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ORIGINAL RESEARCH

Low *PLCE1* levels are correlated with poor prognosis in hepatocellular carcinoma

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Background: Previous reports show that phospholipase C epsilon-1 (*PLCE1*) expression is positively correlated with esophageal squamous cell carcinoma and gastric cardia adenocarcinomas; however, the expression of *PLCE1* in hepatocellular carcinoma (HCC) and its correlation with clinical outcome still remain unclear. The aim of this study was to explore the expression of *PLCE1* in HCC tissue and to determine whether *PLCE1* was a prognostic factor for HCC patients.

Materials and methods: *PLCE1* levels in 20 paired HCC tissues and corresponding para-carcinomatous tissues was investigated by quantitative real-time polymerase chain reaction and Western blot assays. In addition, protein levels of *PLCE1* in one normal liver epithelial cell and four HCC cell lines were examined using Western blot assay. Moreover, immunohistochemistry was applied to determine the expression of *PLCE1* in HCC and corresponding surrounding tissues from 90 patients. Statistical analyses were used to examine the association between *PLCE1* levels and clinicopathological features.

Results: We found that the expression of *PLCE1* in tumor tissues was significantly lower than those in paracarcinomatous tissues at both mRNA and protein levels ($P<0.05$). We also determined that *PLCE1* protein expression levels were lower in HCC cell lines than normal liver epithelial cells ($P<0.05$). Notably, immunohistochemical assay showed that *PLCE1* expression was significantly low in HCC tissues compared with the adjacent normal liver tissues (40% vs 18.9%; $P<0.05$). Besides, *PLCE1* levels were negatively correlated with tumor capsulae, vascular invasion, Edmondson grade, alpha-fetoprotein, and tumor-node-metastasis stage ($P<0.05$). Univariate analysis revealed that lower level expression of *PLCE1* was significantly associated with poorer overall survival (OS) rate ($P<0.001$) and disease-free survival rate ($P<0.001$). Multivariate analysis revealed that low *PLCE1* level was an independent poor prognostic factor of OS and recurrence-free survival ($P<0.001$ and $P=0.003$, respectively).

Conclusion: In brief, our results revealed that decreased *PLCE1* expression was associated with tumor progression in HCC and may function as a promising biomarker for HCC prognosis.

Keywords: *PLCE1*, hepatocellular carcinoma, prognosis, immunohistochemistry

Introduction

Hepatocellular carcinoma (HCC) is the second most cause of cancer-related mortality in males and the sixth lethal among females.[1] It has a gloomy prognosis with a 5-year survival rate of 11%.[2] One of the most important factors for the low survival rate is that the vast majority of HCC patients are detected at an advanced stage, where radical resections are not feasible because of tumor metastasis. Therefore, the examination of identified biomarkers, especially for early stage cancer, is imperative to improve the prognosis of HCC patients.

A novel susceptibility locus (rs2274223: A5780G), located at exon 26 of phospholipase C epsilon-1 (*PLCE1*) has been reported by three large-scale and independent

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genome-wide association studies in the People's Republic of China (PRC) and is strongly correlated with the risk factor for esophageal and gastric cancers in Chinese population.[3–5] *PLCE1* is located in chromosome 10q23, and it is a unique member of the phospholipase family, which encodes a phospholipase that catalyzes the hydrolysis of phosphatidyl-inositol 4,5-bisphosphate to generate two secondary messengers: inositol 1,4,5-triphosphate and diacylglycerol; these secondary messengers subsequently regulate cell growth, differentiation, and gene expression.[6,7] Recently, increasing studies have reported that *PLCE1* may play an essential role in carcinogenesis and progression of numerous human cancers, including cancers of the esophagus, stomach, intestine, head and neck, skin, bladder, and colorectal.[8–14] However, less information has been reported about the expression of *PLCE1* in HCC. In this study, the expression of *PLCE1* in HCC and in surrounding nontumor tissue were detected by Western blotting and quantitative real-time polymerase chain reaction (qRT-PCR) assays. In addition, protein levels of *PLCE1* in one normal liver epithelial cell and four HCC cell lines were examined using Western blot assay. Moreover, immunohistochemical (IHC) staining of *PLCE1* in tumor tissues were performed for 90 patients with HCC, and we explored the association between *PLCE1* levels, clinicopathological features, and postoperative survival.

Materials and methods
Patients and specimens
We obtained the tumor tissues and paracarcinomatous specimens from 90 patients with HCC who had undergone curative hepatectomy from 2006 to 2011 at the Affiliated Provincial Hospital of Anhui Medical University, Hefei, PRC. Patients who had undergone preoperative any other anticancer therapy were eliminated from this study. Additionally, we also obtained the tumor and paracarcinomatous tissues from 20 patients for analyzing the expression of *PLCE1* by Western blotting and qRT-PCR assays. The clinical data and pathological features, such as age, gender, tumor size, tumor capsula, tumor nodule, vascular invasion, cirrhosis, HBsAg status, Edmondson grade, alpha-fetoprotein (AFP), Child–Pugh grade, and tumor-node-metastasis (TNM) stage, were collected by retrospective medical records. Tumor differentiation was defined according to the Edmondson grading system, and tumor pathological stage were defined according to the seventh edition TNM classification of The American Joint Committee on Cancer;[15] we also used Child–Pugh classification to estimate liver function.

Moreover, overall survival (OS) time was calculated from the surgery date to the death or last observation date. Disease-free survival (DFS) time was defined as the period from the surgery day to the date of first diagnosed recurrence. If recurrence was not verified, the survivors were censored on the death date or the last date of follow-up. Every patient had signed the informed consent for their specimens to be used in this study, and the study protocol was approved by the Research Ethics Committee of Anhui Provincial Hospital, and was in accordance with the Declaration of Helsinki.

Quantitative real-time PCR
Total RNA gathered from snap-frozen tissue samples was isolated using Trizol (Life Technologies) according to the manufacturer's protocol. We designed specific primers for *PLCE1* (Primer Designing Tool, NCBI, Bethesda, MD, USA) as follows: forward: 5′-GCCACCAACCAATTTACTGATGA-3′ and reverse: 5′-TAGTAACAAGGGTTCCAGTGC-3′. 18S RNA was used as the internalized control, and the sequences were shown as follows: forward: 5′-CGCTACTACCGATTGGATGG-3′ and reverse: 5′-AGTTCGACCGTCTTCTCAGC-3′. *PLCE1* level was determined by SYBR Green-based RT-PCR performed on a PikoReal RT-PCR system (Thermo Fisher Scientific) in the following conditions: a first denaturation step with 95°C for 10 minutes, followed by 40 amplification cycles involving denaturation for 15 seconds at 95°C, then annealing for 30 seconds at 60°C, and finally elongation for 30 seconds at 72°C. We performed melting-curve analysis to monitor PCR product purity, and the data of relative gene expression were analyzed using the $2^{-\Delta\Delta Ct}$ method.

Western blot
We used radioimmunoprecipitation assay lysis buffer (Beyotime Institute of Biotechnology, Shanghai, PRC) to lyse snap-frozen tumor and corresponding paracarcinomatous tissues, and the bicinchoninic acid protein assay was employed to estimate protein concentration. The same amount of protein samples were separated by 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transferred to the polyvinylidene difluoride membranes (Millipore, Billerica, MA, USA). Membranes were incubated overnight at 4°C with rabbit primary antibodies anti-*PLCE1* (1:300, Abcam PLC, Cambridge, UK) and human antibody of β-actin (1:5,000, Zhongshan Golden Bridge Biotechnology Co Ltd, Beijing, PRC). Following a wash with Tris-buffered saline/0.1% Tween for three times, each time for 5 minutes, we incubated the membranes with secondary antibodies

at room temperature for 2 hours. The blots were captured and visualized by Alpha-EaseFC imaging system (Alpha Innotech, San Leandro, CA, USA). Using the Alpha-EaseFC software, the integrated density value (IDV) of each band was detected by drawing a rectangle outlining the band. A total IDV by summation of each band IDV was employed when a protein had double bands. Results were normalized to the internal control, β-actin.

Cell culture

HCC cell lines SK-Hep1, PLC, HepG2, and Hep3B, as well as the immortalized normal liver epithelial cell THLE3 obtained from ATCC were cultured in Dulbecco's Modified Eagle's Medium (Gibco, Invitrogen Corporation, Carlsbad, CA, USA), while THLE3 cells were cultured in bronchial epithelial growth medium (Lonza/Clonetics Corporation, Walkersville, MD, USA). The cells were maintained at 37°C with 5% CO_2 in a humidified atmosphere.

Immunohistochemistry

Serial tissue sections (4 μm thick) were deparaffinized with xylene, then rehydrated through grade alcohols and subjected to autoclave antigen retrieval in ethylenediaminetetraacetic acid buffer (pH 8.0) at 100°C for 5 minutes. Endogenous peroxidase activity was blocked by 3% hydrogen peroxide for 10 minutes. Next, tissue sections were incubated at 4°C overnight with anti-*PLCE1* rabbit polyclonal antibody (Abcam PLC) at a dilution of 1:100 in phosphate-buffered saline (PBS) with 1% bovine serum albumin, then washed with PBS and incubated with horseradish peroxidase-conjugated secondary antibody (Zhongshan Golden Bridge Biotechnology) for 30 minutes. Immunoreactivity was visualized with chromogen 3,3′-diaminobenzidine. Next, all sections were counterstained with hematoxylin and then dehydrated in grade alcohols, and finally mounted with neutral balsam. Under the same conditions, PBS was used as the negative control for the primary antibody, and a known immunopositive section served as the positive control. We consulted a previously reported method to evaluate semi-quantitatively tumor expression of *PLCE1*[16] as follows: the percentage of staining cell scores: 0, no staining; 1 point, <10%; 2 points, 10%–30%; and 3 points, >30%. The staining intensity scores: 0 point, negative; 1 point, weak intensity; 2 points, moderate intensity; and 3 points, strong intensity. Sum scores with ≥3 points were considered positive, whereas sum scores with <3 points were regarded as negative. All scores were assessed independently by two pathologists, and the variability of the interobserver was <3%.

Statistical analysis

All data analyses were carried out with SPSS 22.0 software (SPSS Inc, Chicago, IL, USA). Data were presented as mean ± standard deviation. Differences of *PLCE1* mRNA and protein levels between HCC tissues and corresponding paracarcinomatous tissues samples were examined with the independent Student *t*-test. Categorical variables were analyzed by the χ^2 test or Fisher's exact test, and the Kaplan–Meier method was used to determine survival analysis, whereas the differences among survival rates were evaluated by the log-rank test. Cox proportional hazard model was used to evaluate the prognostic factors, which were significant in univariate analyses. *P*-values <0.05 were served as statistically significant.

Results

PLCE1 is downregulated in HCC tissues

To investigate whether *PLCE1* could function as a new prognostic factor for HCC patients, we first determined its levels in 20 paired HCC tissues and paracarcinomatous tissues using qRT-PCR and Western blot assay. qRT-PCR assay revealed that *PLCE1* levels were significantly lower in tumor sample tissues than that in adjacent nontumor tissues (0.51±0.49 vs 1.46±1.10, *P*<0.05), and 85% of samples (17/20 paired) have shown that tumor tissues have a lower expression (Figure 1). Western blot assay suggested that *PLCE1* levels in tumor sample tissues and adjacent paracarcinomatous tissues samples were 0.93±0.39 and 1.22±0.40, respectively (Figure 2A and B). And 18 paired samples (18/20) were remarkably lower in the HCC tumor samples, which showed a similar pattern to qRT-PCR assay. In addition, we analyzed *PLCE1* expression in HCC cells and normal liver cells. Western blot assay found that *PLCE1* was downregulated in all four HCC
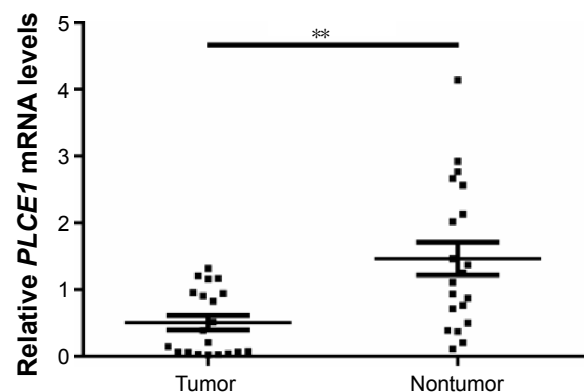

Figure 1 Real-time polymerase chain reaction analysis of *PLCE1* expression in 20 pairs of hepatocellular carcinomas and matched adjacent nontumorous tissues.
Note: **P*<0.01.
Abbreviations: mRNA, messenger RNA; *PLCE1*, phospholipase C epsilon-1.
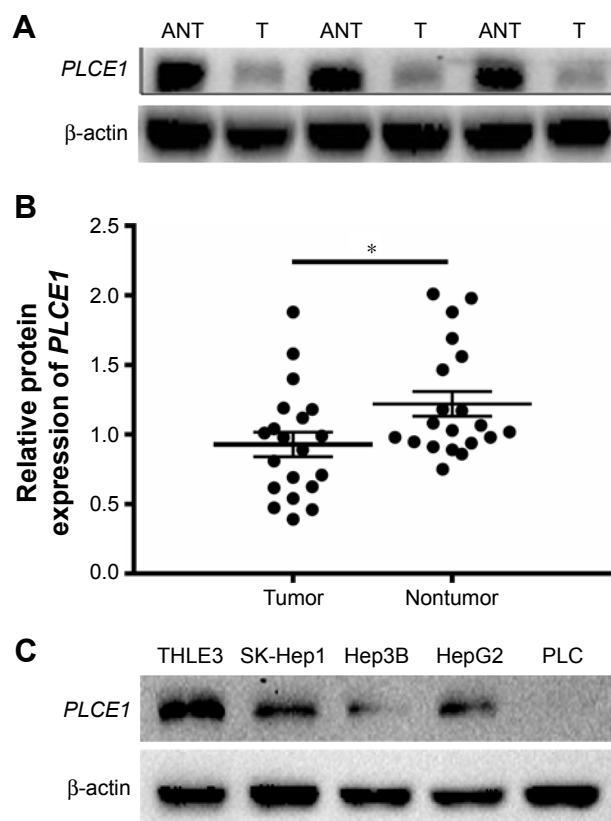
Figure 2 Western blotting analysis of *PLCE1* expression in tissues from HCC tumorous tissues or cell lines and adjacent nontumorous tissues or normal liver epithelial cells.
Notes: (**A**) Representative results of *PLCE1* protein expression in paired HCC tumorous tissues (T) and the matched ANT from 3 patients. *PLCE1*-protein expression was normalized to β-actin. (**B**) All of the paired HCC tissues and the matched adjacent noncancerous tissues from 20 patients. *P<0.05. (**C**) *PLCE1* protein expression in THLE3 and 4 cultured HCC cell lines. β-actin was used as internal control.
Abbreviations: ANT, adjacent nontumorous tissues; HCC, hepatocellular carcinoma; *PLCE1*, phospholipase C epsilon-1.

cell lines compared with THLE3 (Figure 2C). Besides, IHC assay showed that *PLCE1* was mainly located in the cytoplasm and cytomembrane of cancer cells and *PLCE1* levels were downregulated in HCC tissues (40%, 36/90) compared with adjacent normal liver tissues (18.9%, 17/90; Figure 3).

These findings suggested that *PLCE1* was downregulated in HCC tissues and cells.

The relationship between *PLCE1* levels and clinicopathological features

Among 90 patients in this study, 77 were males and 13 were females, and the mean age was 52 (range 19–80) years. Statistical analyses suggested that downregulation expression of *PLCE1* was significantly positively correlated with tumor capsula statues, Edmondson grade, vascular invasion, AFP, and TNM stage. But there was no association between *PLCE1* levels and age, gender, hepatitis B surface antigen status, cirrhosis, or Child–Pugh grade (Table 1).

PLCE1 is a favorable prognostic factor for HCC patients

Follow-up data of this study were gathered for all the patients. Median follow-up was 31 months (range 2–120 months). Kaplan–Meier curve with univariate analysis (log-rank) showed that the OS time of the patients who had the negative tumor score of *PLCE1* expression was shorter than those who had the score positive (15 months, 95% confidence interval [CI]: 11.080–18.920 vs 51 months, 95% CI: 36.200–65.800; $P<0.001$). Similarly, compared with the parents with positive *PLCE1* expression (28 months, 95% CI: 23.202–32.798), the parents who had negative score of *PLCE1* expression had a shorter DFS time (9 months, 95% CI: 7.831–10.169; $P<0.001$; Table 2; Figure 4). Univariate analysis also demonstrated tumor size, tumor capsulae, serum AFP, vascular invasion, Edmondson grade, and TNM stage had markedly prognostic value on OS, and tumor size, tumor capsulae, vascular invasion, Edmondson grade, and TNM stage had markedly prognostic value on disease-free survival. Furthermore, we used multivariate analysis to determine whether *PLCE1* was an independent prognostic factor for HCC and found that *PLCE1* level, tumor capsulae, serum AFP, and
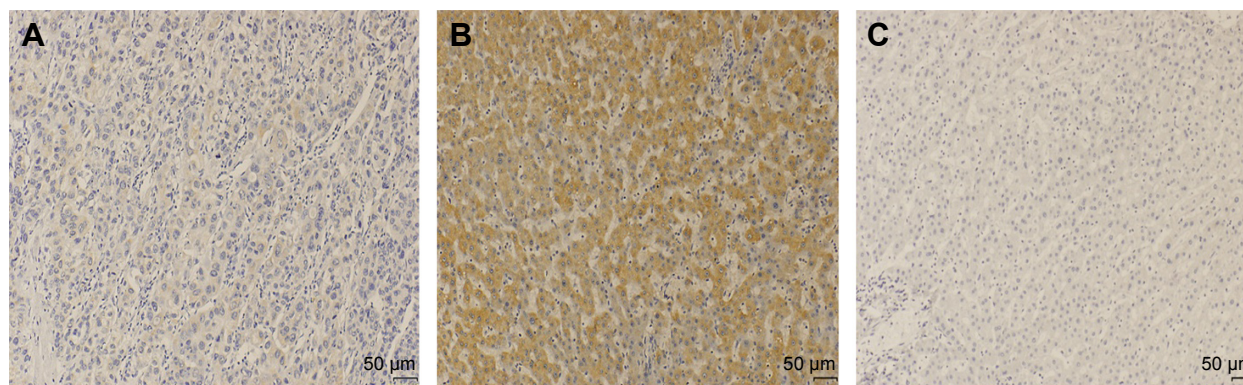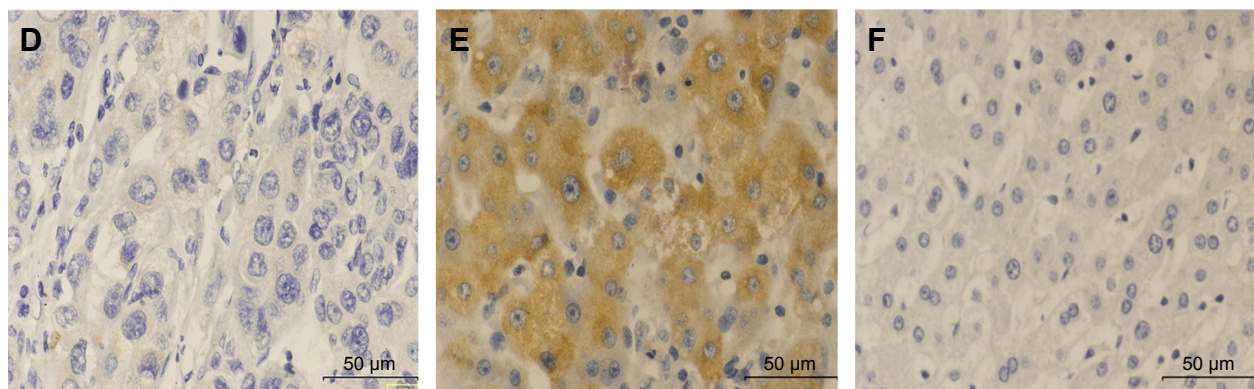

Figure 3 (*Continued*)

Figure 3 Representative immunohistochemical staining of *PLCE1* in HCC and matched adjacent noncancerous liver tissues.
Notes: (**A**, **D**) Low expression of *PLCE1* in HCC tissue cells. (**B**, **E**) Positive expression of *PLCE1* in matched adjacent noncancerous tissue cells. (**C**, **F**) Low expression of *PLCE1* in adjacent noncancerous tissue cells. (**A–C**) Magnification 100×; (**D–F**) Magnification 400×. Bar =50 μm.
Abbreviations: HCC, hepatocellular carcinoma; *PLCE1*, phospholipase C epsilon-1.

Table 1 Expression of *PLCE1* in relation to clinicopathologic features

Variable	Total	PLCE1 expression		χ^2 test	P-value
		Positive	Negative		
Age (years)				0.166	0.684
≤50	37	19	18		
>50	53	35	18		
Gender				2.937	0.087
Male	77	49	28		
Female	13	5	8		
Tumor capsular				5.030	0.025
Absent	42	20	22		
Present	48	34	14		
Tumor size (cm)				3.117	0.077
≤5	35	25	10		
>5	55	29	26		
Vascular invasion				7.500	0.006
No	30	24	6		
Yes	60	30	30		
Edmondson grade				6.750	0.009
I–II	50	36	14		
III–IV	40	18	22		
AFP (ng/mL)				6.433	0.011
≤20	53	26	27		
>20	37	28	9		
HBsAg				2.411	0.121
Negative	20	15	5		
Positive	70	39	31		
Cirrhosis				2.141	0.143
Absent	16	7	9		
Present	74	47	27		
Child–Pugh grade				0.010	0.921
A	67	40	27		
B	23	14	9		
TNM stage				6.750	0.009
I–II	58	36	14		
III–IV	32	18	22		
Tumor nodule				0.100	0.752
I	71	42	29		
≥2	19	12	7		

Abbreviations: AFP, alpha-fetoprotein; HBsAg, hepatitis B surface antigen; *PLCE1*, phospholipase C epsilon 1; TNM, tumor-node-metastasis.

vascular invasion were the independent prognostic factor for OS, whereas *PLCE1* expression, vascular invasion, TNM stage, and tumor capsulae were the independent prognostic factor for RFS (Table 3).

Discussion

PLCE1 had different functions compared with the other molecules of the PLC family,[17] which served as a vital effector of Ras family proteins that regulate cell growth, differentiation, apoptosis, and angiogenesis.[18] Therefore, recent studies had demonstrated that the abnormal expression of *PLCE1* in human tissues may be related to initiation and development of cancer. For example, through their semi-quantitative analysis study, Chen et al had indicated that the overexpression of *PLCE1* was significantly correlated with esophageal squamous cell carcinoma and non-small cell lung cancer (NSCLC),[13,19] whereas in the study of Wang et al, *PLCE1* can serve as a cancer suppressor gene in colon cancer, which inhibited the proliferation of tumor cells.[20] Furthermore, Li et al found that knockdown of *PLCE1* can markedly increase the expression of p53 in esophageal cancer cells, indicating that overexpression of *PLCE1* may promote the formation and development of tumor cells.[21] Otherwise, an animal examination showed that knockdown of *PLCE1* generated the resistance of intestinal tumor formation.[8] However, there are few explorations about the expression of *PLCE1* and its clinical significance in HCC.

In this study, our IHC analysis showed that protein levels of *PLCE1* were markedly low in HCC tissues compared with paracarcinomatous tissues, and the negative expression of *PLCE1* in HCC tissues was significantly associated with tumor capsulae, AFP, vascular invasion, Edmondson grade, and TNM stage in comparison to the positive expression in HCC samples. Moreover, *PLCE1* in 20 paired HCC samples

Table 2 Univariate analysis of parameters associated with OS and DFS

Features	OS			DFS		
	Median survival time (months)	95% CI	*P*-value	Median survival time (months)	95% CI	*P*-value
PLCE1			<0.001			<0.001
Positive	51	36.200–65.800		28	23.202–32.798	
Negative	15	11.080–18.920		9	7.831–10.169	
Age (years)			0.857			0.230
≥50	33	25.867–40.133		21	16.924–25.076	
<50	29	19.466–38.534		21	16.247–25.753	
Gender			0.413			0.334
Male	32	25.793–38.207		21	17.875–24.125	
Female	27	11.734–42.266		21	0.000–45.661	
Tumor size (cm)			0.023			0.014
>5	27	22.156–31.844		18	12.550–23.450	
≤5	51	34.951–67.049		32	20.409–43.591	
Tumor nodule			0.294			0.174
Single	29	23.500–34.500		32	16.534–47.466	
Multiple	47	18.334–75.666		20	16.247–23.753	
Tumor capsulae			<0.001			<0.001
Present	52	24.471–79.529		27	22.150–31.850	
Absent	17	7.473–26.527		9	3.713–14.287	
Vascular invasion			<0.001			<0.001
Yes	23	18.624–29.376		15	9.213–20.787	
No	49	19.254–79.267		38	22.443–53.557	
TNM stage			<0.001			<0.001
I–II	52	27.581–76.419		29	21.298–36.702	
III-IV	18	10.562–25.438		9	7.767–10.233	
Edmondson grade			0.006			0.016
I–II	38	23.610–52.390		25	19.089–30.911	
III–IV	23	15.765–30.235		17	11.394–22.606	
Cirrhosis			0.956			0.902
Present	31	25.971–36.029		20	15.785–24.215	
Absent	24	10.933–37.067		21	14.467–27.533	
AFP (ng/mL)			0.008			0.640
≥20	27	21.905–32.095		20	15.930–24.070	
<20	51	33.538–68.462		23	19.027–26.973	
Child–Pugh grade			0.748			0.937
A	31	23.043–38.957		21	17.505–24.495	
B	31	18.479–43.521		21	16.417–25.583	
HBsAg			0.803			0.478
Positive	29	22.426–35.574		21	16.902–25.098	
Negative	31	24.898–37.102		21	16.639–25.361	

Abbreviations: AFP, alpha-fetoprotein; CI, confidence interval; DFS, disease-free survival; HBsAg, hepatitis B surface antigen; OS, overall survival; *PLCE1*, phospholipase C epsilon 1; TNM, tumor-node-metastasis.

were examined by qRT-PCR and Western blot, and the results were consistent with those in IHC examination. Therefore, our data suggested that low expression of *PLCE1* in HCC was unfavorable for clinical outcome and may be a potential valuable prognostic factor.

The regulatory mechanism of *PLCE1* in cancer is not clear until now. Bunney et al found that *PLCE1* contains several Ras-binding domains for small G-protein of the Ras family and is the downstream of the Ras superfamily guanosine triphosphatases (GTPases), which was responsible for regulating cell growth, differentiation, apoptosis, and angiogenesis.[22] Li et al reported that *PLCE1* serves cancerogenic function in intestinal carcinogenesis through augmentation of inflammatory signaling pathways and angiogenesis.[8] It is reported that *PLCE1* may function as a Ras receptor, which plays an important role in promoting apoptosis of tumor cells, thereby serving as a tumor resister.[20] On the basis of these findings and our results, we speculated
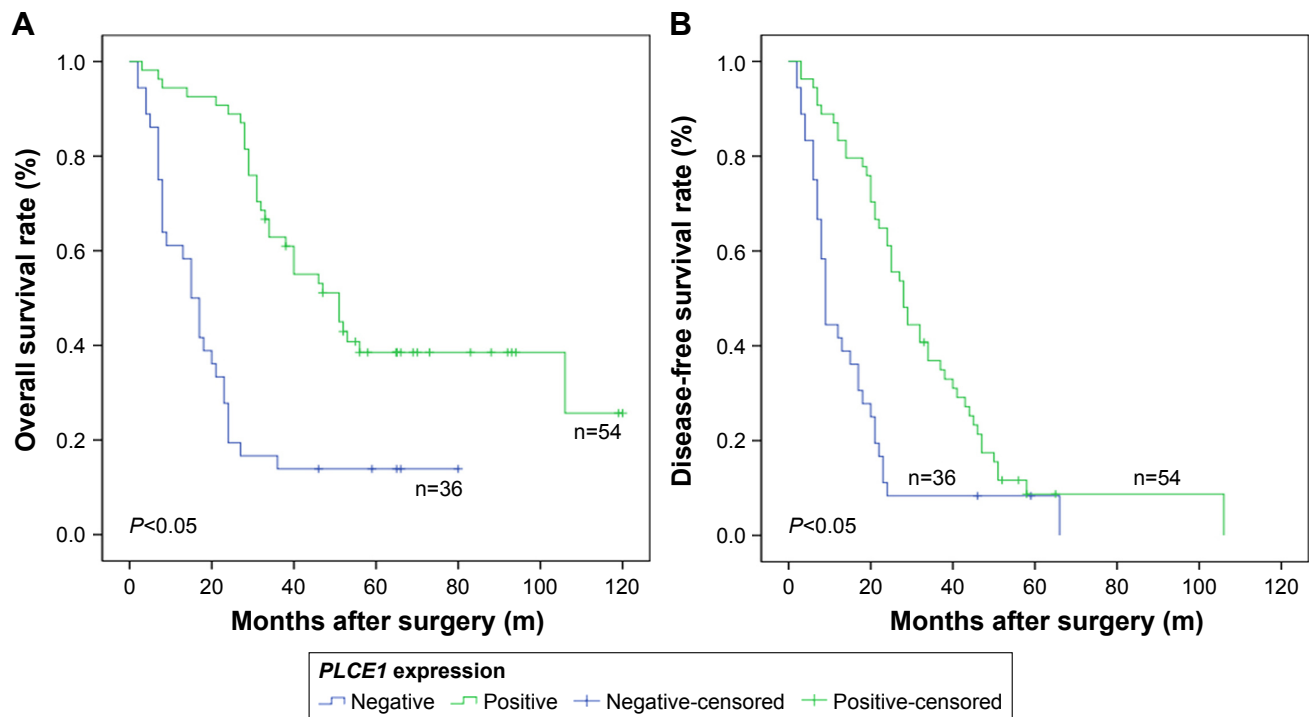
A

B

PLCE1 expression
Negative　　Positive　　Negative-censored　　Positive-censored

Figure 4 Kaplan–Meier analysis of OS and DFS curves of patients with HCC based on *PLCE1* expression as positive or negative.
Notes: (**A**) OS curve of patients with HCC based on *PLCE1* expression; (**B**) DFS curve of patients with HCC based on *PLCE1* expression. HCC patients with *PLCE1*+ showed markedly better OS and DFS rates than those with *PLCE1*−.
Abbreviations: DFS, disease-free survival; HCC, hepatocellular carcinoma; OS, overall survival; *PLCE1*, phospholipase C epsilon-1.

that *PLCE1* may function as a suppressor in HCC by altering the motility of some matters in Ras-signaling pathway. However, the concrete mechanism of cancerogenic function by *PLCE1* in HCC requires further research.

Present researches have indicated the prognostic significance of *PLCE1* in other malignancies. Yu et al reported that *PLCE1*-positive expression was significantly related to the lower 5-year survival rate in gastric cancer.[23] Moreover, Chen et al reported that the low expression of miR-1976 (which can regulate mRNA expression directly by targeting the 3′-untranslated region [UTR] of *PLCE1*) is correlated with poor clinical prognosis in NSCLC parents.[19] In our study,

we demonstrated that HCC parents with negative *PLCE1* expression had a markedly shorter OS and RFS than those with positive *PLCE1* expression by log-rank test. Moreover, using the Cox proportional hazard regression model, we demonstrate *PLCE1* as an independent factor for the prognosis of HCC patients who had undergone surgical resection.

In summary, we provided that the first evidence of *PLCE1* is downregulated in HCC cells and demonstrated that *PLCE1* is related to aggressive clinicopathological features. In addition, we also found that negative expression of *PLCE1* is an independent prognostic predictor of poor OS and RFS for HCC parents who had undergone surgical resection. Our

Table 3 Multivariate analysis of prognostic features associated with OS and DFS

Features	OS			DFS		
	HR	95% CI	*P*-value	HR	95% CI	*P*-value
PLCE1 (positive vs negative)	3.356	1.847–6.096	<0.001	2.335	1.349–4.043	0.003
AFP, ng/mL, (≥20 vs <20)	0.528	0.299–0.930	0.027	–	–	–
Tumor size, cm, (>5 vs ≤5)	1.300	0.734–2.301	0.368	1.364	0.826–2.254	0.226
Vascular invasion (no vs yes)	3.718	1.852–7.464	<0.001	2.670	1.531–4.655	0.001
TNM stage (I–II vs III–IV)	1.677	0.921–3.053	0.091	1.825	1.055–3.158	0.032
Edmondson grade (I–II vs III–IV)	1.149	0.651–2.028	0.633	0.872	0.507–1.498	0.620
Tumor capsluae (absent vs present)	2.228	1.177–4.216	0.014	2.124	1.208–3.732	0.009

Abbreviations: AFP, alpha-fetoprotein; CI, confidence interval; DFS, disease-free survival; HR, hazard ratio; OS, overall survival; *PLCE1*, phospholipase C epsilon 1; TNM, tumor-node-metastasis.

results suggest that *PLCE1* may be a mark that can predict the outcome and recurrence of HCC parents who receive radical resection. However, further investigations need to explore the exact mechanism of decreased expression of *PLCE1* in HCC.

Disclosure

The authors report no conflicts of interest in this work.

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