Original Article

Phosphotriesterase-related protein as a novel prognostic predictor for hepatocellular carcinoma patients

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Background: Hepatocellular carcinoma (HCC) is the sixth incidence of cancer and the third leading cause of cancer mortality in the world. Facing the ever-increasing population of HCC patients, there is still an urgent need to find good diagnostic and prognostic markers to explore new therapeutic targets. Phosphotriesterase-related (PTER) protein, an expressed protein in the liver and injured or ploycystic kidneys, was reported to be correlated with serum aspartate aminotransferase (AST) and alanine transaminase (ALT). Our study aimed to investigate the expression of PTER protein in HCC patients and the association between PTER protein expression with clinicopathological features of HCC.

Methods: Western blot analysis and immunohistochemistry (IHC) were performed in paired para-tumor and liver tumor tissues and HCC tissue microarray (TMA) to detect PTER protein expression. Correlation between PTER protein and prognostic factors were analyzed through univariate and multivariate analysis.

Results: We identified that PTER protein was significantly up-regulated in HCC tumors. Our data revealed that high PTER protein expression was associated with aggressive clinicopathological features of HCC, such as advanced tumor staging, vascular invasion, recurrence, and shorter overall survival (OS) and disease-free survival (DFS) time. Besides, in multivariate analyses, PTER protein was an independent predictor for OS (P=0.004) and DFS (P=0.013) for HCC patients. Meanwhile, the prognosis of patients with high PTER protein is much worse than those with low PTER protein expression.

Conclusions: PTER protein expression is raised in HCC tissues and may be a potential prognostic predictor for HCC patients.

Keywords: Phosphotriesterase-related protein (PTER protein); hepatocellular carcinoma (HCC); prognosis

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Introduction

Background

Hepatocellular carcinoma (HCC) is the sixth incidence of cancer and the third leading cause of cancer mortality in the world (1). Currently, Hepatectomy remains the first-choice only in early stages of HCC, but only 10–20% patients with early-stage HCC are diagnosed. In the intermediate stages, transarterial chemoembolization (TACE) is the preferred therapeutic option, while chemotherapy administration is the eligible treatment in advanced HCC. Although the application of TACE and targeted drugs has led to an improvement in the 5-year survival rate of patients with HCC, the prognosis of this disease is still poor (2). The main reasons for the poor prognosis of HCC patients are the high incidence of tumor recurrence or distant metastasis after surgical resection as well as resistance to chemotherapy (3). Therefore, it is an urgent issue to strengthen the research on the molecular pathological mechanisms of HCC and find new therapeutic targets and treatments.

Rationale and knowledge gap

Although new targeted therapy drugs such as sorafenib, lenvatinib, regorafenib, cabozantinib, ramucirumab, and immune checkpoint inhibitors have prolonged the survival time of HCC patients, the problems of recurrence, metastasis, and drug resistance have compromised the efficacy of these treatments (3). Facing the ever-increasing population of HCC patients, there is still an urgent need to find good diagnostic and prognostic markers to explore new therapeutic targets.

Highlight box

Key findings

- PTER mRNA and protein was upregulated in HCC.
- PTER protein was correlated with poor prognosis of HCC.

What is known and what is new?

- Good diagnostic and prognostic markers were urgently needed to explore new therapeutic targets of HCC.
- PTER protein expression was raised in HCC tissues and correlated with poor prognosis of HCC.

What is the implication, and what should change now?

- PTER protein might be a potential prognostic predictor for HCC patients.

Objective

Phosphotriesterase-related (PTER) protein is encoded by human chromosome 10p12, it is homologous to phosphotriesterase (PTE) in mice and rats (4). There are very few published studies on the function of PTER protein, and even fewer related to cancer as well as HCC. PTER protein expression may be associated with tissue damage and inflammation. A previous study reported that PTER protein is ubiquitously expressed in the liver (5), and it was correlated with aspartate aminotransferase (AST) and alanine transaminase (ALT), which are secreted by the liver (6). The downregulation of PTER protein in the lake trout liver may be associated with persistent expression of inflammatory factors due to parasitism by the sea lamprey (7). Through ribonucleic acid sequencing (RNA-seq) analysis of HCC tissues and para-tumor tissues, we observed that PTER protein was highly expressed in HCC samples. Nevertheless, there is no related study reporting whether PTER protein expression is associated with the tumorigenesis and progression of HCC. Therefore, we investigated the expression of PTER protein in HCC tissues, and analyzed its relationship with clinicopathological features and prognosis of HCC patients. We present this article in accordance with the REMARK reporting checklist (available at https://cco.amegroups.com/article/view/10.21037/cco-23-42/rc).

Methods

Patient information and clinical specimens

A retrospective study with 263 patients diagnosed as HCC from the Eastern Hepatobiliary Surgery Hospital (Shanghai, China) during 2005 to 2008. The patients were enrolled following conditions: (I) pathological diagnosis of HCC according to World Health Organization (WHO) criteria; (II) no radiation therapy or chemotherapy prior to curative resection; (III) indication for surgical resection; (IV) preoperative status 0–1, Child-Pugh class A, absence of ascites; (V) surgical resection was defined as complete resection of all tumor nodules with no cancer on histological sections (8). The tumor tissues were formalin-fixed, paraffin-embedded, and underwent tissue microarray (TMA) analysis. Exclusion criteria were patients that did not sign the informed consent and patients that have undergone certain treatments. All the information were collected from the clinical record of the patients. The study was conducted

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in accordance with the Declaration of Helsinki (as revised in 2013) and approved by the ethics committee of Eastern Hepatobiliary Surgery Hospital, Naval Medical University (protocol code: EHBHKY2017-K-006). Written informed consent was obtained from each patient.

Follow-up

Patients were followed up every 3–6 months for 5 years after surgery. The median follow-up duration of the subjects was 8.2 months. Detailed history, serum alpha-fetoprotein (AFP), liver function, and abdominal ultrasound were executed in each follow-up examination. Positron emission tomography-computed tomography (PET-CT) or magnetic resonance imaging (MRI) was also performed in each follow-up examination, especially when recurrence or metastasis was found. Overall survival (OS) was defined as the time between hepatectomy and death or the last follow-up. Disease-free survival (DFS) was defined as the time between hepatectomy and recurrence or last follow-up.

TMA and immunohistochemistry (IHC) analysis

The tumor tissues were formalin-fixed, paraffin-embedded, and undergone TMA. TMA slides were constructed after screening hematoxylin and eosin-stained slides for optimal tumor content (Biochip, Shanghai, China). IHC was performed as previously reported (9). The detailed protocols are as follows. Tissue sections were first baked in a 60 ℃ oven for 1h and then deparaffinized by xylene and 100%, 95%, 85%, and 75% alcohol. Distilled water was washed once. The peroxidase in the tissue sections was inactivated with 3% hydrogen peroxide at room temperature for 20 minutes. Citrate acid repair solution was boiled to repair the tissue sections for 2 minutes. Double-distilled water was washed once. One percent bovine serum albumin (BSA) was used to seal the tissue sections for 30 min at 37 ℃. Then the sealing solution was aspirated and discarded, and the primary antibody against PTER protein (Ab106526, Abcam, USA) was added dropwise to the tissue sections and incubated overnight at 4 ℃ for 8 h. The tissue sections were washed with phosphate buffer saline (PBS) buffer for five minutes four times. The secondary antibody was incubated at 37 ℃ for 30 min and washed with PBS buffer for 5 minutes for 4 times. Use DAB kit (11299366A, Dako, USA) to develop the color for 3–10 minutes. Wash with double-distilled water for five minutes for two times. Hematoxylin re-staining of cell nuclei for 10 min, ethanol hydrochloride differentiation for one time. Tap water running rinses returned blue for 20 minutes. Tissue sections were dehydrated through a series of concentration gradients of alcohol (75%, 85%, 95%, 100%) and xylene. After air-drying, the tissue sections were dripped with neutral resin, covered with coverslips, dried and stored, and photographed under a microscope. Stained sections were assessed in a blinded manner by three researchers without prior knowledge of the clinical details. The staining intensity was defined as "0" (negative), "1" (weak), "2" (moderate) or "3" (strong). Scores of 0 and 1 were defined as low expression of PTER protein, while scores of 2 and 3 were defined as high expression of PTER protein. Cases with different scores were discussed together with other researchers until agreements were reached.

RNA isolation, complementary DNA synthesis, and quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted from HCC specimens using TRIzol reagent (Invitrogen, USA). A total of 2 μg of RNA per sample was used for complementary DNA synthesis using the Oligo (dT) primer kit (Promega, USA). We randomly selected 26 pairs of para-tumor and HCC tissues that were tested by qRT-PCR assay to detect the messenger ribonucleic acid (mRNA) level of PTER. The qRT-PCR assay was carried out in Light Cycler® using a Roche SYBR® Green Master kit according to the instructions (Roche, Switzerland), the results were normalized to 18S control. The primer sequences are as follows: PTER, forward primer 5'-GTAGAGCCAAGCAAACTGGG-3' and reverse primer 5'-TGGACAGTAACAGCAGTCAAAG-3'; 18S, forward primer 5'-CGGCTACGACATCCAAGGAA-3' and reverse primer 5'-GCTGGAATTAGCGCGGCT-3'.

Western blotting

Tissues were collected and lysed in Radio-Immunoprecipitation Assay (RIPA) buffer (Beyotime, China). Western blotting assay was performed as previously described (9). The detailed protocols are as follows. Tissues were homogenized using an ultrasonic crusher. The supernatant was obtained by centrifugation at 12,000 rpm for 15 minutes. The protein concentration was determined by BCA (Bicinchoninicacid) kit (23225, Thermo fisher scientific, Massachusetts, USA), and then prepared into a certain concentration of protein with SDS loading buffer and RIPA buffer, and then the

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protein was boiled at 100 ℃ for 5 min. Then protein was separated by sodium dodecyl sulfonate-polyacrylamide gel electrophoresis (SDS-PAGE). The proteins were transferred to nitrocellulose filter membrane. The membrane was incubated with primary antibody against PTER protein (Ab106526, Abcam, USA) and Actin (81115-1-RR, proteintect, Wuhan, China) at 4 ℃ overnight for 8 h, and the membrane was washed with Tris Buffered Saline Tween (TBST) buffer for 5 min for 3 times. Then incubate the fluorescent secondary antibody at room temperature for 1 h, and wash the membrane with TBST buffer for 5 min for 3 times. The membrane was scanned with an Odyssey fluorescence scanner (LiCor, Lincoln, Neb) for imaging. We used ImageJ software to quantify the immunoblot bands, and the PTER protein bands in para-tumor and tumor tissues were quantified relative to their own actin, respectively, and the ratios of PTER protein/actin were statistically analyzed by Graphpad prism 8.0 software.

The Cancer Genome Atlas (TCGA) analysis

mRNA transcriptional data of HCC and normal liver tissues were downloaded from TCGA (https://portal.gdc.cancer.gov/). We were only able to download 50 pairs of para-tumor tissue and HCC tissue from the database. The transcriptional level of PTER in HCC and normal liver were compared by Student's *t*-test with the R software, version 4.0.3. Patients were divided into the high-transcriptional group or the low-transcriptional group according to the median value of the mRNA transcription level in all 375 HCC samples.

Data processing for Gene Set Enrichment Analysis (GSEA)

GSEA curves were built by GSEA software using the public HCC data in the website of TCGA (https://portal.gdc.cancer.gov/). Student's *t*-tests were used to evaluate consistent changes in differentially expressed genes (DEGs) in signaling pathways of interest. A 1,000-fold permutation test was applied to identify significantly altered pathways, and possible false-positive results were controlled by correcting P values using the Benjamini and Hochberg false discovery rate method (10).

Statistical analysis

Statistical analysis of all data was performed by GraphPad Prism 8.0 software. Data are presented as mean ± standard deviation. Statistical significance was defined using Student's *t*-test when comparing two groups. OS and DFS were obtained using the Kaplan-Meier analysis and log-rank test by SPSS version 20.0. Univariate and multivariate analysis was done using the COX proportional hazard model and a forward stepwise method was used to bring variables into the model to identify independent risk factors on OS and DFS: age, sex, hepatitis B surface antigen (HBsAg), serum AFP, AST, liver cirrhosis, largest tumor size, tumor foci, tumor differentiation, tumor encapsulation, distant metastasis, portal vein tumor thrombus (PVTT), microscopic portal vein tumor thrombus (MI-PVTT), Barcelona-Clinic Liver Cancer (BCLC) stage, tumor node metastasis (TNM) stage, PTER protein expression. The correlation between PTER protein expression and clinical characteristics was assessed using the chi-square test, and the P values are shown in the tables. P<0.05 was considered statistically significant.

Results

PTER protein was significantly up-regulated in HCC tissues

To understand the significance of PTER protein in HCC development, we examined the expression of PTER protein in paired para-tumor and liver tumor tissues. Western blotting in 9 paired para-tumor and HCC specimens showed that PTER protein was up-regulated in HCC tissues (*Figure 1A,1B*). qRT-PCR assays in 26 paired para-tumor and HCC specimens also revealed that the mRNA levels of PTER protein in HCC tissues were higher than that in para-tumor tissues (*Figure 1C*). Further, we explored the mRNA expression levels of PTER protein in HCC from the TCGA database. Analyses revealed that the mRNA levels of PTER in HCC tissues are higher than para-tumor in both unpaired (*Figure 1D*) and 50 paired specimens (*Figure 1E*). Altogether, these data demonstrated that PTER protein is significantly up-regulated in HCC tissues.

High PTER protein expression was associated with aggressive clinicopathological features of HCC

To gain insights into the relationship between PTER protein expression and clinical features of HCC, we detected the expression of PTER protein in HCC TMA including 263 cases by IHC. IHC showed that PTER protein was expressed in both the cytoplasm and the

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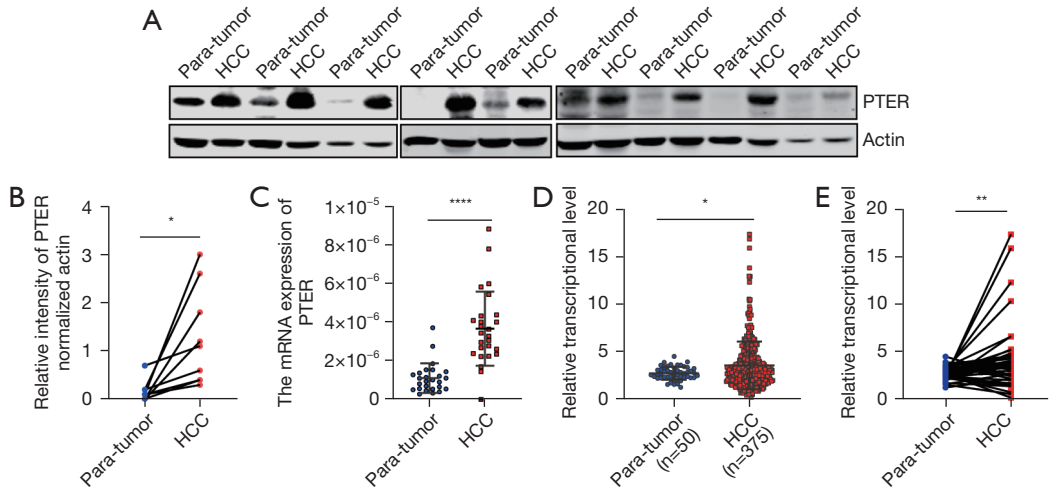

Figure 1 PTER mRNA and protein was significantly up-regulated in HCC tissues. (A) Western blotting analysis of PTER protein expression in 9 paired para-tumor tissues and HCC tumor tissues. (B) Quantification of (A). (C) qRT-PCR analysis of PTER mRNA expression in 26 paired para-tumor tissues and HCC tumor tissues. (D) The mRNA expression of PTER in 50 cases of para-tumor tissues and 375 cases of HCC tissues from the TCGA database. (E) PTER mRNA expression in 50 paired HCC tumors and para-tumor tissues from the TCGA database. P values were determined by a two-tailed *t*-test. *, P<0.05; **, P<0.01; ****, P<0.0001. HCC, hepatocellular carcinoma; PTER, phosphotriesterase-related; qRT-PCR, quantitative real-time polymerase chain reaction; mRNA, messenger ribonucleic acid; TCGA, The Cancer Genome Atlas.

nucleus of HCC cells, and is not found in peri-tumor tissues. Patient characteristics are shown in *Table 1*. In all the patients (n=263), 87.5% of the patients (n=230) were male; 93.2% of the patients (n=245) were hepatitis type B virus (HBV) positive; 70.7% (n=186) had elevated serum AFP; 92% of the patients (n=242) had single tumor nodule; 59.3% of the patients (n=156) had PVTT; and 31.6% of the patients (n=83) had distant metastasis.

Similar results on the immunostaining intensity of PTER protein analysis were gained from three different pathologists. The results showed that 72.6% (191/263) para-tumor tissues presented low-expression (score 0 and 1) and 27.4% (72/263) presented high-expression (score 2 and 3) of PTER protein, while the tumor tissues showed 54.4% (143/263) low-expression and 45.6% (120/263) high-expression (*Figure 2A*).

Patients were divided into two groups according to PTER protein expression in tumors (*Figure 2A*). Next, the relationship between PTER protein expression and HCC clinicopathological features was analyzed. The result showed that PTER protein was positively correlated with AFP (P=0.030), tumor foci (P=0.018), tumor encapsulation (P=0.021), MI-PVTT (P=0.000), BCLC stage (P=0.012), TNM stage (P=0.035), and recurrence (P=0.034) features (*Table 2*). Moreover, the correlation between PTER protein levels and survival was analyzed by Kaplan-Meier analysis. The result revealed the PTER protein high-expressed patients had a significantly shorter OS than the PTER protein low-expressed patients (high-PTER versus low-PTER: 5.8 versus 13.5 months; 95% CI: 4.567–7.033 versus 7.125–19.875, P<0.001, *Figure 2B*). Furthermore, patients in the PTER protein high-expressed group had a much shorter DFS than those in the PTER protein low-expressed group (high-PTER versus low-PTER: 2.0 versus 5.37 months; 95% CI: 1.951–2.049 versus 3.045–7.695, P<0.001, *Figure 2C*). Likewise, we analyzed the clinical relevance of PTER protein through the Kaplan-Meier plotter online website and found that patients with PTER high mRNA expression had a much shorter OS (logrank P=0.0015, *Figure 2D*) and DFS (logrank P=0.0049, *Figure 2E*) (11,12). Together, these data indicated that PTER mRNA and protein high expression was associated with aggressive clinicopathological features and poor prognosis of HCC.

Univariate and multivariate analysis of prognostic factors

Next, cox regression analysis was conducted to further evaluate the prognostic factors. Univariate analysis showed that AST, largest tumor size, tumor foci, tumor differentiation, tumor encapsulation, distant metastasis,

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Table 1 Patient characteristics (n=263)

Characteristic	No. patients (%)
Age (years)	
Mean ± SD	48.3±10.188
Median [range]	48 [26–75]
Sex	
Male	230 (87.5)
Female	33 (12.5)
HBsAg	
Negative	18 (6.8)
Positive	245 (93.2)
Serum AFP (ng/mL)	
≤400	77 (29.3)
>400	186 (70.7)
ALT (U/L)	
≤40	77 (29.3)
>40	186 (70.7)
AST (U/L)	
≤40	66 (25.1)
>40	197 (74.9)
Liver cirrhosis	
Negative	77 (29.3)
Positive	186 (70.7)
Largest tumor size (cm)	
≤5	51 (19.4)
>5	212 (80.6)
Tumor number	
Single	242 (92.0)
Multiple	21 (8.0)
Tumor foci	
Negative	142 (54.0)
Positive	121 (46.0)
Tumor differentiation stage	
I–II	7 (2.7)
III–IV	256 (97.3)
Tumor encapsulation	
No	168 (63.9)
Incomplete	34 (12.9)
Complete	61 (23.2)
Distant metastasis	
Negative	180 (68.4)
Positive	83 (31.6)
PVTT	
Negative	107 (40.7)
Positive	156 (59.3)
MI-PVTT	
Negative	27 (10.3)
Positive	236 (89.7)
BCLC stage	
A	37 (14.1)
B	70 (26.6)
C	156 (59.3)
TNM stage	
I/II	86 (32.7)
III/IV	177 (67.3)
Recurrence	
Negative	67 (25.5)
Positive	196 (74.5)

SD, standard deviation; HBsAg, hepatitis B surface antigen; AFP, alpha-fetoprotein; ALT, alanine transaminase; AST, aspartate aminotransferase; PVTT, portal vein tumor thrombus; MI-PVTT, microscopic portal vein tumor thrombus; BCLC, Barcelona Clinic Liver Cancer; TNM, tumor node metastasis.

PVTT, MI-PVTT, BCLC stage, TNM stage, and PTER protein expression were unfavorable predictors for OS and DFS. In addition, serum AFP was associated with OS, and gender was correlated with DFS (*Table 3*).

Based on the results of univariate analysis, we recruited univariate variables closely related to OS and DFS with P value less than 0.15 for multivariate analysis. The result demonstrated that PTER protein was an independent prognostic factor for both OS (P=0.004) and DFS (P=0.013) of HCC patients (*Table 4*).

Hallmark pathways enriched by GSEA in PTER protein differentially expressed HCC

Considering the substantial role of PTER protein in predicting HCC prognosis, we further explored by which

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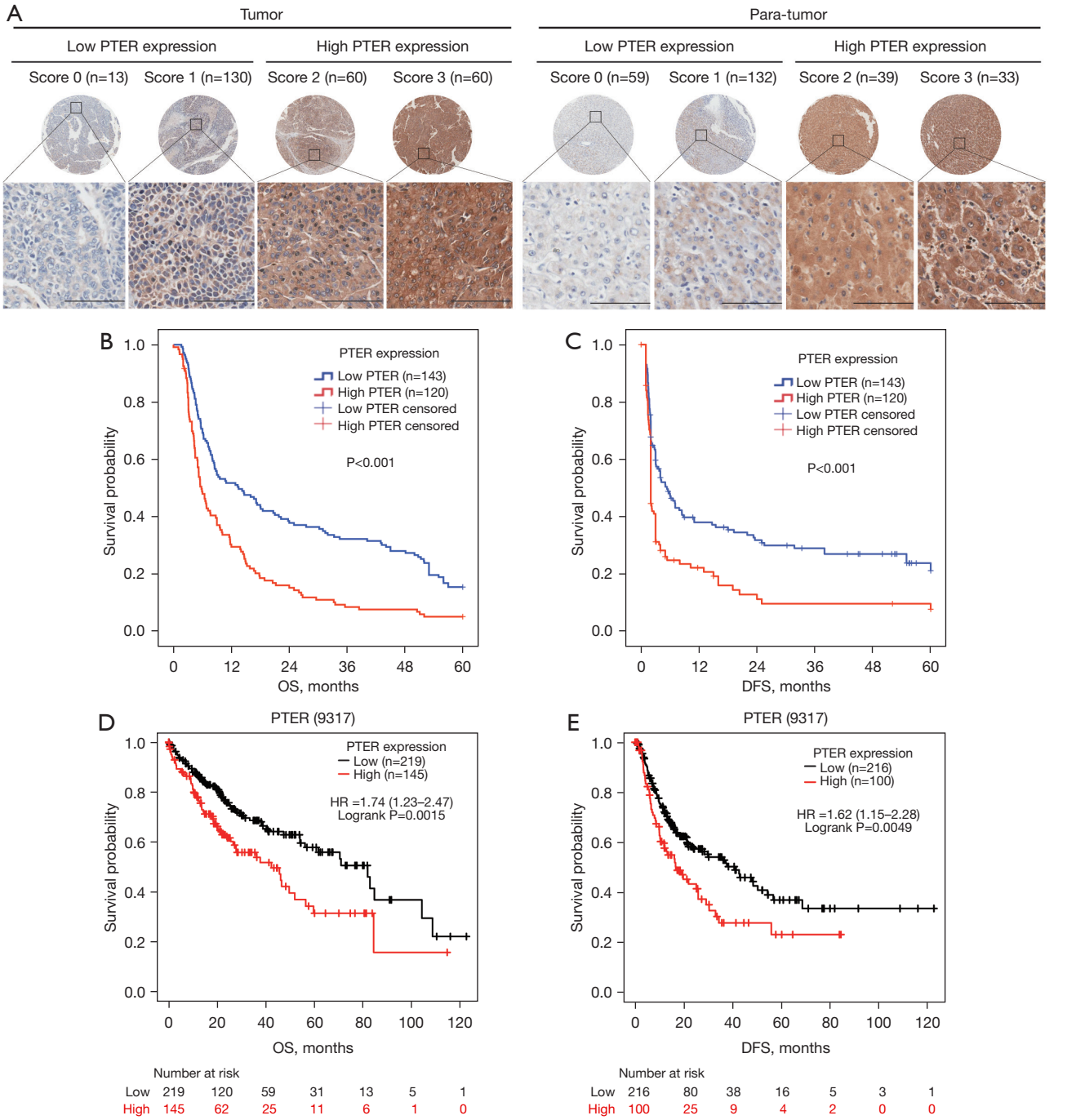

Figure 2 High PTER protein expression was associated with aggressive clinicopathological features and poor prognosis of HCC. (A) IHC and statistical analysis of PTER protein expression (score 0, 1, 2, 3) in HCC TMA, including tumor and para-tumor tissues. (B) Kaplan-Meier analysis of overall survival of HCC patients in PTER protein low and high expression group. (C) Kaplan-Meier analysis of disease-free survival of HCC patients. (D) Overall survival curve of HCC patients in Kaplan-Meier plotter online website, this result was obtained based on RNA-seq data from liver cancer patients in this website. (E) Disease-free survival of HCC patients in Kaplan-Meier plotter online website, this result obtained was based on RNA-seq data from liver cancer patients in this website. Scale bars, 100 μm. P values were determined by the log-rank test. PTER, phosphotriesterase-related; OS, overall survival; DFS, disease-free survival; HR, hazard ratio; HCC, hepatocellular carcinoma; IHC, immunohistochemistry; TMA, tissue microarray; RNA-seq, ribonucleic acid sequencing.

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Table 2 Association between PTER protein expression and clinicopathological characteristics (n=263)

Characteristic	No. patients [N=263 (%)]	PTER immunoreactivity		P value
		Low [n=143 (54.4%)]	High [n=120 (45.6%)]	
Age (years)				0.457
≤49	142 (54.0)	74	68	
>49	121 (46.0)	69	52	
Sex				0.576
Male	230 (87.5)	127	103	
Female	33 (12.5)	16	17	
HBsAg				0.465
Negative	18 (6.8)	8	10	
Positive	245 (93.2)	135	110	
Serum AFP (ng/mL)				0.030
≤400	77 (29.3)	50	27	
>400	186 (70.7)	93	93	
ALT (U/L)				0.417
≤40	77 (29.3)	45	32	
>40	186 (70.7)	98	88	
AST (U/L)				0.777
≤40	66 (25.1)	37	29	
>40	197 (74.9)	106	91	
Liver cirrhosis				0.176
Negative	77 (29.3)	47	30	
Positive	186 (70.7)	96	90	
Largest tumor size (cm)				0.349
≤5	51 (19.4)	31	20	
>5	212 (80.6)	112	100	
Tumor number				
Single	242 (92.0)	131	111	0.824
Multiple	21 (8.0)	12	9	
Tumor foci				0.018
Negative	142 (54.0)	87	55	
Positive	121 (46.0)	56	65	
Tumor differentiation stage				0.460
I–II	7 (2.7)	5	2	
III–IV	256 (97.3)	138	118	

Table 2 *(continued)*

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Table 2 *(continued)*

Characteristic	No. patients [N=263 (%)]	PTER immunoreactivity		P value
		Low [n=143 (54.4%)]	High [n=120 (45.6%)]	
Tumor encapsulation				0.021
No	168 (63.9)	81	87	
Incomplete	34 (12.9)	24	10	
Complete	61 (23.2)	38	23	
Distant metastasis				0.063
Negative	180 (68.4)	105	75	
Positive	83 (31.6)	38	45	
PVTT				0.059
Negative	107 (40.7)	66	41	
Positive	156 (59.3)	77	79	
MI-PVTT				0.000
Negative	27 (10.3)	24	3	
Positive	236 (89.7)	119	117	
BCLC stage				0.012
A	37 (14.1)	28	9	
B	70 (26.6)	39	31	
C	156 (59.3)	76	80	
TNM stage				0.035
I & II	86 (32.7)	55	31	
III & IV	177 (67.3)	88	89	
Recurrence				0.034
Negative	67 (25.5)	44	23	
Positive	196 (74.5)	99	97	

PTER, phosphotriesterase-related; HBsAg, hepatitis B surface antigen; AFP, alpha-fetoprotein; ALT, alanine transaminase; AST, aspartate aminotransferase; PVTT, portal vein tumor thrombus; MI-PVTT, microscopic portal vein tumor thrombus; BCLC, Barcelona Clinic Liver Cancer; TNM, tumor node metastasis.

pathway PTER protein probably affects HCC progression using TCGA database. HCC expression profiles were divided into two groups by PTER mRNA level, and GSEA was performed. The result suggested that gene sets related to Cancer, Wnt/β-catenin, mitogen-activated protein kinase (MAPK), ubiquitin-mediated proteolysis, endocytosis, and apoptosis signaling pathways were positively enriched in PTER protein high expression group, while gene sets related to ribosomes and oxidative phosphorylation were enriched in PTER protein low-expression group (*Figure 3*). Therefore, it is speculated that PTER protein high expression may be associated with HCC tumorigenesis and progression, which needs further exploration and verification.

Discussion

Key findings

HCC is a highly malignant tumor with a poor prognosis, although existing treatment options have led to a significant

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Table 3 Univariate Cox regression analyses of factors associated with overall survival and disease-free survival (n=263)

Variables	OS		DFS	
	Hazard ratio (95% CI)	P value	Hazard ratio (95% CI)	P value
Age (years) (≤49 *vs.* >49)	0.783 (0.605–1.013)	0.063	0.941 (0.711–1.247)	0.673
Sex (male *vs.* female)	0.732 (0.490–1.094)	0.128	0.567 (0.353–0.911)	0.019
HBsAg (negative *vs.* positive)	1.527 (0.889–2.622)	0.125	1.667 (0.906–3.065)	0.1
Serum AFP (ng/mL) (≤400 *vs.* >400)	1.418 (1.064–1.889)	0.017	1.329 (0.975–1.812)	0.072
ALT (U/L) (≤40 *vs.* >40)	1.068 (0.804–1.417)	0.652	1.129 (0.829–1.537)	0.441
AST (U/L) (≤40 *vs.* >40)	1.628 (1.197–2.215)	0.002	1.587 (1.138–2.213)	0.007
Liver cirrhosis (no *vs.* yes)	1.303 (0.979–1.735)	0.07	1.127 (0829–1.532)	0.444
Largest tumor size (cm) (≤5 *vs.* >5)	2.109 (1.490–2.984)	<0.0001	2.884 (1.898–4.381)	<0.0001
Tumor number (single *vs.* multiple)	1.162 (0.734–1.839)	0.521	1.240 (0.743–2.071)	0.411
Tumor foci (no *vs.* yes)	1.815 (1.400–2.353)	<0.0001	1.459 (1.096–1.942)	0.01
Tumor differentiation (I–II *vs.* III–IV)	2.756 (1.133–6.701)	0.025	4.939 (1.223–19.949)	0.025
Tumor encapsulation (no *vs.* incomplete *vs.* complete)	1.453 (1.235–1.709)	<0.0001	1.368 (1.149–1.629)	<0.0001
Distant metastasis (no *vs.* yes)	1.288 (0.977–1.697)	0.073	2.136 (1.601–2.850)	<0.0001
PVTT (no *vs.* yes)	0.452 (0.345–0.593)	<0.0001	1.802 (1.342–2.418)	<0.0001
MI-PVTT (no *vs.* yes)	2.442 (1.540–3.873)	<0.0001	3.402 (1.888–6.130)	<0.0001
BCLC stage (A *vs.* B *vs.* C)	1.837 (1.522–2.217)	<0.0001	1.682 (1.372–2.063)	<0.0001
TNM (I + II *vs.* III + IV)	2.233 (1.673–2.981)	<0.0001	2.121 (1.545–2.910)	<0.0001
PTER protein expression (low *vs.* high)	1.879 (1.446–2.443)	<0.0001	1.742 (1.308–2.320)	<0.0001

OS, overall survival; DFS, disease-free survival; CI, confidence interval; HBsAg, hepatitis B surface antigen; AFP, alpha-fetoprotein; ALT, alanine transaminase; AST, aspartate aminotransferase; PVTT, portal vein tumor thrombus; MI-PVTT, microscopic portal vein tumor thrombus; BCLC, Barcelona Clinic Liver Cancer; TNM, tumor node metastasis; PTER, phosphotriesterase-related.

improvement in the five-year survival time of HCC patients (2), there is still an urgent need to tap into effective molecular targets to improve diagnostic, therapeutic and prognostic approaches for HCC. Herein, we observed PTER mRNA and protein was up-regulated in HCC tissues through our RNA-seq analysis. However, the roles of PTER protein in HCC have rarely been reported. In this study, we provided the expression data of PTER mRNA and protein in HCC specimens and explored the relationship with clinicopathological features. We demonstrated that PTER mRNA and protein was up-regulated in HCC and associated with tumor staging, vascular invasion, and recurrence. HCC patients with high PTER protein expression have shorter OS and DFS. Meanwhile, PTER protein acted as an independent predictor of OS and DFS of HCC patients. Briefly, HCC patients with PTER protein high expression processed poor prognosis, high recurrence rate, and short OS. These results suggested that PTER mRNA and protein might be a promising predictor for HCC prognosis.

Strengths and limitations

Our results highlight the potential use of PTER protein as a predictive biomarker in HCC to improve the clinical landscape of this liver tumor. A strength of our study is the larger cohort size. Nevertheless, ~93% of the patients within the cohort are HBV+, and so a limitation would be that the findings ideally need to be validated in HCC patients with more diverse aetiologies of background disease. In addition, our study provides a useful insight into the likely role played by this PTER protein as well as the possible tumor-associated pathways that could be modulated by PTER protein by GSEA analysis, but the findings here

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Table 4 Multivariate Cox regression analyses of factors associated with overall survival and disease-free survival (n=263)

Variables	OS		DFS	
	Hazard ratio (95% CI)	P value	Hazard ratio (95% CI)	P value
Age (years) (≤49 *vs.* >49)	0.878 (0.664–1.160)	0.361	n.a.	–
Sex (male *vs.* female)	0.808 (0.527–1.239)	0.328	0.688 (0.417–1.135)	0.143
HBsAg (negative *vs.* positive)	1.624 (0.921–2.866)	0.094	1.755 (0.938–3.283)	0.078
Serum AFP (ng/mL) (≤400 *vs.* >400)	0.908 (0.660–1.250)	0.555	1.044 (0.754–1.445)	0.797
AST (U/L) (≤40 *vs.* >40)	1.295 (0.939–1.785)	0.115	1.403 (0.993–1.983)	0.055
Liver cirrhosis (no *vs.* yes)	1.018 (0.743–1.395)	0.912	n.a.	–
Largest tumor size (cm) (≤5 *vs.* >5)	1.354 (0.861–2.129)	0.19	1.941 (1.182–3.188)	0.009
Tumor foci (no *vs.* yes)	1.152 (0.849–1.562)	0.364	0.987 (0.714–1.364)	0.936
Tumor differentiation (I–II *vs.* III–IV)	1.582 (0.614–4.077)	0.343	1.610 (0.367–7.064)	0.528
Tumor encapsulation (no *vs.* incomplete *vs.* complete)	1.146 (0.957–1.373)	0.138	1.174 (0.975–1.414)	0.09
Distant metastasis (no *vs.* yes)	1.004 (0.749–1.345)	0.98	1.611 (1.191–2.179)	0.002
PVTT (no *vs.* yes)	0.931 (0.451–1.921)	0.846	1.582 (1.264–1.281)	0.179
MI-PVTT (no *vs.* yes)	1.332 (0.778–2.280)	0.296	1.921 (0.988–3.735)	0.054
BCLC stage (A *vs.* B *vs.* C)	1.379 (0.787–2.416)	0.262	1.6432 (0.769–2.665)	0.258
TNM (I + II *vs.* III + IV)	1.068 (0.647–1.761)	0.798	1.320 (0.742–2.349)	0.344
PTER expression (low *vs.* high)	1.528 (1.146–2.038)	0.004	1.466 (1.084–1.983)	0.013

OS, overall survival; DFS, disease-free survival; CI, confidence interval; HBsAg, hepatitis B surface antigen; AFP, alpha-fetoprotein; AST, aspartate aminotransferase; PVTT, portal vein tumor thrombus; MI-PVTT, microscopic portal vein tumor thrombus; BCLC, Barcelona Clinic Liver Cancer; TNM, tumor node metastasis; PTER, phosphotriesterase-related; n.a., not applicable.

observed are preliminary, the mechanisms by which PTER protein functions need to be explored in depth.

Comparison with similar research

Previous study revealed PTER, a highly expressed gene in the liver, was correlated with serum AST and ALT (6), and it was expressed in kidney proximal tubular cells, especially in the injured and ploycystic kidneys presenting with an abnormally high expression (5,13). The upregulation of PTER protein was associated with membranous nephropathy and involved in proteinuria-mediated activation of proximal tubular cells, which ultimately leading to end-stage renal disease, silencing the expression of PTER protein by Ribonucleic Acid interference diminished albuminuria-induced inflammatory and pro-fibrotic cytokines production (14), suggesting that PTER protein may play a role in inflammation. Meanwhile, in a genome-wide association data study of 1,380 Europeans with early-onset and morbid adult obesity and 1,416 age-matched normal-weight controls, PTER protein was detected to be significantly associated with obesity (15). Furthermore, a high-risk allele for obesity in the PTER single nucleotide polymorphism (SNP) was associated with being small for gestational age (16). These dates suggest that aberrant expression of PTER protein may contribute to disease.

Our study reveals the potential use of PTER protein as a predictive biomarker in HCC. We have found several new therapeutic targets in HCC, such as RMP and RPRD1A. RMP was a significant oncoprotein highly expressed in HCC, which promoted the progression of HCC and predicted the therapeutic value of TACE (17). RPRD1A was also increased in HCC and correlated with poor prognosis of HCC, which rely on activation of Nrf2 (18). PTER protein was similar to the two oncoproteins, as its expression was increased in HCC and predicted poor prognosis. Amounts of research focused on new biomarkers for HCC, such as HM13, SIX4, SPINDOC, NRP1 and FOXO3, which were upregulated in HCC and related with prognosis (19-23). SIX4 was involved in the HGF-SIX4-c-MET

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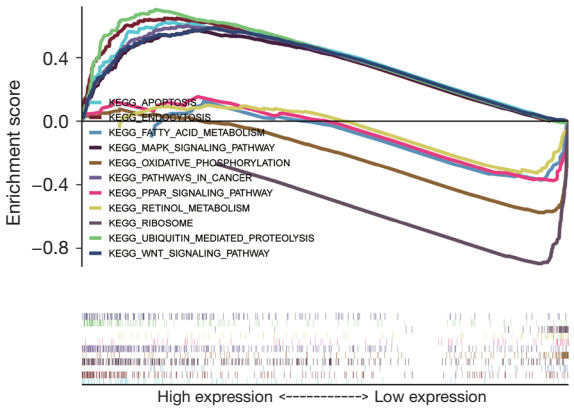

Figure 3 Hallmark pathways enriched by GSEA in PTER protein differentially expressed HCC. GSEA of HCC expression profiles in TCGA database (PTER high *vs.* PTER low). Pathways including cancer, Wnt/β-catenin, MAPK, ubiquitin-mediated proteolysis, endocytosis, and apoptosis signaling were positively enriched in PTER protein high expression group, while ribosomes and oxidative phosphorylation pathways were negatively enriched. KEGG, Kyoto Encyclopedia of Genes and Genomes; GSEA, Gene Set Enrichment Analysis; PTER, phosphotriesterase-related; HCC, hepatocellular carcinoma; TCGA, the Cancer Genome Atlas; MAPK, mitogen-activated protein kinase.

positive feedback loop and might be a promising therapy target for SIX4-driven HCC metastasis (20). However, the underlying mechanism of PTER mRNA and protein upregulation in HCC and its potential therapeutic value was still ambiguous.

Explanations of findings

Through GSEA analysis, we found that Wnt/β-catenin, MAPK, Cancer, and ubiquitin-mediated proteolysis pathways were positively enriched in PTER protein high expression group. The Wnt/β-catenin signaling is involved in various physiological processes such as cell proliferation, differentiation, apoptosis, migration, invasion, and tissue homeostasis (24). Growing evidence suggests that dysregulation of the Wnt/β-catenin signaling contributes to the development and progression of hematological malignancies and some solid tumors (25-27). MAPK pathway mainly includes extracellular protein kinases (ERK1/2), Jun N-terminal kinase (JNK) and p38, which convert the extracellular signals into an extensive range of cellular responses. Given that these vital roles of MAPK signaling pathways in critical cellular activities, such as cell proliferation, differentiation, survival or death, and inflammation, dysregulation of MAPK signaling pathways has been implicated in the pathogenesis of many human diseases, including neurodegenerative diseases and various types of cancers (28). Maintenance of a stable proteome through precisely regulated protein synthesis and degradation mechanisms is critical for cell survival (29). The ubiquitin-proteasome system profoundly regulates cell proliferation and differentiation by controlling the abundance of key cyclins, modulates immune and inflammatory responses, and controls various signal transduction pathways (30,31). Unscheduled proteolysis of cell cycle regulators contributes to tumorigenesis in many human cancers (31). Abnormal mutation of the enzymes of the ubiquitin-proteasome system or the motifs that recognize specific substrates leads to the loss of the ability to regulate target proteins, which generates oncoprotein aggregation, abnormal degradation of tumor suppressor proteins, blocked apoptosis and proliferation of mutant cells, eventually leading to tumorigenesis (32). Therefore, it is speculated that PTER protein high expression may be associated with HCC tumorigenesis and progression, which needs further exploration and verification.

Implications and actions needed

Tumor recurrence and metastasis are the major problems limiting patient survival. The high expression of PTER protein was positively correlated with HCC aggressive features, including more tumor numbers, metastases, higher BCLC or TNM stage, and higher recurrence rate. Therefore, PTER protein may facilitate HCC tumorigenesis and progression, while the exact role and underlying mechanism of PTER protein need further study.

Conclusions

We demonstrated that PTER mRNA and protein was significantly up-regulated in HCC tumors. High PTER protein expression was associated with aggressive clinicopathological features of HCC. Besides, we identified PTER protein expression was an independent predictor of OS and DFS of HCC patients. Thus, PTER protein may serve as a potential prognostic biomarker for HCC.

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Footnote

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://cco.amegroups.com/article/view/10.21037/cco-23-42/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013) and approved by the ethics committee of Eastern Hepatobiliary Surgery Hospital, Naval Medical University (protocol code: EHBHKY2017-K-006). Written informed consent was obtained from each patient.

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References

1. Sung H, Ferlay J, Siegel RL, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin 2021;71:209-49.
2. Yang JD, Hainaut P, Gores GJ, et al. A global view of hepatocellular carcinoma: trends, risk, prevention and management. Nat Rev Gastroenterol Hepatol 2019;16:589-604.
3. Llovet JM, Montal R, Sia D, et al. Molecular therapies and precision medicine for hepatocellular carcinoma. Nat Rev Clin Oncol 2018;15:599-616.
4. Alimova-Kost MV, Imreh S, Buchman VL, et al. Assignment1 of phosphotriesterase-related gene (PTER) to human chromosome band 10p12 by in situ hybridization. Cytogenet Cell Genet 1998;83:16-7.
5. Hou X, Maser RL, Magenheimer BS, et al. A mouse kidney- and liver-expressed cDNA having homology with a prokaryotic parathion hydrolase (phosphotriesterase)-encoding gene: abnormal expression in injured and polycystic kidneys. Gene 1996;168:157-63.
6. Kim HY, Cho S, Yu J, et al. Analysis of copy number variation in 8,842 Korean individuals reveals 39 genes associated with hepatic biomarkers AST and ALT. BMB Rep 2010;43:547-53.
7. Goetz F, Smith SE, Goetz G, et al. Sea lampreys elicit strong transcriptomic responses in the lake trout liver during parasitism. BMC Genomics 2016;17:675.
8. Poon RT, Ng IO, Lau C, et al. Tumor microvessel density as a predictor of recurrence after resection of hepatocellular carcinoma: a prospective study. J Clin Oncol 2002;20:1775-85.
9. Dong LW, Hou YJ, Tan YX, et al. Prognostic significance of Beclin 1 in intrahepatic cholangiocellular carcinoma. Autophagy 2011;7:1222-9.
10. Subramanian A, Tamayo P, Mootha VK, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci U S A 2005;102:15545-50.
11. Lánczky A, Győrffy B. Web-Based Survival Analysis Tool Tailored for Medical Research (KMplot): Development

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and Implementation. J Med Internet Res 2021;23:e27633.
12. Menyhárt O, Nagy Á, Győrffy B. Determining consistent prognostic biomarkers of overall survival and vascular invasion in hepatocellular carcinoma. R Soc Open Sci 2018;5:181006.
13. Davies JA, Buchman VL, Krylova O, et al. Molecular cloning and expression pattern of rpr-1, a resiniferatoxin-binding, phosphotriesterase-related protein, expressed in rat kidney tubules. FEBS Lett 1997;410:378-82.
14. Cheng CW, Chang LC, Tseng TL, et al. Phosphotriesterase-related protein sensed albuminuria and conferred renal tubular cell activation in membranous nephropathy. J Biomed Sci 2014;21:32.
15. Meyre D, Delplanque J, Chèvre JC, et al. Genome-wide association study for early-onset and morbid adult obesity identifies three new risk loci in European populations. Nat Genet 2009;41:157-9.
16. Morgan AR, Thompson JM, Murphy R, et al. Obesity and diabetes genes are associated with being born small for gestational age: results from the Auckland Birthweight Collaborative study. BMC Med Genet 2010;11:125.
17. Zhang J, Jiang TY, Jiang BG, et al. RMP predicts survival and adjuvant TACE response in hepatocellular carcinoma. Oncotarget 2015;6:3432-42.
18. Feng X, Jiang T, Yang C, et al. RPRD1A stabilizes NRF2 and aggravates HCC progression through competing with p62 for TRIM21 binding. Cell Death Dis 2021;13:6.
19. Liu J, Li W, Wu L. Pan-cancer analysis suggests histocompatibility minor 13 is an unfavorable prognostic biomarker promoting cell proliferation, migration, and invasion in hepatocellular carcinoma. Front Pharmacol 2022;13:950156.
20. He Q, Lin Z, Wang Z, et al. SIX4 promotes hepatocellular carcinoma metastasis through upregulating YAP1 and c-MET. Oncogene 2020;39:7279-95.
21. Tong W, Yang L, Liu L, et al. SPINDOC is Highly Expressed in Pan-Cancer Samples and Can Promote the Proliferation, Invasion and Migration of Hepatocellular Carcinoma Cells by Activating Wnt/β-Catenin Signaling Pathway. Onco Targets Ther 2022;15:555-70.
22. Fernández-Palanca P, Payo-Serafín T, Fondevila F, et al. Neuropilin-1 as a Potential Biomarker of Prognosis and Invasive-Related Parameters in Liver and Colorectal Cancer: A Systematic Review and Meta-Analysis of Human Studies. Cancers (Basel) 2022;14:3455.
23. Fondevila F, Fernández-Palanca P, Méndez-Blanco C, et al. Association of FOXO3 Expression with Tumor Pathogenesis, Prognosis and Clinicopathological Features in Hepatocellular Carcinoma: A Systematic Review with Meta-Analysis. Cancers (Basel) 2021;13:5349.
24. Zhang Y, Wang X. Targeting the Wnt/β-catenin signaling pathway in cancer. J Hematol Oncol 2020;13:165.
25. Zhan T, Rindtorff N, Boutros M. Wnt signaling in cancer. Oncogene 2017;36:1461-73.
26. Gajos-Michniewicz A, Czyz M. WNT Signaling in Melanoma. Int J Mol Sci 2020;21:4852.
27. Ge X, Wang X. Role of Wnt canonical pathway in hematological malignancies. J Hematol Oncol 2010;3:33.
28. Kim EK, Choi EJ. Pathological roles of MAPK signaling pathways in human diseases. Biochim Biophys Acta 2010;1802:396-405.
29. Wolska-Washer A, Smolewski P. Targeting Protein Degradation Pathways in Tumors: Focusing on their Role in Hematological Malignancies. Cancers (Basel) 2022;14:3778.
30. Ciechanover A, Orian A, Schwartz AL. Ubiquitin-mediated proteolysis: biological regulation via destruction. Bioessays 2000;22:442-51.
31. Bashir T, Pagano M. Aberrant ubiquitin-mediated proteolysis of cell cycle regulatory proteins and oncogenesis. Adv Cancer Res 2003;88:101-44.
32. Weissman AM, Shabek N, Ciechanover A. The predator becomes the prey: regulating the ubiquitin system by ubiquitylation and degradation. Nat Rev Mol Cell Biol 2011;12:605-20.

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