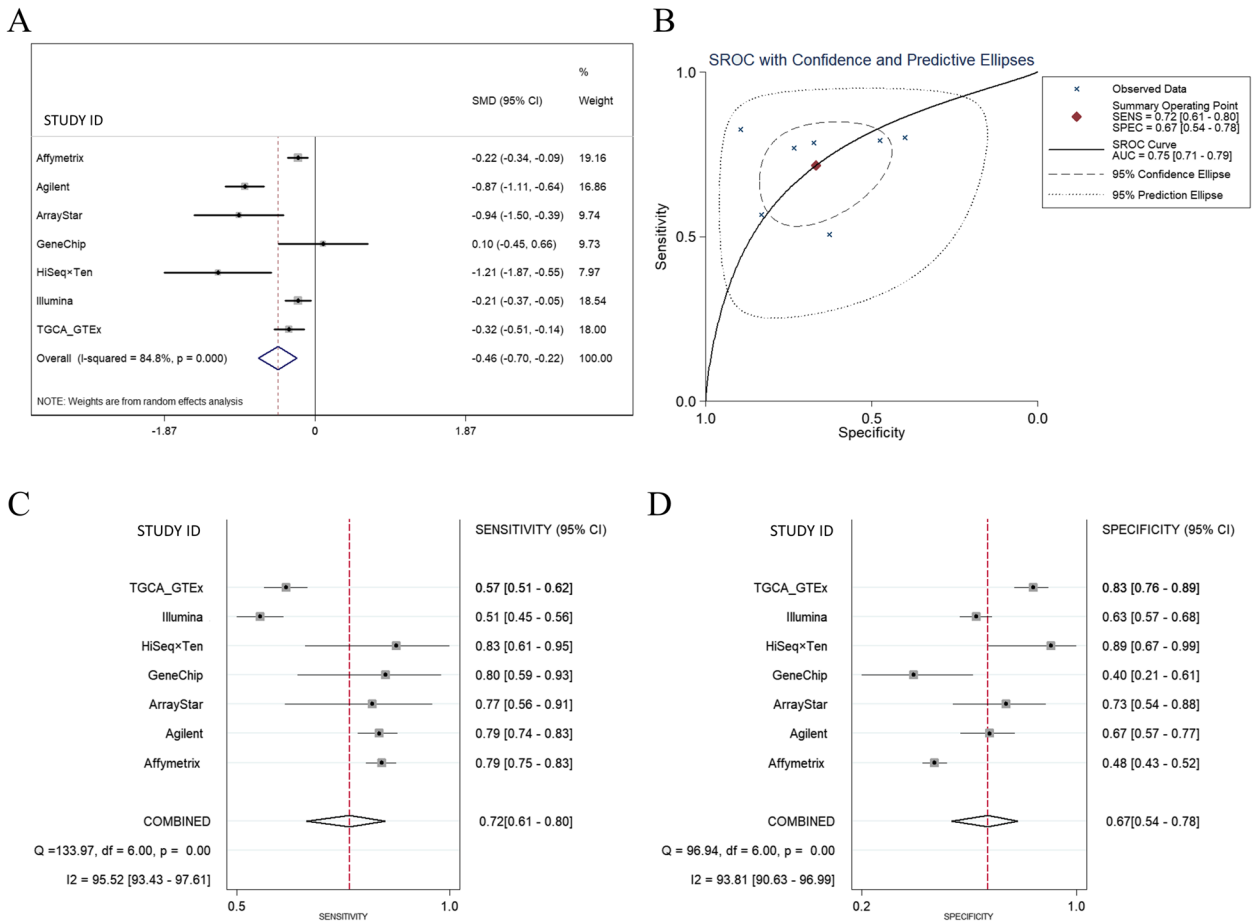

Fig. 2 Expression and clinical potential of PLIN1 based on public databases. **A** Forest plot for evaluating PLIN1 expression on different platforms. **B** SROC curve demonstrating the performance of PLIN1 in diagnosing HCC. **C** Pooled sensitivity analysis of PLIN1. **D** Pooled specificity analysis of PLIN1

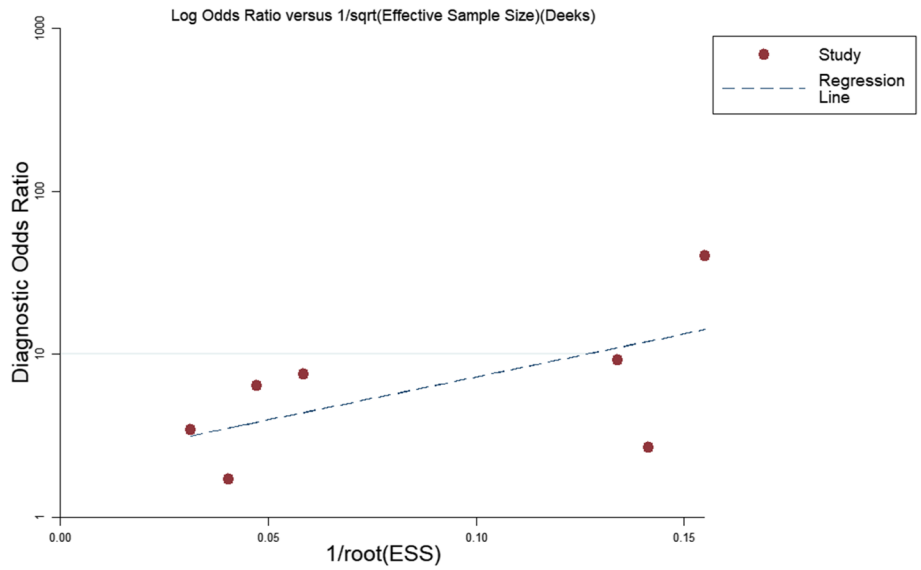

Fig. 3 Publication bias tested in this study using a Deek funnel plot

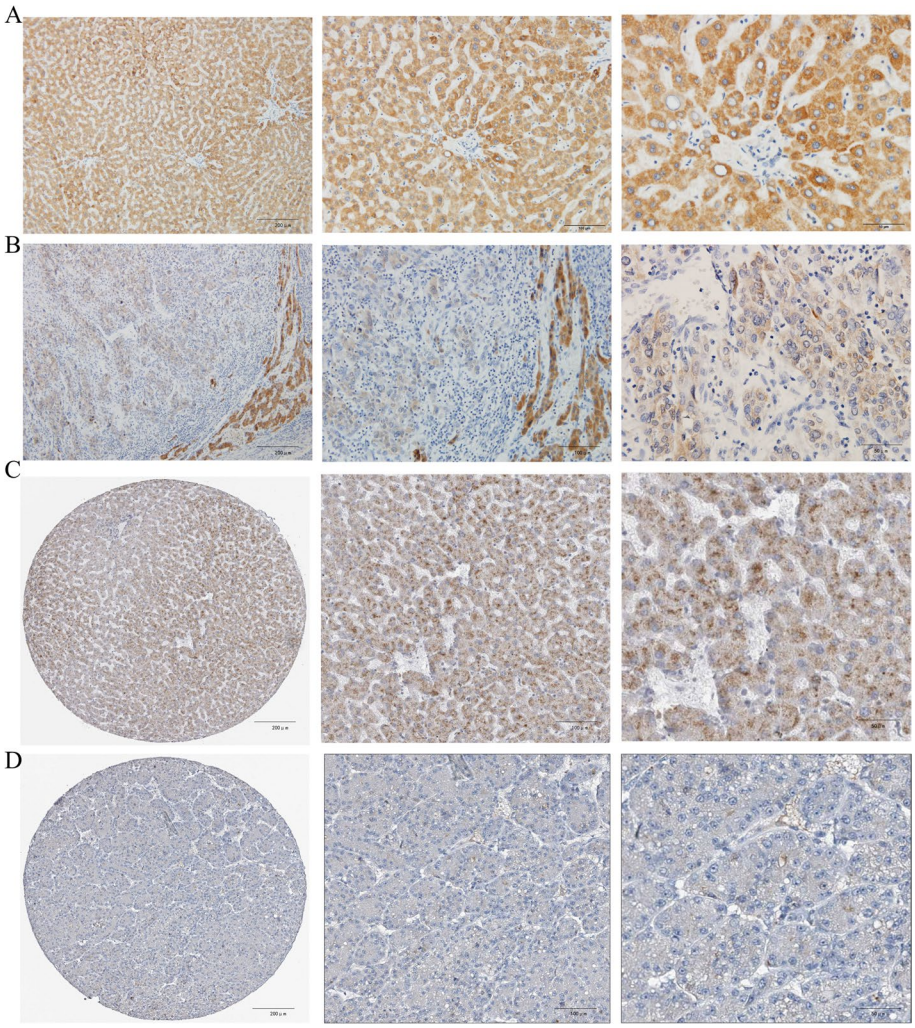

Fig. 4 PLIN1 protein expression in HCC tissues and adjacent non-HCC tissues (magnification: × 100, × 200, × 400). **A** In-house IHC analysis of PLIN1 expression in non-HCC tissues. **B** In-house IHC analysis of PLIN1 expression in HCC tissues. **C** PLIN1 expression in non-HCC tissues from the HPA database. **D** PLIN1 expression in HCC tissues from the HPA database. Note: From left to right, the scales in Figures **A** to **D** are 200 μm, 100 μm, and 50 μm, respectively. The brown staining is positive for cytoplasm staining. The purple staining indicates nuclear staining

tissues from non-tumour liver tissues ($P < 0.05$, Fig. 5A). Additionally, PLIN1 mRNA levels were also lower in the Huh7 and SK cell lines than in the L-02 cell line ($P < 0.05$, Fig. 5B). The decrease in the PLIN1 mRNA level was validated to be consistent with the PLIN1 protein level in HCC. Moreover, we analyzed the relationship between PLIN1 mRNA levels and the clinicopathological characteristics of 84 HCC patients. PLIN1 was found to be associated with AFP levels and extrahepatic metastasis. Patients with high AFP levels and extrahepatic metastasis had significantly lower PLIN1 expression ($P < 0.05$, Table 1 and Fig. 5C, D), which indicates that PLIN1 plays an essential role in HCC progression. Kaplan–Meier survival analysis revealed that HCC patients with lower PLIN1 levels were associated with worse overall survival outcomes ($P = 0.039$, Fig. 5E), which was validated by computational biology methods based on the GEPIA2 database ($P = 0.038$, Fig. 5F). The above results revealed that PLIN1 is markedly downregulated in HCC, and patients with lower PLIN1 expression had a poorer prognosis.

Construction of a lentivirus stable transmutation strain

To investigate the biological functions of PLIN1 in HCC cells, we transfected PLIN1-overexpressing lentiviral vector and empty vector into SK and Huh7 cells, which have relatively low PLIN1 expression (Fig. 5B). Under the fluorescent microscope, HCC cells transfected with lentivirus showed green fluorescence, indicating that the transfection was successful (Fig. 6A, B). PLIN1 overexpression

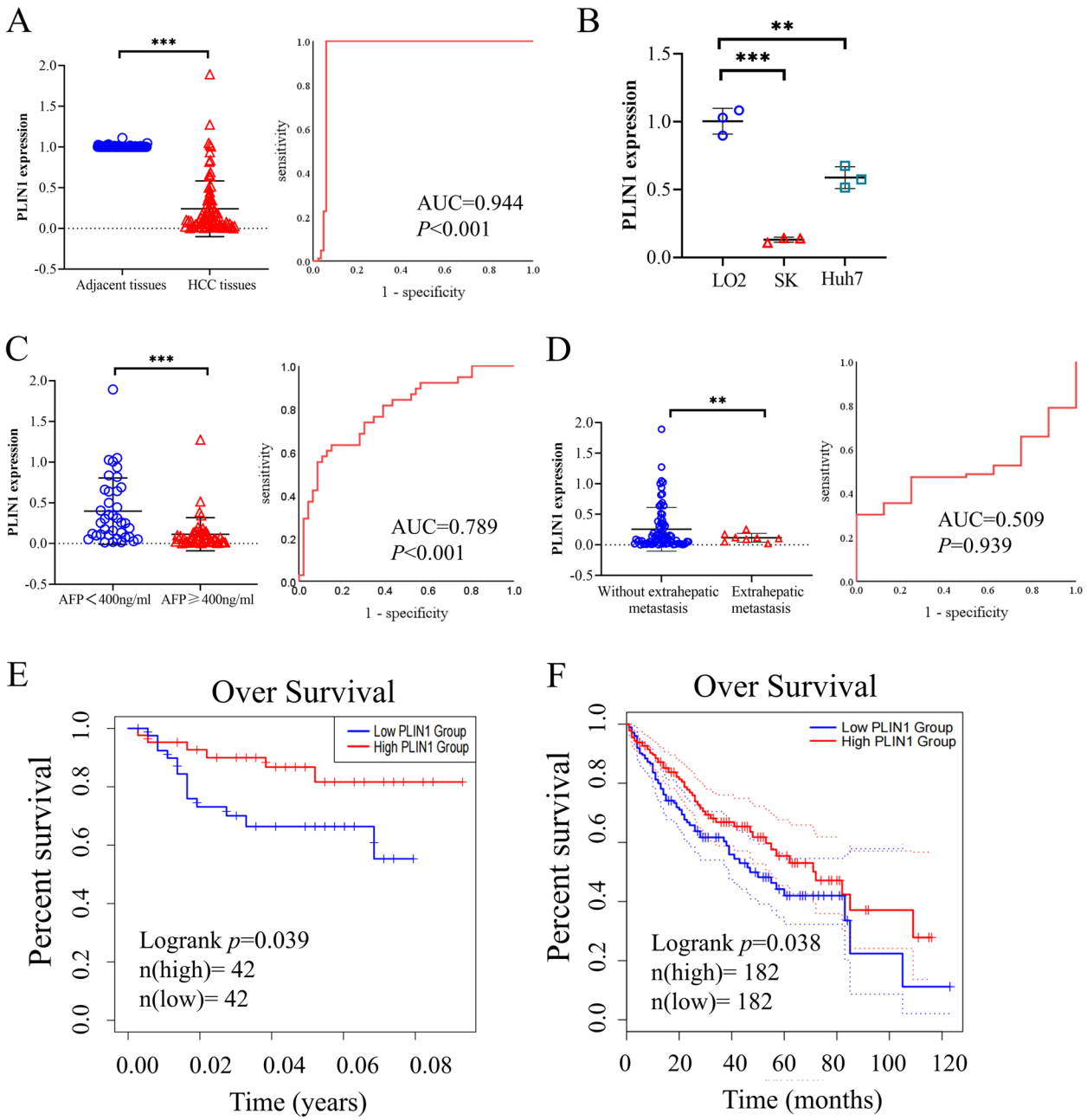

Fig. 5 Expression and clinical significance of PLIN1 mRNA in HCC (** $P < 0.01$, *** $P < 0.001$). **A** Expression of PLIN1 in different organizational types and ROC curve. **B** Expression of PLIN1 mRNA in cell lines. **C** Expression of PLIN1 in AFP levels and ROC curve. **D** Expression of PLIN1 in patients with extrahepatic metastasis and ROC curve. **E** Kaplan–Meier curve of PLIN1 in HCC based on in-house RT-qPCR results. **F** Kaplan–Meier curve of PLIN1 in HCC based on GEPIA2 database

efficiency was verified using RT-qPCR. The results showed that PLIN1 mRNA levels in PLIN1 overexpressing SK cells ($P < 0.001$) and PLIN1 overexpressing Huh7 cells ($P < 0.001$) were significantly higher than those in the empty vector group (Fig. 6C, D). As can be observed, PLIN1 overexpressing lentivirus-transformed cell lines were successfully established in SK and Huh7 cells.

Overexpression of PLIN1 inhibited HCC cell proliferation

Following the overexpression of PLIN1 in HCC cells, the proliferation of SK and Huh7 cells was dramatically

Table 1 Correlations between PLIN1 mRNA levels and clinicopathological features in HCC patients based on RT–qPCR

Clinicopathological feature		Case no	PLIN1 expression t-test of data		
			Mean ± SD	t	*P*-value
Tissues	**HCC**	84	0.241 ± 0.342	-20.457	< 0.0001
	Non-HCC	84	1.006 ± 0.014		
Gender	**Male**	72	0.217 ± 0.291	1.618	0.109
	Female	12	0.877 ± 0.557		
Age	**< 60**	69	0.234 ± 0.350	-0.396	0.693
	≥ 60	15	0.273 ± 0.310		
Hypersplenism	**Yes**	4	0.097 ± 0.170	0.857	0.393
	No	80	0.248 ± 0.347		
AFP (ng/ml)	**≥ 400**	38	0.113 ± 0.204	-3.909	< 0.0001
	< 400	46	0.397 ± 0.408		
HBV-DNA (IU/ml)	**≥ 500**	59	0.250 ± 0.300	-0.353	0.725
	< 500	25	0.221 ± 0.430		
Tumor size (cm)	**≥ 5**	54	0.247 ± 0.287	-0.221	0.825
	< 5	30	0.230 ± 0.429		
Tumor nodule	**Single**	59	0.237 ± 0.305	-0.175	0.861
	Several	25	0.251 ± 0.424		
Microvascular cancer embolus	**Yes**	42	0.288 ± 0.383	-1.269	0.208
	No	42	0.194 ± 0.292		
Macrovascular invasion	**Yes**	30	0.34 ± 0.24	-0.142	0.887
	No	54	0.37 ± 0.34		
Extrahepatic metastasis	**Yes**	8	0.116 ± 0.072	2.878	0.006
	No	76	0.254 ± 0.357		
Microsatellite loci	**Yes**	12	0.277 ± 0.526	-0.394	0.694
	No	72	0.235 ± 0.306		
Edmenson grade	**1–2**	57	0.259 ± 0.372	0.686	0.494
	3–4	27	0.204 ± 0.272		
BCLC stage	**0-A**	39	0.280 ± 0.426	0.942	0.350
	B-C	45	0.207 ± 0.248		

suppressed (Fig. 7A, B). The distribution of the cell cycle in each group was determined using flow cytometry. The proportion of G0/G1 phase SK and Huh-7 cells in the PLIN1 overexpression group dramatically increased compared to that in the empty vector group; however, the number of S phase cells significantly decreased (Fig. 8A, B), suggesting that PLIN1 overexpression can block the cell cycle of SK and Huh7 cells in the G0/G1 phase.

Overexpression of PLIN1 suppressed HCC cell invasion and migration

Wound healing assays were performed to examine the migration capacity of HCC cells in each group. Compared with that in the empty vector group, the lateral transfer ability of SK and Huh7 cells in the PLIN1 overexpression group was noticeably weakened (Fig. 9A, B). We also performed a transwell assay to further determine the effect of PLIN1 on the migration ability of HCC cells. The findings showed that the vertical migration ability of HCC cells decreased significantly with the overexpression of PLIN1 (Fig. 10A). The results of the invasion experiment demonstrated that the capacity of HCC cells to invade was decreased (Fig. 10B). In summary, the aforementioned studies demonstrated that PLIN1 overexpression can impair the migration and invasion of SK and Huh7 cells.

Recognition of PLIN1-related functional pathways and molecular mechanisms in HCC

From 7 different platforms, 2,544 DEGs (1,256 upregulated and 1,288 downregulated genes) and 18,478 PLIN1 co-expressed genes were identified. A total of 1438 DCGs were produced by crossing these two genes. We discovered that PLIN1-related DCGs are involved in various functional pathways related to fatty acid metabolism. As shown in Fig. 11A-C and Supplementary Table 2, the three most significantly enriched GO cellular component (CC) iterms were collagen-containing extracellular

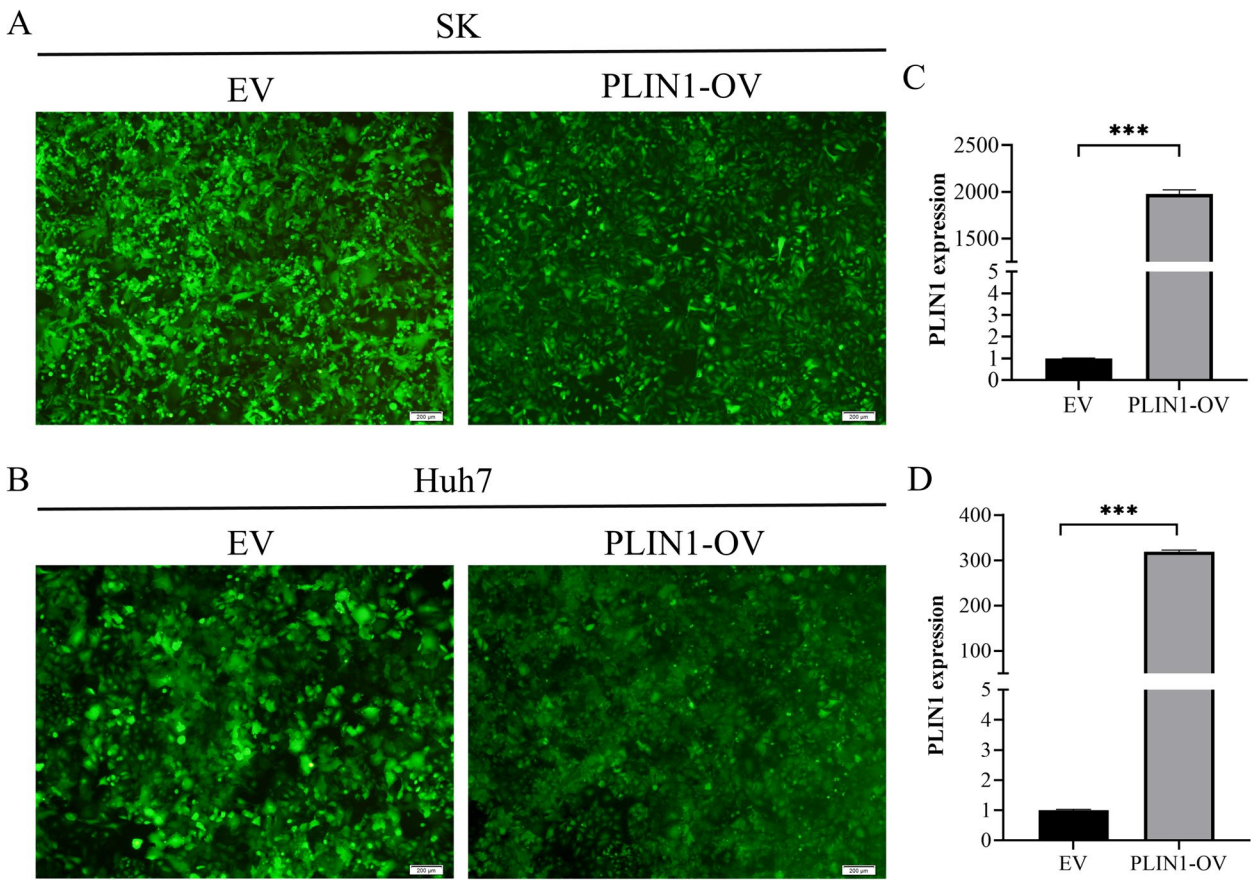

Fig. 6 Fluorescence images of PLIN1 overexpression lentiviruses in HCC cell lines (magnification: × 100; ***$P < 0.001$). **A** Fluorescence images of SK cells. **B** Fluorescence images of Huh7 cells. **C** PLIN1 mRNA levels increased in PLIN1 overexpressing SK cells. **D** PLIN1 mRNA levels increased in PLIN1 overexpressing Huh7 cells

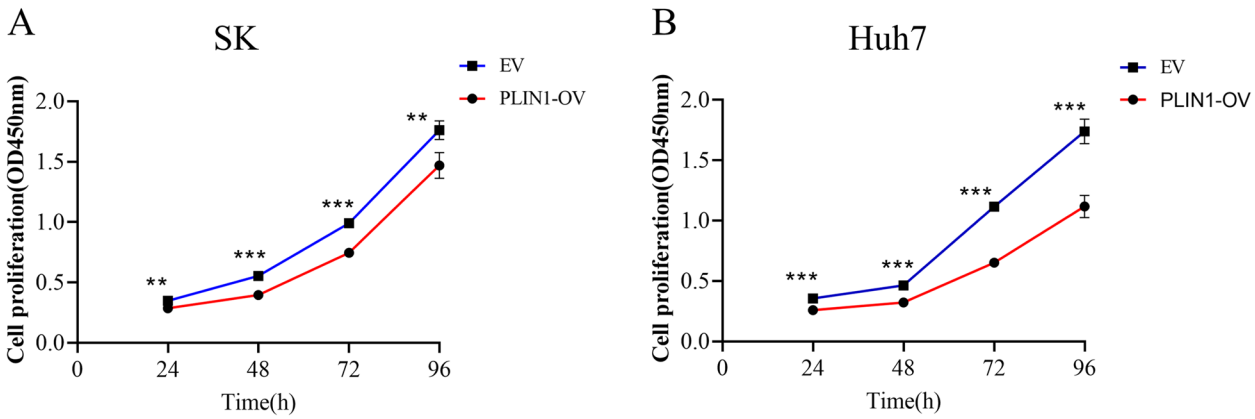

Fig. 7 Effect of PLIN1 overexpression on the proliferation of HCC cells (**$P < 0.01$, ***$P < 0.001$). **A** Effects of PLIN1 overexpression on the proliferation of SK cells. **B** Effects of PLIN1 overexpression on the proliferation of Huh7 cells

matrix, cytoplasmic vesicle lumen, and vesicle lumen; The three most significantly enriched GO biological process (BP) iterms were organic acid catabolic process, carboxylic acid catabolic process, and carboxylic acid biosynthetic process. The three most significantly enriched GO molecular function (MF) iterms were monooxygenase activity, steroid hydroxylase activity, and heme binding. KEGG analysis revealed that

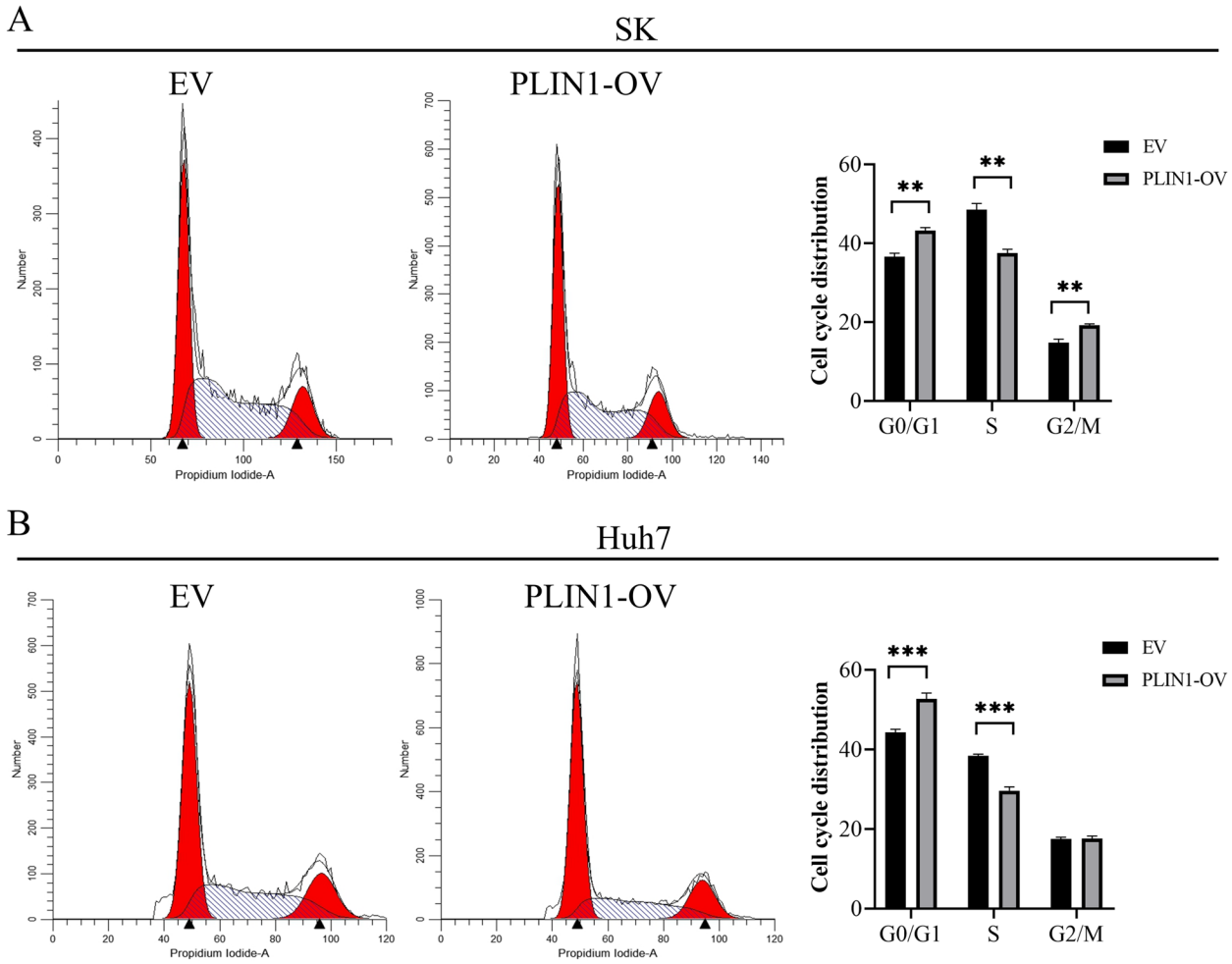

Fig. 8 Effect of forced PLIN1 expression on the cell cycle distribution of HCC cells (**$P < 0.01$, ***$P < 0.001$). **A** Effects of PLIN1 overexpression on the cell cycle in SK cells and histogram of the cell cycle distribution ratio in different periods. **B** Effects of PLIN1 overexpression on the cell cycle in Huh7 cells and histogram of the cell cycle distribution ratio in different periods

PLIN1-related DCGs were related mainly to Valine, leucine and isoleucine degradation, Fatty acid degradation, and Cell cycle (Fig. 11D and Supplementary Table 3).

Discussion

More than half of all new cases of liver cancer worldwide are reported in China each year, which poses a major risk to people's lives and health [22]. Owing to the unique position of the liver, HCC is highly invasive, metastatic, and exceedingly heterogeneous, and only 14% of patients with advanced HCC survive for five years [23]. Consequently, an in-depth study of the mechanisms underlying the development and progression of HCC may aid in the search for novel therapeutic and diagnostic approaches that will improve the overall prognosis of HCC patients.

In this study, we used comprehensive integration and analysis of public microarray profiles to elucidate PLIN1 expression and its clinical significance in HCC. PLIN1 was weakly expressed in HCC tissues, and patients who had low PLIN1 levels had shorter overall survival times. This result was also validated by in-house tissue samples. We used in-house immunohistochemistry and RT–qPCR to detect PLIN1 expression and discovered that PLIN1 was considerably downregulated in HCC tissues and cell lines. Moreover, PLIN1 expression may be able to predict the prognosis of HCC patients by combining serum AFP levels and extrahepatic metastasis, according to the results of the correlation analysis between PLIN1 levels and clinicopathological parameters. Furthermore, Kaplan–Meier analysis revealed that low PLIN1 expression is associated with poor prognosis, suggesting that PLIN1 may be a useful prognostic biomarker for HCC. Consistent with our study, a study using immunohistochemistry and electron microscopy to analyse the expression of lipid droplet-associated proteins of the PAT family in HCC and para-cancerous tissues showed that PLIN1

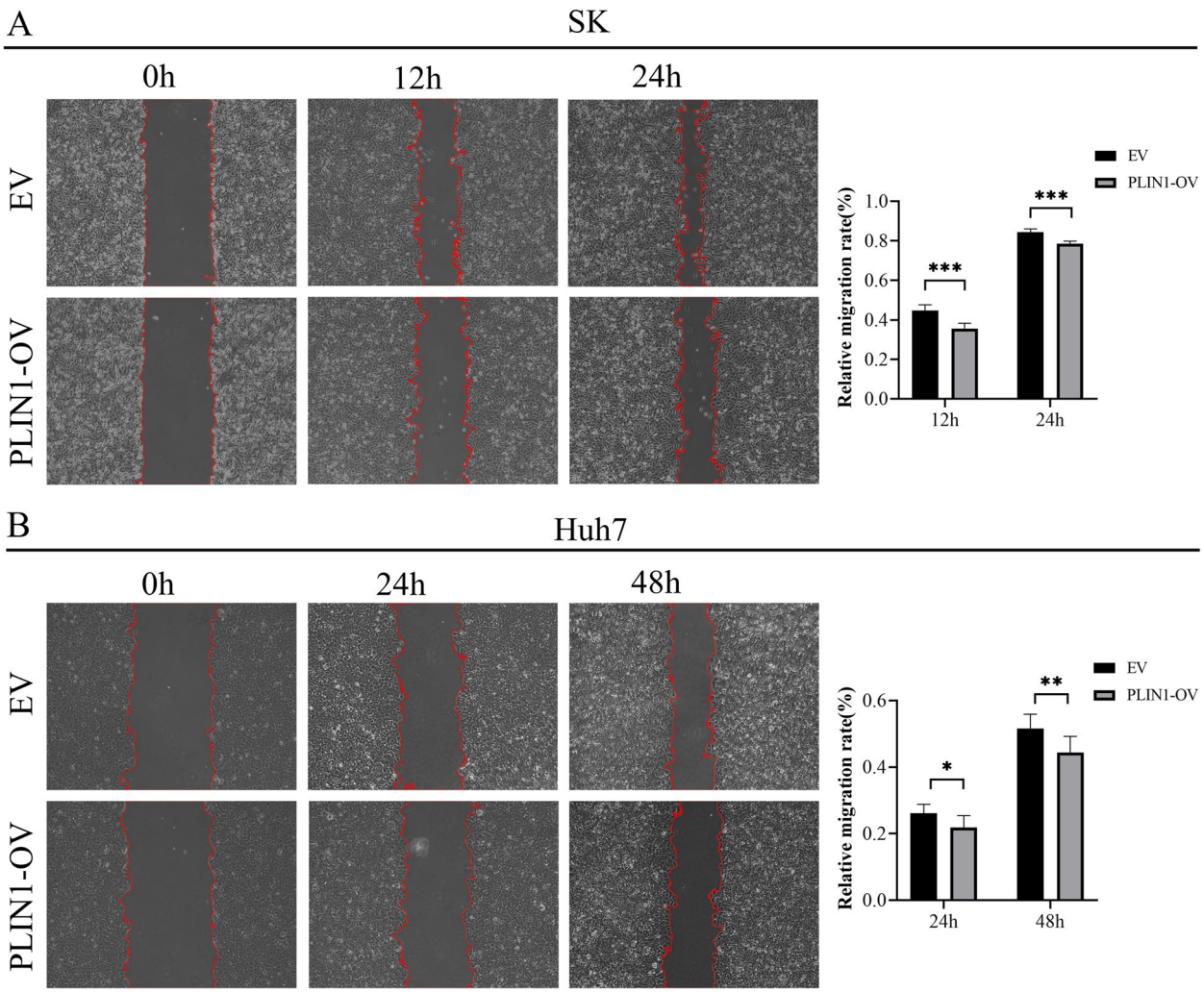

Fig. 9 Effect of forced PLIN1 expression on the migration of HCC cells (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). **A** Effects of PLIN1 overexpression on the migration of SK cells and histogram of the migration rate at different time points. **B** Effects of PLIN1 overexpression on the migration of Huh7 cells and histogram of the migration rate at different time points

expression was absent in HCC tissues [24]. Apart from PLIN1, other members of the perilipin family, including PLIN2, PLIN3, PLIN4, and PLIN5, are also present on the lipid droplet surface and function as "molecular switches" to maintain lipid homeostasis. They have been investigated in several human cancers, such as HCC [25], colorectal cancer [26], pancreatic ductal adenocarcinoma [27], gliomas [28], and prostate cancer [29]. There are few reports on the function of PLIN1 in cancer.

Our findings showed that PLIN1 was considerably down-expressed in HCC tissues and was associated with increased AFP levels, extrahepatic metastasis following surgery, and a worse prognosis for survival. As a result, it was postulated that PLIN1 might contribute to the emergence and progression of HCC in an oncogenic manner. Therefore, we investigated how PLIN1 affects the biological activity of HCC cells. Overexpression of PLIN1 in HCC cell lines suppressed HCC cell proliferation and reduced migration and invasion. Moreover, HCC cells in the G0/G1 phase were blocked. It is postulated that PLIN1-mediated lipid metabolism is pivotal for the development of HCC in conjunction with the function of PLIN1 in lipid droplet synthesis. This is essentially a disease of cellular growth and proliferation, requiring proteins, lipids, and nucleic acids—building blocks of cells. Consequently, we carried out a preliminary analysis of the potential mechanisms by which PLIN1 controls several biological behaviors, including the invasion, migration, and proliferation of HCC cells, in a follow-up study. The involvement of PLIN1 in HCC is not autonomous

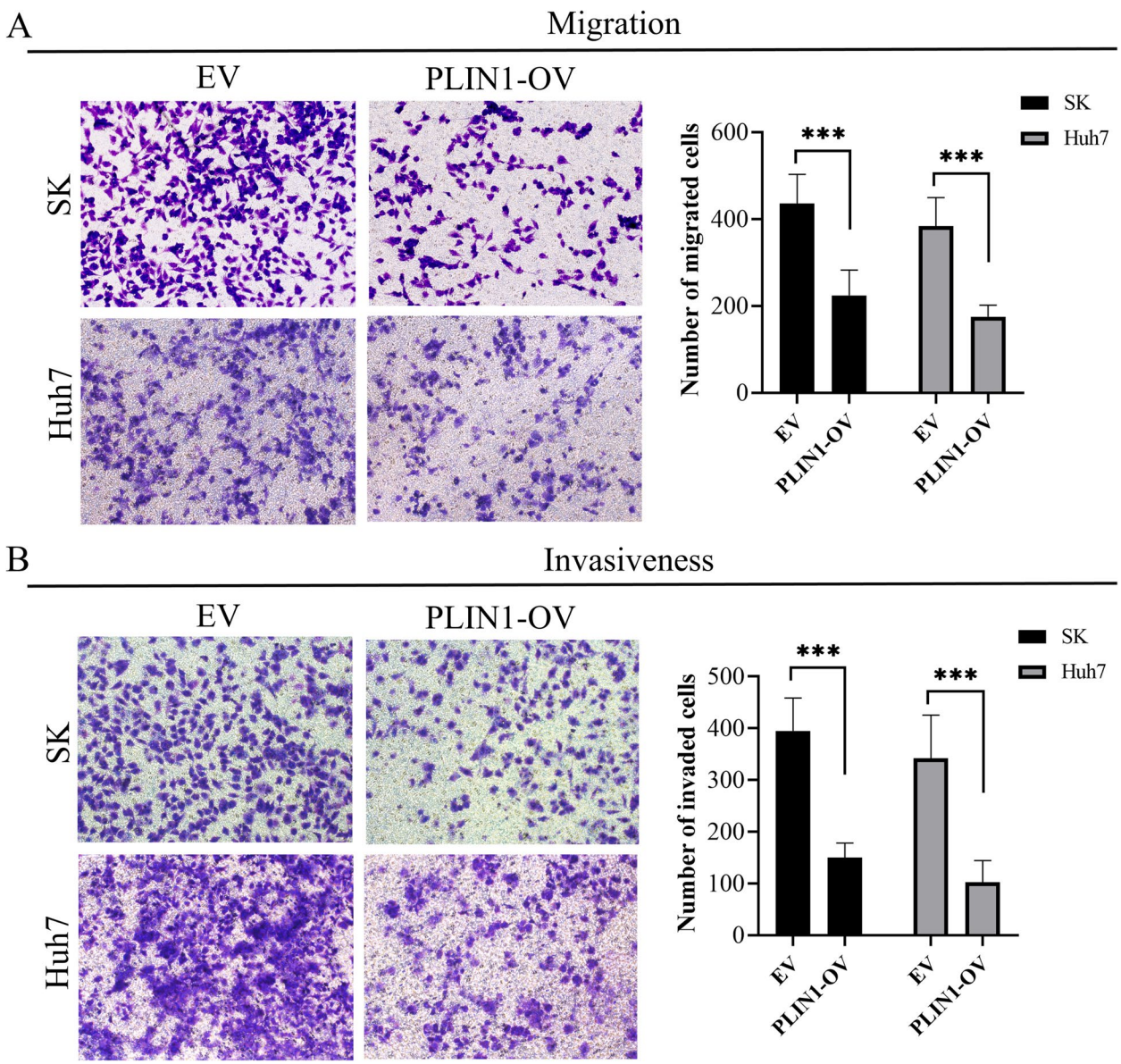

Fig. 10 Migration and invasion abilities of HCC cells in different groups (***$P < 0.001$). **A** Migration results of SK and Huh7 cells and histogram of the migration ability. **B** Invasiveness results of SK and Huh7 cells and histogram of the invasion ability

but rather contributes to disease onset and progression through interactions with several proteins and molecules that influence various signalling pathways [30]. Therefore, we first obtained PLIN1-related DCGs and enriched these genes for analysis.

According to the GO functional annotation, PLIN1-related DCGs are involved in a variety of biological processes that are connected to the conversion of energy, including the organic acid catabolic process, carboxylic acid catabolic process, and carboxylic acid biosynthetic process. Research has indicated that metabolites of organic acids, including carboxylic acid, pyruvate, glutamine, and fatty acids, are crucial for controlling the proliferation, invasion, and metastasis of tumors [31]. According to research by Jessalyn M. Ubellacker et al. [32], the lymphatic system promotes the survival and metastatic ability of melanoma cells by supplying them with higher levels of oleic acid. According to the CC article, DCGs may play a role in the development of HCC by regulating the composition of the extracellular matrix, including the collagen-containing extracellular matrix, cytoplasmic vesicle lumen, and secretory granule lumen. Accumulating evidence indicates that tumor cells accelerate the development of metastasis by secreting

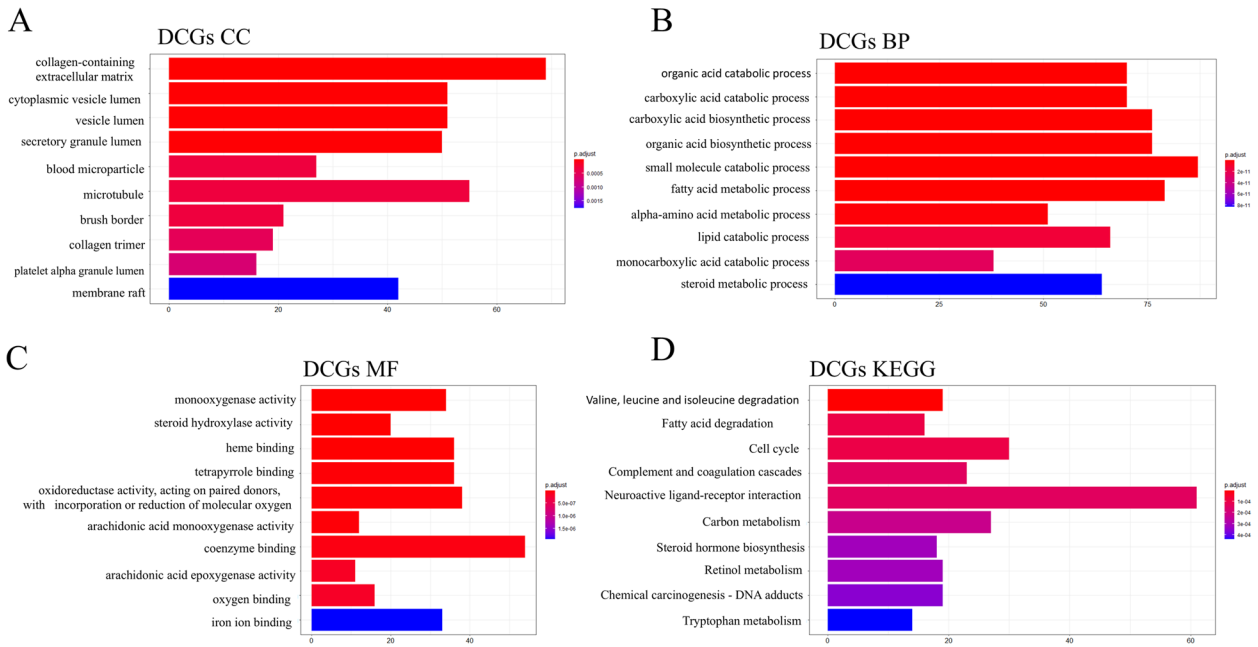

Fig. 11 Bioinformatics analysis of the potential mechanism of PLIN1 in HCC. **A** GO CC analysis. **B** GO BP analysis. **C** GO MF analysis. **D** KEGG pathway analysis

specific factors and extracellular vesicles, which enable the formation of an environment suitable for the survival of tumor cells in distant organs [33–35]. By interfering with this process, these DCGs prevent the progression of HCC. Additionally, the activity of a number of enzymes, including monooxygenase, steroid hydroxylase, oxidoreductase, and arachidonic acid monooxygenase, can be regulated by certain DCGs. These enzymes have been shown to mediate the occurrence and progression of certain malignant tumors, including HCC, through the regulation of lipid metabolism [36, 37].In conclusion, the relevant enzyme activities, aberrant gene expression, and an increase or reduction in metabolites may affect the biological behavior of HCC cells by regulating lipid synthesis and catabolism. According to our findings, PLIN1-related DCGs have a wide range of biological activities that are intimately connected to the metabolism of lipids and organic acids.

KEGG pathway analysis confirmed that PLIN1-related DCGs are primarily enriched in metabolism-related signalling pathways. Cancer is characterized mainly by dysregulated metabolic activities [38]. To date, the "Warburg effect" and glutamine metabolism have been the most extensively researched metabolic anomalies in cancer cells. The aberrant metabolism of fatty acids in tumor cells has steadily gained attention in the last several years. The augmentation of fatty acid de novo synthesis and associated lipid synthesis, which produces the intermediates needed for tumor cell growth, is the primary manifestation of abnormal lipid metabolism in tumor cells. On the other hand, inverse modifications occur in the lipid metabolism of tumor hosts, characterized by increased catabolism of adipose tissue and decreased consumption of exogenous lipids [39, 40].

PLIN1, a hyperphosphorylated protein localized on the surface of lipid droplets, is phosphorylated in a manner that both depends on and does not need hormone-sensitive lipase to increase hormone-sensitive lipase-mediated lipolysis [41]. On the one hand, adipocyte lipolysis yields fatty acids, which serve as building blocks for the creation of phosphoglycerides and sphingomyelin to maintain cell membrane stability in tumor cells. On the other hand, tumor cells cause adipocytes to undergo lipolysis to produce energy sources for their unrestricted proliferation. These alterations are strongly linked to the activation of metabolic enzyme activities and lipid metabolism-related pathways, and the aberrant expression of lipid metabolism-related genes is one of the main variables involved in the regulation of enzyme activities and pathway activation. Thus, lipid metabolism-related genes are potential targets for anticancer drug therapy in tumors.

In HCC, metabolic stress induced by glucose deficiency and a hypoxic microenvironment result in a series of adaptive metabolic alterations. Changes in lipid metabolism are among the key metabolic pathways that are essential for the growth and survival of HCC cells [42]. In breast cancer, fine tumor activity prompts enhanced lipolysis and a large-scale release of fatty acids from

adipocytes around the tumor. Breast cancer cells absorb these fatty acids, which causes tumor cells to undergo significant metabolic remodelling and become more invasive [43]. Similarly, Zhou et al. [44] proposed that PLIN1 overexpression plays a tumor suppressor role in breast cancer progression by mediating lipid metabolism, which influences the cyclic distribution of breast cancer cells, promotes apoptosis, and inhibits their proliferative and migratory abilities. Cui et al. [45] demonstrated that the expression of miR-205, which is upregulated in glucose-deficient environments, could accelerate the catabolism of triglycerides in lipid droplets and promote the fatty acid oxidation process to supply energy for HCC by downregulating the expression of ACSL. Yamashita et al. [46] showed that the activation of the adipogenic pathway caused by SREBF-1 overexpression was correlated with cellular proliferation and poor prognosis in HCC patients. The aforementioned research showed how essential lipids are for the essential functions of tumor cells, and the changes in lipid metabolism are linked to the growth, survival, and invasion of HCC. Remarkably, we discovered that a few DCGs are also involved in cell cycle signalling pathways. Cyclin and its partner CDKs are key combinations involved in regulating the cell cycle at different stages, and their aberrant expression has been proven to mediate tumorigenesis and progression [47]. Targeting cell cycle pathways has emerged as an emerging avenue for cancer therapy [48]. The activation of different cell cycle protein-CDK complexes at particular stages of the cell cycle and the phosphorylation of their target proteins in turn accelerate cell cycle progression [49]. Thus, in combination with the role of PLIN1 in lipid and energy homeostasis, our researches imply that PLIN1-related DCGs may fundamentally block pathways that provide material and energy sources for HCC cell cycle progression, as well as proliferation and metastasis, by synergistically regulating the aforementioned metabolism-related signalling pathways, thereby exerting an anti-HCC effect.

The current study has several limitations. Firstly, PLIN1 expression was found to be associated with extrahepatic metastasis in HCC patients. However, the number of patients with metastases was significantly lower compared to the number of patients without metastasis, and this discrepancy may affect the statistical analysis. In the future, collecting more tissue specimens for validation will help to reduce the impact of the difference in numbers between groups. Second, there is a lack of experiments related to the effect of PLIN1 on the biological behaviour of HCC cells in vivo. Third, in-depth studies on the relationship between PLIN1 and lipid metabolism-related pathways have not yet been conducted. Further studies are needed to validate the findings.

Conclusion

PLIN1 is lowly expressed in HCC tissues, and this study provides the first evidence of the potential clinical utility of PLIN1 in predicting overall survival and postoperative metastatic risk in HCC patients. PLIN1 prevents HCC cells from migrating and invading by modulating metabolism-related signalling pathways and obstructing cell cycle progression. These findings provide new avenues for tumor prognostic assessment and clinical treatment.

Abbreviations

AFP	Alpha-fetoprotein
AUC	Area under the curve
BCLC	Barcelona clinic liver cancer
BP	Biological progress
CC	Cellular component
CCK8	Cell counting kit-8
DCGs	Differently co-expressed genes
DEGs	Differently expressed genes
GEO	Gene expression omnibus
GO	Gene ontology
GTEx	Genotype-tissue expression
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HCC	Hepatocellular carcinoma
HR	Hazard ratio
IHC	Immunohistochemistry
KEGG	Kyoto encyclopedia of genes and genomes
MF	Molecular function
OS	Overall survival
PLIN1	Perilipin1
PLINs	Perilipins
RT-qPCR	Real-time quantitative reverse transcription polymerase chain reaction
SMD	Standardized mean difference
SROC	Summarized receiver operating characteristics

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12885-024-12842-1.

Additional file 1: Supplementary Table 1. The information of 45 datasets included in this study.

Additional file 2: Supplementary Table 2. Top 10 GO functional annotations involved by genes differentially co-expressed with PLIN1 in HCC.

Additional file 3: Supplementary Table 3. Top 10 KEGG pathways involved by genes differentially co-expressed with PLIN1 in HCC.

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Not applicable.

Authors' contributions

Jiang-hua Huang, Yan Wei, and Zhen Fang contributed equally to this work. Jiang-hua Huang and Yan Wei: research design, data collection, investigation, and writing–original draft. Zhen Fang: research design, data analysis, and writing–revise and edit. Cong Yu: data collection and data analysis. Rui Zhang: data analysis. Zhen-Bo Feng: supervision, validation, and funding acquisition; Li-Ping Zeng: methodology, supervision, and funding acquisition. All authors prepared the manuscript.

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Availability of data and materials

The data that support the findings of this study are available from the authors but restrictions apply to the availability of these data, which were used under license from the First Affiliated Hospital of Guangxi Medical University (China) for the current study, and so are not publicly available. Data are, however, available from the authors upon reasonable request and with permission from the Department of Pathology at First Affiliated Hospital of Guangxi Medical University.

Declarations

Ethics approval and consent to participate

This study was approved by the ethics committee of the First Affiliated Hospital of Guangxi Medical University (number 2018-KY-E-099). All the subjects provided appropriate informed consent.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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