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High-expressed PTPN1 promotes tumor proliferation signature in human hepatocellular carcinoma

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ABSTRACT

Hepatocellular carcinoma (HCC) is a highly prevalent malignant tumor that is associated with substantial morbidity and mortality rates. Despite the progress made in diagnostic technology, the survival rate of HCC patients remains unsatisfactory due to the complex nature and extensive metastasis of the disease. Consequently, the discovery of new molecular targets is of great practical significance for the diagnosis and treatment of HCC. Protein tyrosine phosphatases (PTPs) play a crucial role in cell signal transduction by catalyzing the dephosphorylation of tyrosine residues in proteins. The present study has revealed that the upregulation of protein tyrosine phosphatase non-receptor type 1 (PTPN1) is a characteristic feature of HCC and is associated with a poor prognosis. Additionally, our investigation into the functional roles of PTPN1-regulated genes in HCC has demonstrated that alterations in PTPN1 expression disrupt normal cell cycle progression metabolism. Additionally, the capacity for proliferation and migration of HCC cells was notably diminished subsequent to PTPN1 silencing, resulting in the prevention of cell entry into the S phase from the G1 phase. Our investigation indicates that PTPN1 may facilitate the onset and progression of HCC by disrupting the cell cycle, thereby presenting a promising molecular target for the diagnosis and treatment of liver cancer.

1. Introduction

Protein tyrosine phosphatases (PTPs) are a large family of enzymes that can regulate the phosphorylation level of protein tyrosine.

Abbreviation: ADH1C, Alcohol dehydrogenase 1C; APOC3, Apolipoprotein C3; ATP1A1, ATPase Na+/K+ transporting subunit alpha 1; DFS, Disease-free survival; Drp1, Dynamin-related protein 1; DSS, Disease-specific survival; DTYMK, Deoxythymidylate kinase; ECM, Extracellular matrix; GEPIA, Gene Expression Profiling Interactive Analysis; HCC, Hepatocellular carcinoma; HPD, 4-hydroxyphenylpyruvate dioxygenase; HR, Hazard Ratio; ICGC, International Cancer Genome Consortium; ITGB1, Integrin subunit beta 1; MMP2, Matrix metallopeptidase 2; MMP9, Matrix metallopeptidase 9; NRAS, Neuroblastoma RAS viral oncogene homolog; NSCLC, Non-small cell lung cancer; OS, Overall survival; PFS, Progression-free survival; PTP1B, Protein tyrosine phosphatase 1B; PTPN, Non-receptor PTP; PTPN1, Protein tyrosine phosphatase non-receptor type 1; PTPs, Protein tyrosine phosphatases; SEC24C, SEC24 homolog C; TCGA, The Cancer Genome Atlas.

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The abnormal activity of PTPs is related to the progress of many diseases [1]. There is evidence that PTPs play a crucial role in metabolism and can act as regulatory factors of liver homeostasis [2]. According to substrate specificity and protein structure, PTPs can be divided into four categories. Non-receptor PTP (PTPN) are the most well-known subfamilies, which play an important role in many life activities, especially in the processes related to metabolism in the human body [2]. PTPN1 is an important family member involved in developing many diseases, including obesity, diabetes, cardiovascular diseases, and cancer [3].

PTPN1 is a special member of tyrosine phosphatase, which is involved in the regulation of many signal cascade reactions. For example, PTPN1 played a significant role in JAK-STAT signal transduction and was considered an essential regulator between metabolic diseases and inflammation and immune response, and cancer [4]. The expression of PTPN1 was related to immunity, and the expression of PTPN1 was increased in tumor T cells. The increase of PTPN1 in T cells limited the expansion and cytotoxicity of T cells and promoted tumor growth. Because of its important role in immune cells, PTPN1 was regarded as an intracellular checkpoint and immunotherapy target [5]. PTPN1 also played an important role in regulating metabolic pathways, which affected the development of type 2 diabetes mellitus and obesity by regulating insulin and leptin signal transduction [6]. Baburajeev et al. conducted prior research indicating that a synthesized small molecule inhibitor of protein tyrosine phosphatase 1B (PTP1B, also known as PTPN1) demonstrated significant efficacy in suppressing cell invasion and reducing tumor volume in HCC [7]. Furthermore, the inhibition of PTP1B through sorafenib presents a promising therapeutic approach for the treatment of HCC [8]. In recent years, PTPN1 inhibitors have been considered a potential therapeutic strategy for treating type 2 diabetes mellitus. In addition, PTPN1 was widely expressed in various cells and tissues and is regarded as one of the primary negative regulators of the insulin receptor signaling pathway [9].

In addition, some studies identified PTPN1 as a critical participant in cancer, which was both a tumor suppressor and a tumor promoter [4]. Glioma was a common primary brain tumor. It was reported that PTPN1 was overexpressed in glioma. By knocking down the expression of PTPN1 in glioma cells, it was found that PTPN1 was silenced, which reduced the expression of matrix metallopeptidase 2 (MMP2) and matrix metallopeptidase 9 (MMP9), thus significantly reducing the proliferation and migration ability of glioma. Moreover, further experiments in vitro showed that PTPN1 promoted the occurrence and development of glioma by activating MAPK/ERK and PI3K/AKT pathways [10]. In addition, the role of PTPN1 has also been reported in other cancers. Pancreatic cancer is a highly malignant cancer. It was found that PTPN1 was highly expressed in pancreatic cancer, and it was related to the tumor stage. Silent PTPN1 significantly inhibited tumor cells' growth, migration, and colony-forming ability. The mechanism study showed that PTN1 might regulate cell growth through the PKM2/AMPK/mTOC1 signaling pathway [11]. In addition, the abnormal expression of PTPN1 was also related to the development of ovarian and breast cancer [12,13].

HCC is one of the most common malignant tumors, and its incidence has increased in recent years [14]. PTPN1 is a crucial factor in cancer, but there is little research on liver cancer. Studies have found that the expression of PTPN1 is increased in HCC [15]. However, the mechanism of PTPN1 in HCC needs to be clarified. Combining bioinformatics with experiments in vitro, our study not only found the influence of PTPN1 on the prognosis of patients with liver cancer but also explored the possible mechanism of PTPN1 in promoting the progress of HCC. Our study provided evidence for PTPN1 as a potential therapeutic target for liver cancer and provided help for liver cancer research and clinical drug development.

2. Materials and methods

2.1. Data collection

The data relating to liver cancer in this study were basically from The Cancer Genome Atlas (TCGA, n = 371 patients) and International Cancer Genome Consortium (ICGC, n = 445 patients) databases.

2.2. cBioPortal

The cBio Cancer Genomics Portal is an open access resource, providing data from more than 5000 tumor samples from 20 cancer studies [16]. This study used cBioPortal resources to explore the mutation rate of the PTPNs family in HCC.

2.3. Gepia

GEPIA (Gene Expression Profiling Interactive Analysis) is a network tool based on TCGA and GTEx data, which provides functions such as gene expression analysis, correlation analysis, and patient survival analysis [17]. In this study, the GEPIA website explored the clinical staging of the PTPNs family in HCC and the prognostic analysis of HCC patients.

2.4. Functional enrichment analysis

The clinical information analysis of PTPN1 from the TCGA and ICGC data set R v4.0.3 software from assistant for clinical bioinformatics. Spearman correlation analysis was used to analyze the correlation between the PTPN1 gene and pathway score.

2.5. Cell culture

The Huh-7 cell line was obtained from American Type Culture Collection (ATCC, Manassas, VA, USA) and authenticated by ATCC with short tandem repeat genotyping analysis. Huh-7 cells in early passages were used to maintain a close resemblance to the original

HCC cancerous cells. This avoided the potential for culture-induced cell instability and selective growth of rapidly growing cells with more significant molecular abnormalities. Huh-7 cells were maintained in tissue culture dishes (diameter 100 mm) in phenol red-free Dulbecco's Modified Eagle Medium (Fisher Scientific, Waltham, MA, USA) that was supplemented with 10% (v/v) heat-inactivated and charcoal-stripped FBS (Fisher Scientific), 1% antibiotics of 50 U/mL penicillin, and 50 μg/mL streptomycin (Invitrogen, Grand Island, NY, USA) at 37 °C and 5% CO_2/95% air. When the initial cells (1×10^5/mL) became ~70% confluent, the cells were starved with medium-low in serum (0.1% v/v FBS) for 16 h before treatments.

HepG2 was obtained and authenticated with STR profile in The Cell Bank of Type Culture Collection of the Chinese Academy of Sciences (www.cellbank.org.cn). Human hepatoma cell HepG2 was cultured in DMEM medium containing 10% fetal bovine serum (FBS) (C2056-1A), 100 units/ml penicillin, and 100 μg/mL streptomycin. They were cultured in a humidified incubator with 37 °C and 5%CO_2.

2.6. siRNA transfection

HepG2 cells were treated and counted, then adjusted to 2×10^6 cells/mL for transfection. For transient small interfering (si) RNA transfection, cells were transfected with siRNA targeting PTPN1 (si-PTPN1-1: GUCGGAUUAAACUACAUCATT, si-PTPN1-2: UGAUGUAGUUUAAUCCGACTT) and control siRNA (si-NC), constructed by Shanghai GenePharma Co., Ltd. After collecting samples and replacing the complete culture medium, samples were collected for analysis after 24–72 h of culture according to different experimental requirements.

2.7. qRT-PCR

Total RNA was extracted from fresh samples and cell lines using TRIzol reagent (Takara Bio, Inc.), and cDNA was prepared using PrimeScript RT reagent kit (Roche Diagnostics), according to the manufacturer's protocols. RT-qPCR was performed on a CFX96 Thermal Cycler Dice™ real-time PCR system (Bio-Rad Laboratories, Inc.) using SYBR™ Green Master Mix (BioTools Pty. Ltd.) under the following cycling conditions: 3 min at 95 °C, followed by 35 cycles of 10 s at 95 °C and 45 s at 58 °C. For the PTP1B gene, the primers were 5′-GCCATTCATTTTCTCCAAAGTGA-3′ (forward) and 5′-CGACCCGACTTCTAACTTCAGTGT-3′ (reverse). For the β-actin gene, the primers were 5′-TCACCCACACTGTGCCCATCTACGA-3′ (forward) and 5′-TCGGTGAGGATCTTCATGAGGTA-3′ (reverse).

2.8. Western blotting analysis

RIPA cell lysate was used to lysate the cells of each group on ice to extract total protein. The protein was quantified by the BCA method. Then SDS-PAGE was performed, and 40 μg protein was added to each well. After electrophoresis, the proteins were transferred to the PVDF membrane surface. PVDF membrane was treated in 5% skim milk powder for 1 h, and TBST was used to clean the PVDF membrane three times for 10 min each time. Rabbit anti-PTPN1 (Sigma, SAB4502525) and Mouse anti-β-actin (Proteintech, 20536-1-AP) were added overnight at 4 °C. After that, the PVDF membrane was cleaned with TBST solution, and corresponding HRP labeled secondary antibodies (rabbit anti-mouse and sheep anti-rabbit) were added and incubated at room temperature for 90min. After TBST solution cleaning, the PVDF membrane was treated with ECL chemiluminescent reagent and developed. Protein bands were analyzed.

2.9. Cell proliferation assay

Cells at the logarithmic growth stage were placed in 96-well plates, and cell proliferation was detected by the CCK-8 kit. The absorbance (A) of each well at the wavelength of 450 nm was measured under the enzyme label, and the proliferation curve was plotted. Cells were cultured at 37 °C, and 5% CO2 and CCK-8 reagents were added to detect cell proliferation at 1, 2, 3, 4, and 5 days, respectively.

2.10. Cell migration assay

Cells at the logarithmic growth stage were taken and spread into a 6-well plate. After the cells were attached to the wall, a line was drawn in the center of the 6-well plate with a sterile gun tip, washed with PBS, and DMEM medium without serum was added. Observation and photos were taken at 0 h and 24 h after the scratch, and the scratch area was calculated.

2.11. Cell cycle detection

The cells of each group were collected, added with 70% ethanol, and fixed overnight at 4 °C. After washing with PBS the next day, each group of cell samples was added with propidium iodide staining solution, kept away from light at 37 °C for 30min, and then detected by flow cytometry.

2.12. Statistical analysis

All experiments were repeated three times, and the data were the mean ± standard deviation (SD) of three or more independent experiments. SPSS performed the statistical analysis, and the student t-test was used to evaluate the wound healing experiment. The

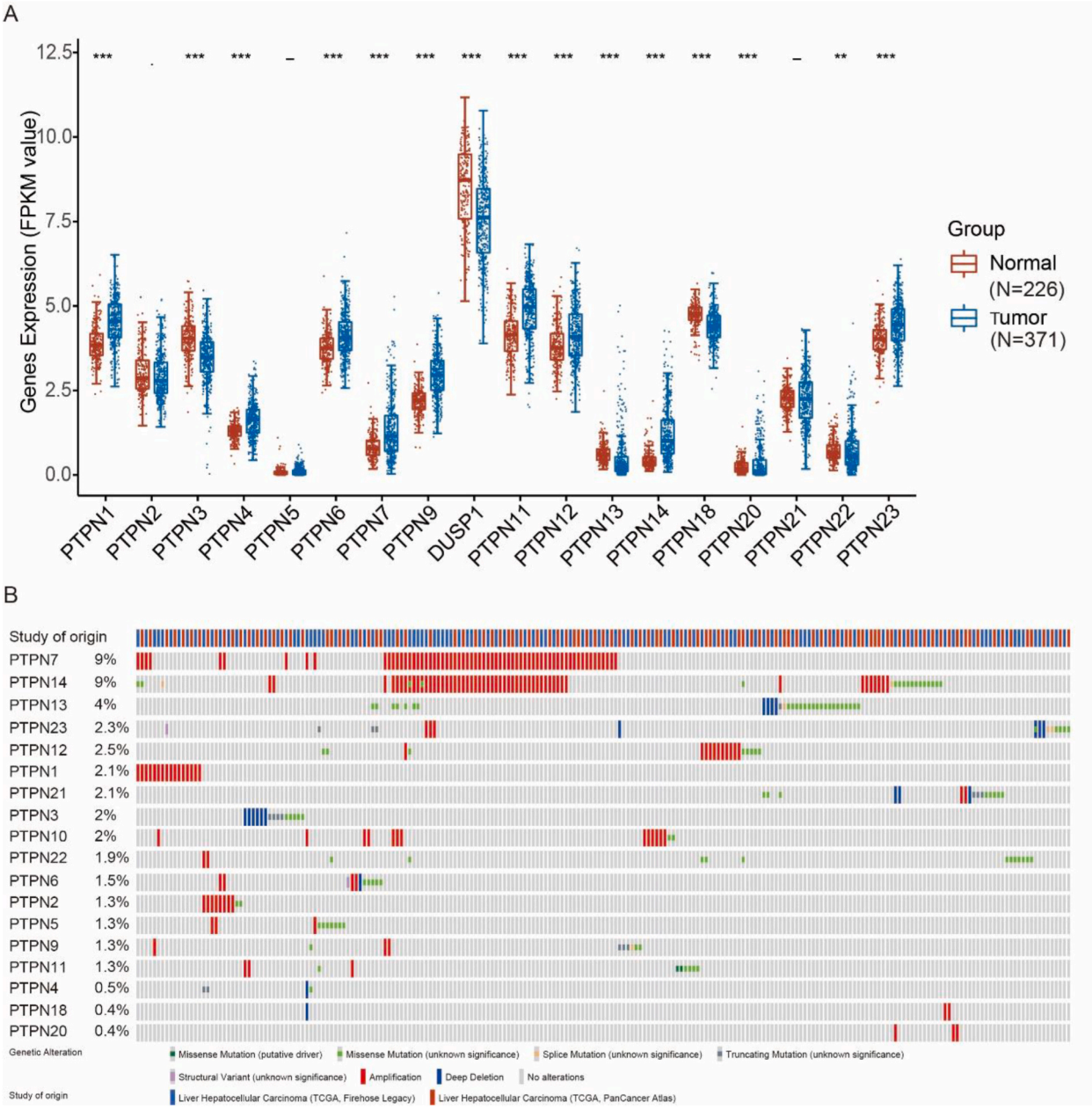

Fig. 1. Differential expression of the PTPNs family in liver cancer. (A) Expression of PTPNs family in 226 healthy controls and 371 patients with HCC in the TCGA database. The red box represents healthy controls, the blue box represents liver cancer patients, and the median line represents the average. ** indicates $P < 0.01$, and *** indicates $P < 0.001$. The expression levels of PTPN1, PTPN4, PTPN6, PTPN7, PTPN9, PTPN11, PTPN12, PTPN14 and PTPN23 in HCC were significantly higher than those in healthy controls ($P < 0.001$). (B) Mutation analysis of PTPNs family in HCC. PTPN1, PTPN2, PTPN3, PTPN4, PTPN5, PTPN6, PTPN7, PTPN9, DUSP1, PTPN11, PTPN12, PTPN13, PTPN14, PTPN18, PTPN20, PTPN21, PTPN22, and PTPN23 in mutation rate of HCC were 2.1%, 1.3%, 0.5%, 1.3%, 1.5%, 9%, 1.3%, 2%, 1.3%, 2%, 1.3%, 2.5%, 4%, 9%, 0.4%, 0.4%, 2.1%, 1.9%, 2.3%.

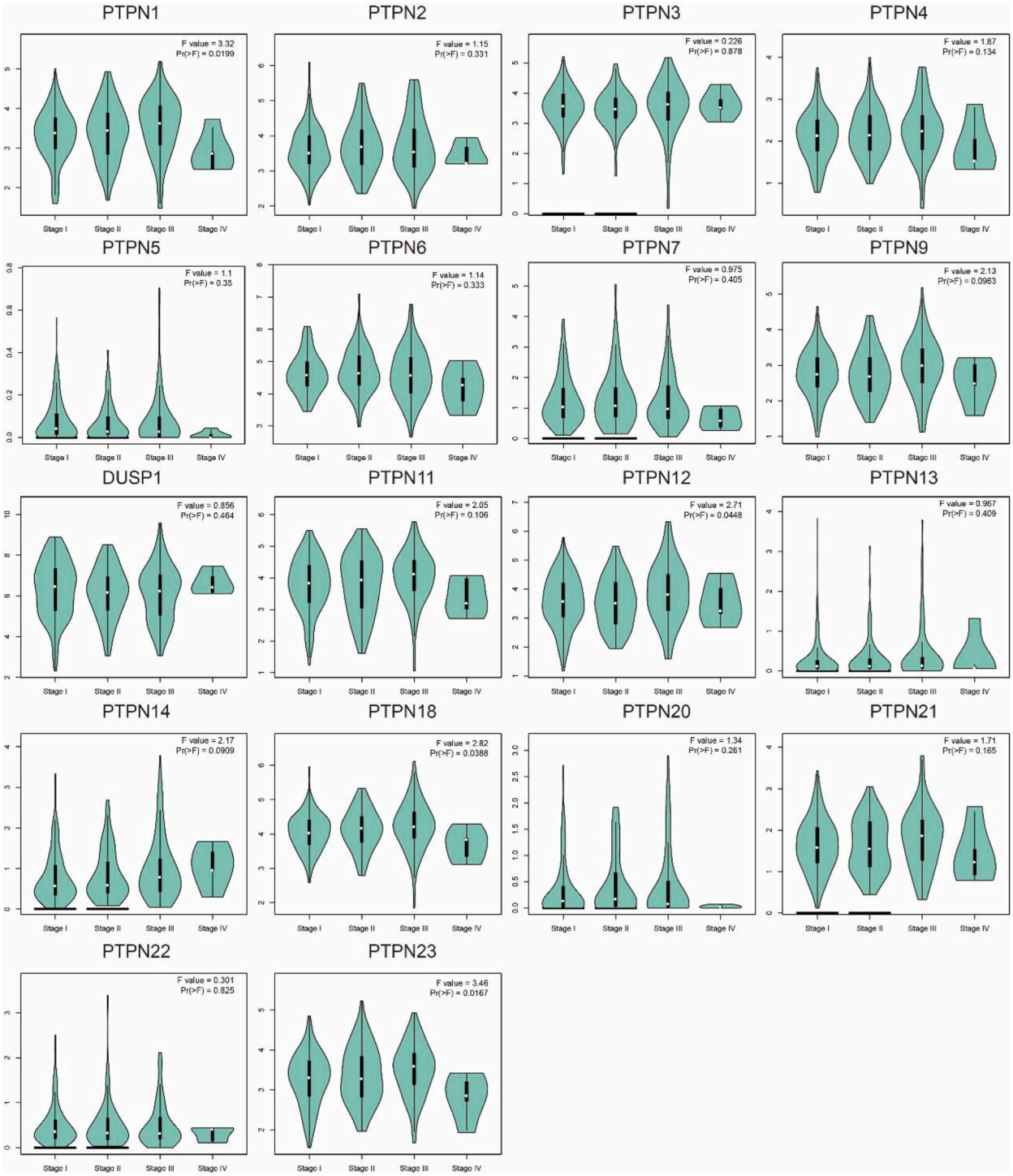

Fig. 2. Correlation analysis between PTPNs family expression and clinical staging in patients with HCC. The expressions of PTPN1, PTPN12, PTPN18, and PTPN23 were significantly correlated with clinical staging.

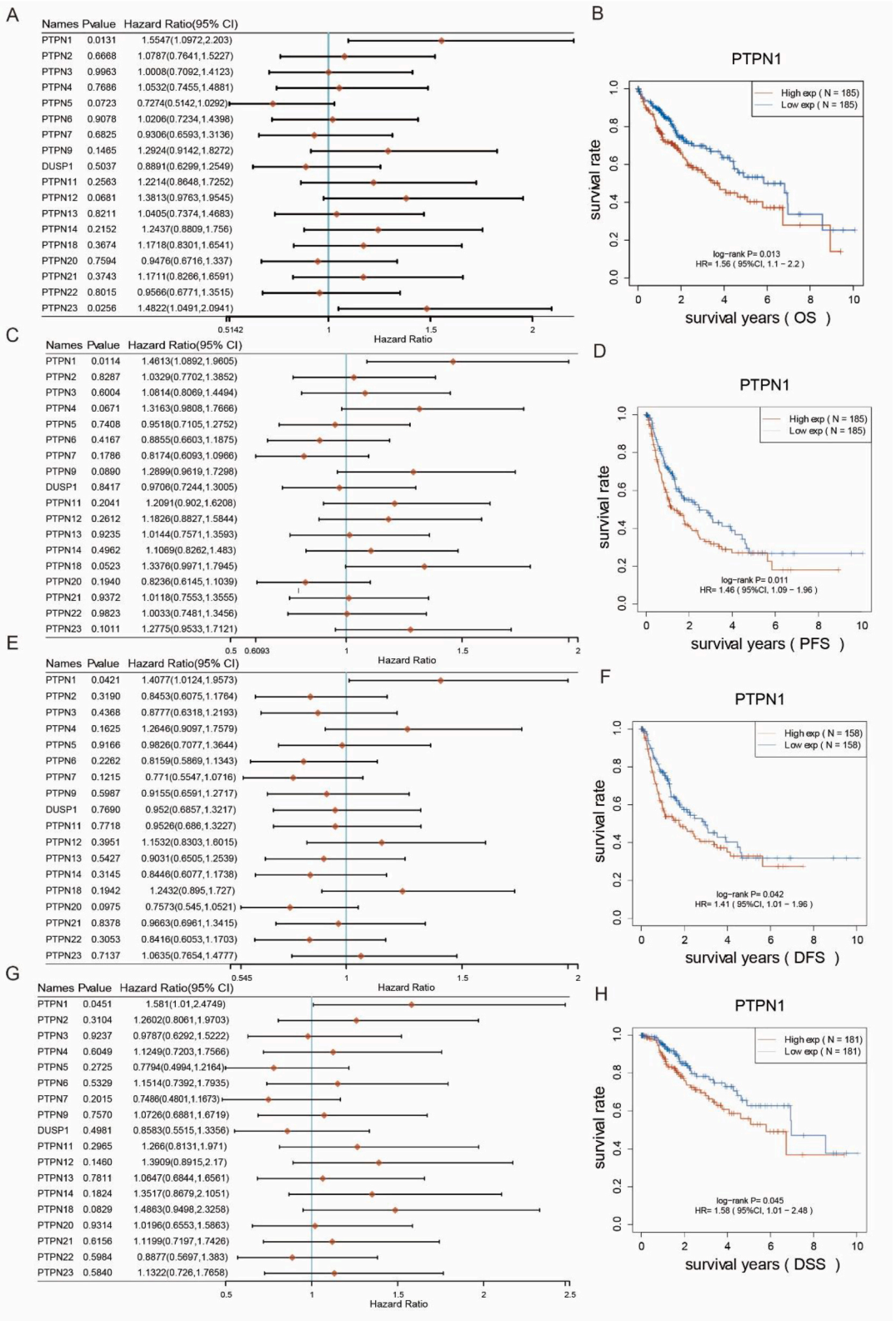

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Fig. 3. Prognostic analysis of PTPN1 expression in patients with liver cancer. (A) Risk ratio analysis based on OS in patients with liver cancer. Gene name, P value, HR and confidence interval are shown in the figure. PTPN1 and PTPN23 were statistically significant ($P < 0.05$). HR > 1, which means that this factor is a risk factor for OS in patients with liver cancer, and the expression of PTPN1 is a risk factor for OS in patients with liver cancer. (B) The difference of PTPN1 expression is significantly related to the OS of patients with liver cancer. The higher the expression of PTPN1, the worse the OS of patients with liver cancer. The red line represents the high expression group of PTPN1 (N = 185), and the blue line represents the low expression group of PTPN1 (N = 185). "log-rank P″ and HR are also shown in figure. (C) Risk ratio analysis based on PFS in patients with liver cancer. HR > 1 means that this factor is a risk factor for PFS in patients with liver cancer, and the expression of PTPN1 is a risk factor for PFS in patients with liver cancer. Only the expression of PTPN1 was statistically significant for PFS in patients with liver cancer ($P < 0.05$). (D) Relationship between the difference of PTPN1 expression level and PFS in patients with liver cancer. The expression of PTPN1 is significantly correlated with the PFS in patients with liver cancer. The higher the expression of PTPN1, the worse the PFS in patients with liver cancer. (E) Based on the risk ratio analysis of patients' DFS, only the expression of PTPN1 was statistically significant ($P < 0.05$), and PTPN1 was a risk factor for DFS in patients with liver cancer. (F) Patients with liver cancer in TCGA database were divided into PTPN1 high expression group (N = 185) and PTPN1 low expression group (N = 185). The higher the expression of PTPN1, the worse the DFS of patients. (G) Risk ratio analysis based on patient DSS. The figure shows that the expression of PTPN1 is a risk factor for DSS in patients with liver cancer. (H) Relationship between PTPN1 expression and DSS in patients. The higher the expression of PTPN1, the worse the DSS of patients.

bioinformatics section used Wilcox tests to assess the differences between the two data sets. The Kruskal-Wallis test was used for the significance of more than two groups of samples. $P < 0.05$ indicated statistical significance.

3. Results

3.1. Different expression levels of the PTPN family in HCC

HCC is a prevalent form of cancer worldwide. The aggressive nature of HCC tumors, characterized by their invasive and metastatic capabilities, contributes to a relatively high mortality rate and diminished quality of life for affected individuals. In this study, we conducted an analysis of the expression patterns of PTPN family members in a cohort consisting of 226 healthy controls and 371 HCC patients, utilizing data from the TCGA database. Our findings, as depicted in Fig. 1A, demonstrate that the expression levels of PTPN1,

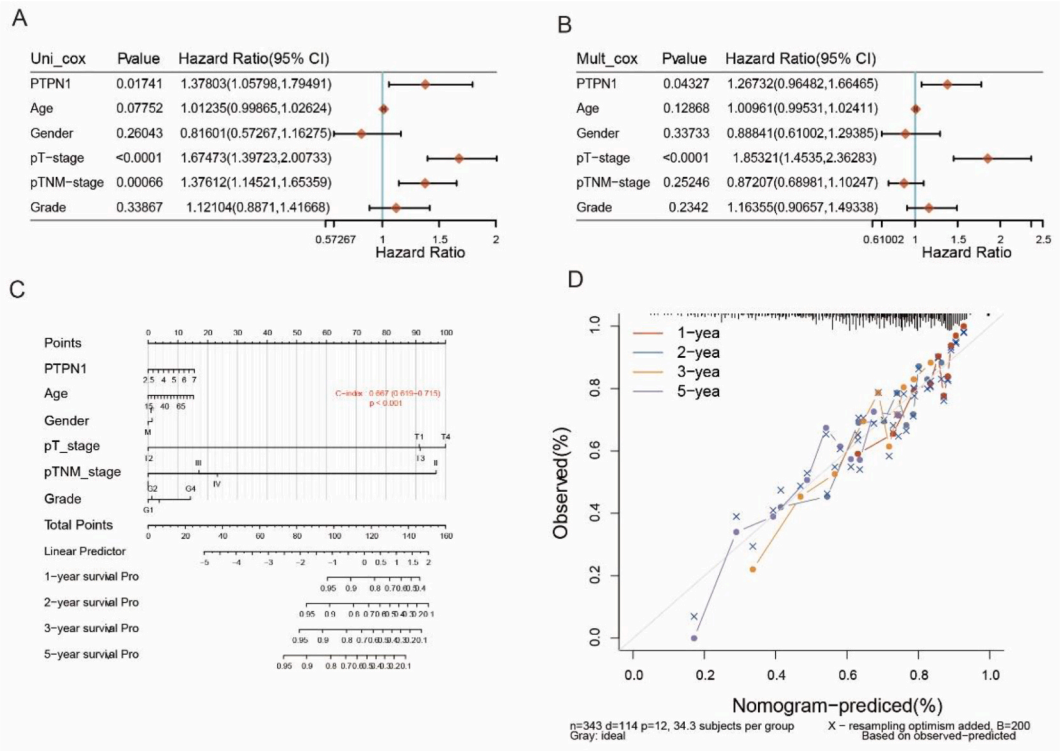

Fig. 4. Advanced prognosis model of PTPN1. (A) Univariate cox analysis of p-value, HR, and confidence intervals for gene expression and clinical features. (B) p-value, HR, and confidence intervals of gene expression and clinical features were analyzed by multivariate cox analysis. (C) The graphs predicted 1 -, 2 -, 3 - and 5-year OS of patients with liver cancer. Draw a straight line vertically on the corresponding axis of each risk factor until you reach the top line marked "points". Add the points for all risk factors and draw a line from the axis of "total points" until it intercepts each axis to determine the probability of survival in 1, 2, 3, and 5 years. (D) Calibration curve of the OS column diagram model in the group. The diagonal dashed lines represent the ideal column lines, and the red, blue, orange, and purple lines represent the observed 1y, 3y, and 5y column lines.

PTPN4, PTPN6, PTPN7, PTPN9, PTPN11, PTPN12, PTPN14, PTPN20, and PTPN23 were significantly elevated in liver cancer patients compared to healthy individuals ($P < 0.001$). The expression levels of PTPN3, DUSP1, PTPN13, PTPN18, and PTPN22 in patients with liver cancer were significantly lower than those in healthy people ($P < 0.001$). There was no significant difference in the expression levels of PTPN2, PTPN5, and PTPN21 between liver cancer patients and healthy people. We further analyzed the mutations of the PTPNs family in liver cancer. PTPN1, PTPN2, PTPN3, PTPN4, PTPN5, PTPN6, PTPN7, PTPN9, DUSP1, PTPN11, PTPN12, PTPN13, PTPN14, PTPN18, PTPN20, PTPN21, PTPN22, and PTPN23 in mutation rate of liver cancer were 2.1%, 1.3%, 0.5%, 1.3%, 1.5%, 9%, 1.3%, 2%, 1.3%, 2%, 1.3%, 2.5%, 4%, 9%, 0.4%, 0.4%, 2.1%, 1.9%, 2.3% and concentrated in Amplification. The mutation rate of PTPN1, PTPN7, PTPN12, PTPN13, PTPN14, PTPN21, and PTPN23 was very high, exceeding 2% (Fig. 1B).

3.2. Establishment of prognostic characteristics of PTPN1

The correlation between the family members of PTPN and clinical staging was examined using the GEPIA website. The analysis revealed that the expressions of PTPN1, PTPN12, PTPN18, and PTPN23 were significantly associated with clinical stages, as depicted in Fig. 2. Additionally, we conducted a comprehensive investigation into the survival and prognosis of patients with PTPN1. Specifically, individuals with liver cancer from the TCGA database were divided into two groups based on their PTPN1 expression levels: the PTPN1 high expression group (N = 185) and the PTPN1 low expression group (N = 185). Overall survival (OS) refers to the time from randomization to death from any cause. Based on the OS of patients, we analyzed the Hazard Ratio (HR) of patients with liver cancer. The figure shows that PTPN1, PTPN2, PTPN4, PTPN6, PTPN9, PTPN11, PTPN12, PTPN13, PTPN14, PTPN18, PTPN21, and PTPN23

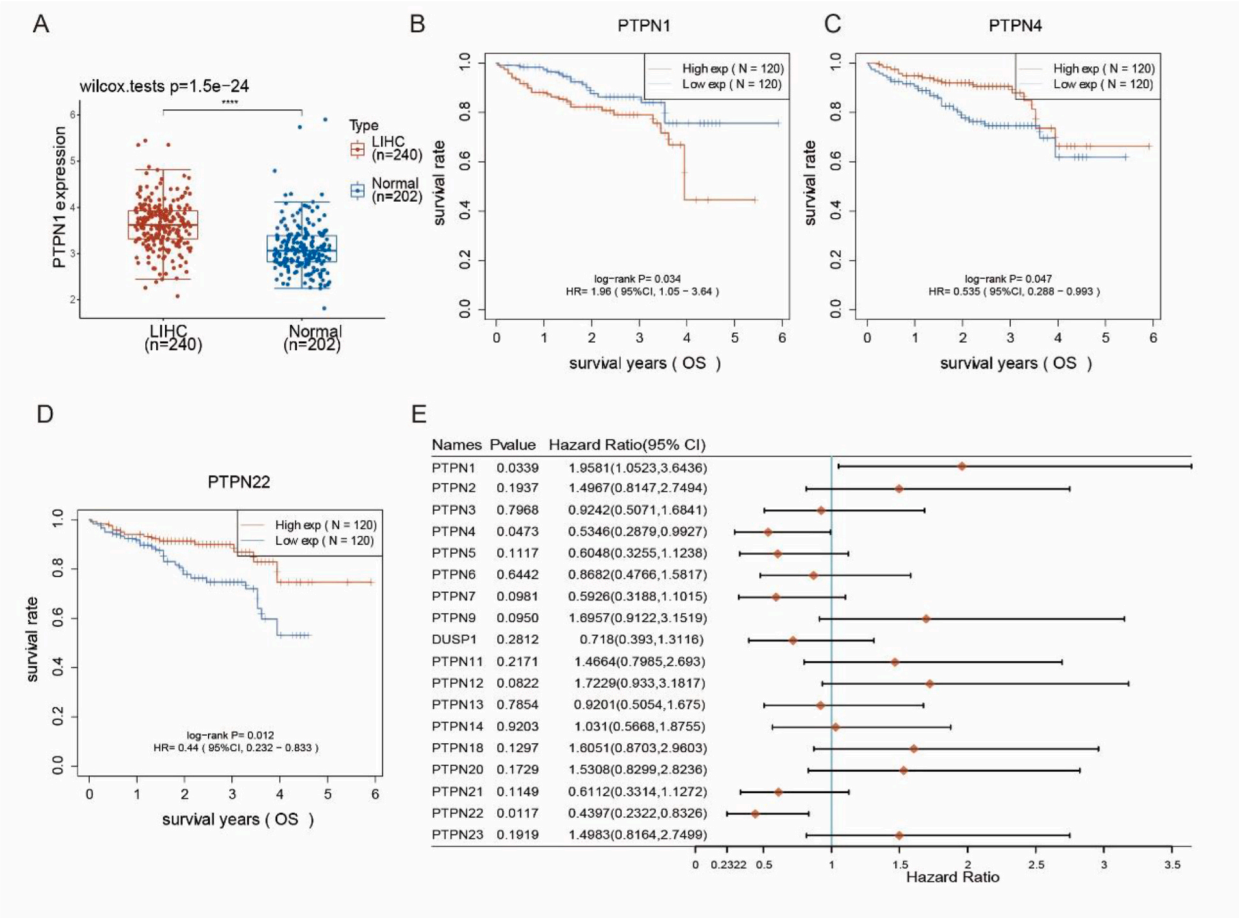

Fig. 5. Expression and prognosis of PTPNs family in liver cancer in ICGC database. (A) The expression of PTPN1 in liver cancer. The red box represents liver cancer patients, the blue box represents healthy controls, and the median line represents the average. The expression level of PTPN1 in 240 patients with liver cancer and 202 healthy controls in ICGC database was significantly higher than that in control group. (B) The effect of PTPN1 expression difference on OS in patients with liver cancer. Liver cancer cases in the ICGC database were divided into PTPN1 high expression group (N = 120) and PTPN1 low expression group (N = 120). As shown in the figure, the higher the expression of PTPN1, the worse the OS of patients. "log-rank P" and "HR" are also shown in figure. (C) The lower the expression of PTPN4, the worse the OS. (D) The lower the expression of PTPN22, the worse the OS. (E) Risk ratio analysis based on patient OS in the ICGC database. HR > 1 indicates that this factor is a risk factor for OS in patients with liver cancer, and HR < 1 indicates that this factor is a protective factor for OS in patients with liver cancer.

are the risk factors affecting the development of liver cancer (Fig. 3A). PTPN1, PTPN23 were statistically significant ($P < 0.05$, Fig. 3A). The figure shows that the OS of patients in the high PTPN1 expression group is worse than that of patients in the low PTPN1 expression group (Fig. 3B). Progression-free survival (PFS) refers to the time from randomization to tumor progression or death from any cause. We also analyzed the PFS of liver cancer patients and divided the liver cancer patients in the TCGA database into two groups: the high-expression group (N = 185) and the low-expression group (N = 185). PTPN1, PTPN2, PTPN3, PTPN4, PTPN9, PTPN11, PTPN12, PTPN13, PTPN14, PTPN18, PTPN21, and PTPN23 are the risk factors for PFS of patients with liver cancer (Fig. 3C). The higher the expression level of PTPN1 in liver cancer patients, the worse the PFS of patients (Fig. 3D). Similarly, we conducted HR analysis based on the PFS of patients with liver cancer. For the influence of PTPN1 on the prognosis of liver cancer, we also analyzed the influence of

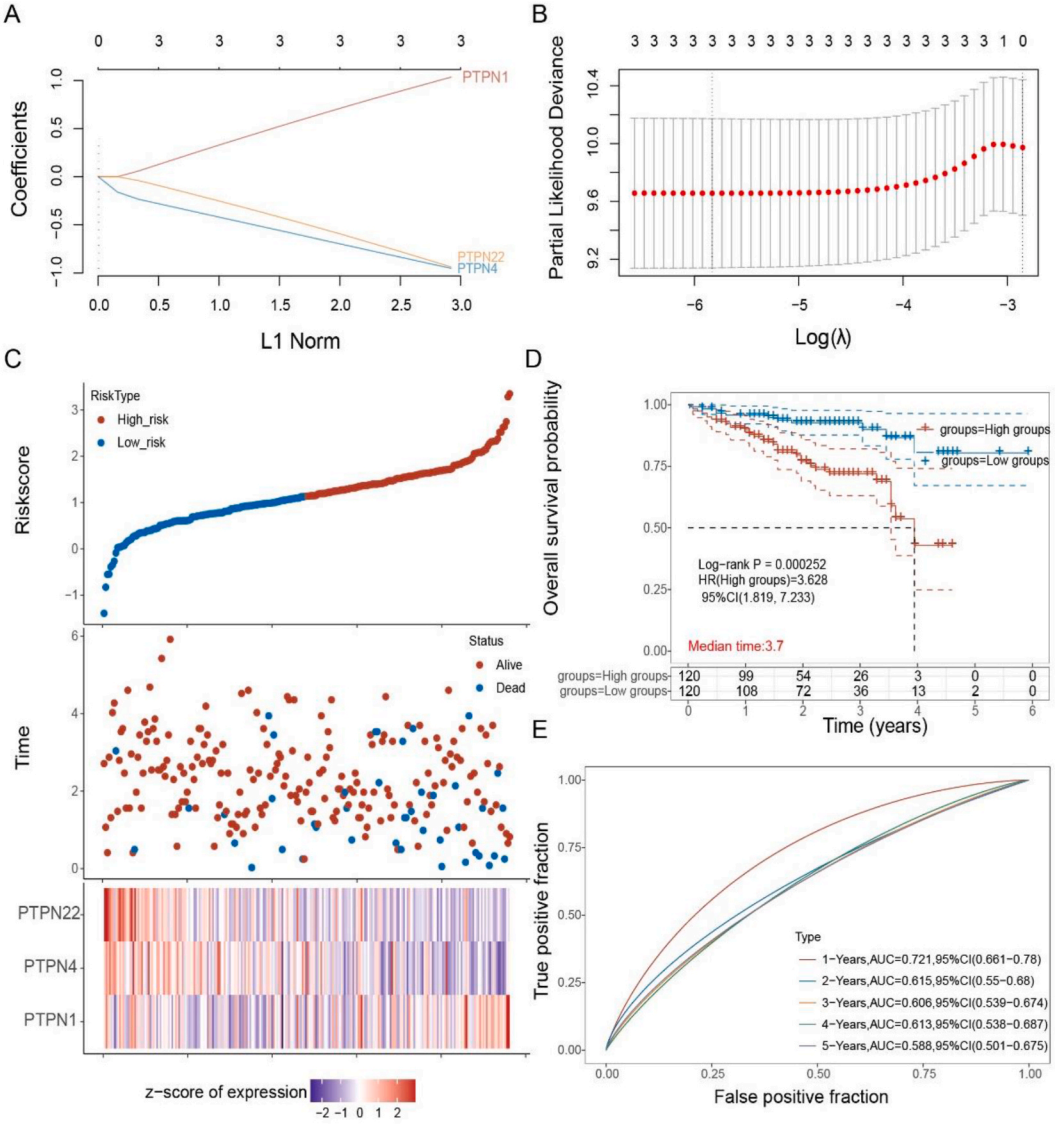

Fig. 6. Advanced prognostic analysis of PTPN1 based on ICGC database. (A) The lambda parameter displays the characteristic coefficients of PTPN1, PTPN4, and PTPN22. The abscissa represents the value of the independent variable lambda, and the ordinate represents the coefficient of the independent variable. (B) The relationship between log (λ) and partial likelihood deviance by Lasso Cox regression model. (C) Relationship between gene expression and survival time and survival status in patients with liver cancer. The bottom figure represents the gene expression heat map of PTPN1, PTPN4, and PTPN22. The figure in the middle represents the scatter plot of survival time and survival state of HCC patients corresponding to the expression of different genes. The top graph represents a scatter plot of risk scores from low to high. Blue represents the low-risk group and red represents the high-risk group. (D) The survival curve of PTPN1, PTPN4, and PTPN22. Log rank P, HR, confidence intervals are shown in the graph. HR > 1 indicates that the combination of PTPN1, PTPN4, and PTPN22 is a risk factor. The higher expression of PTPN1 and the lower levels of PTPN4 and PTPN22 showed the worse the prognosis of patients. Median time represents the median survival time (unit year), that is, the time corresponding to the survival rate of 50% in the low and high expression groups of PTPN1. (E) ROC curve and AUC value of PTPN1, PTPN4, and PTPN22 in 1, 2, 3, 4 and 5 years, the higher the AUC value, the stronger the predictive ability of the gene.

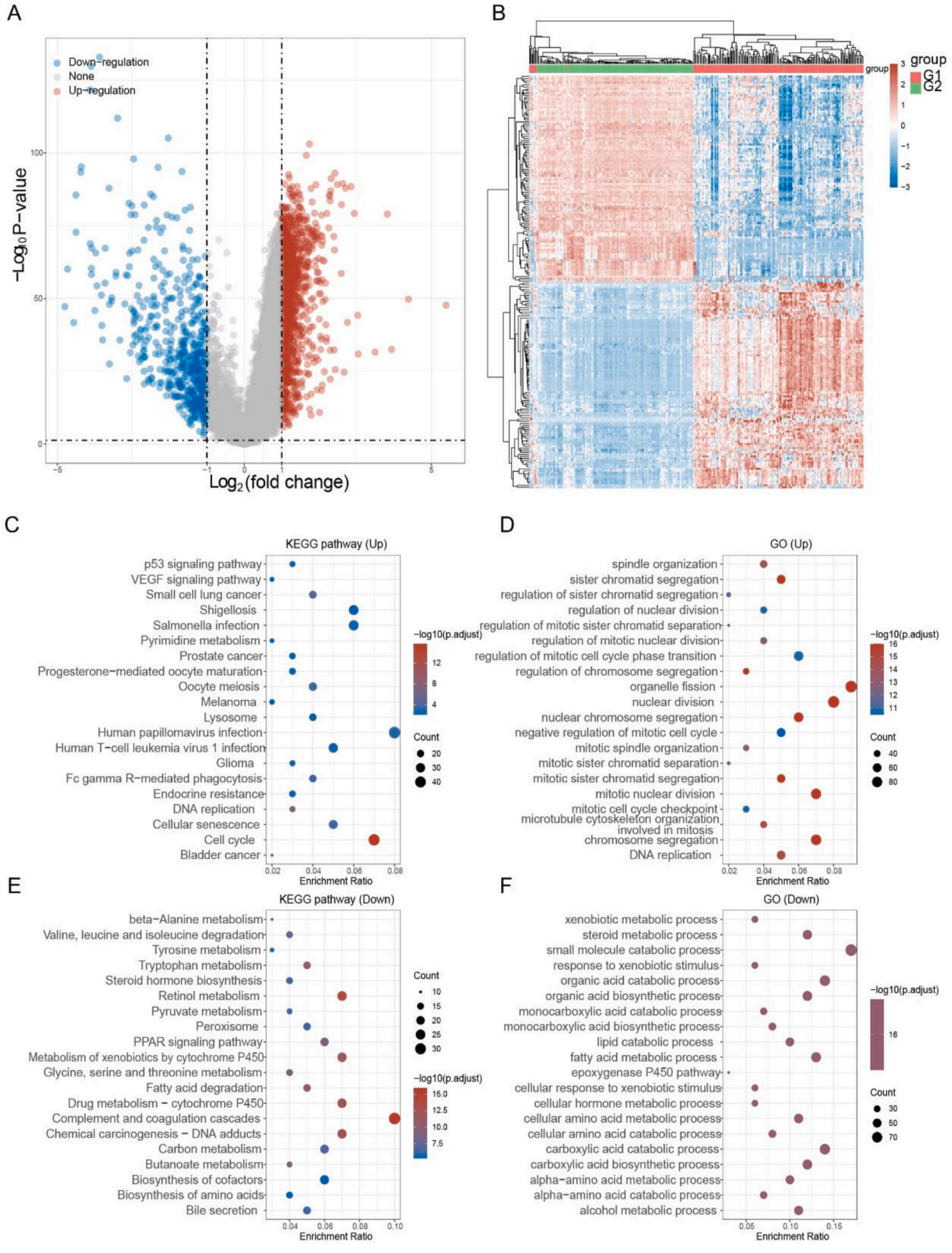

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Fig. 7. Functional analysis of PTPN1 in the ICGC database. (A) Differential expression of PTPN1 regulated genes in liver cancer. Blue dots represent down-regulated genes, red dots represent up-regulated genes, and gray dots represent genes with no change in expression. (B) Heat map of differential expression of top50 genes regulated by PTPN1. (C) KEGG pathway analysis revealed the related functions of genes up-regulated by PTPN1. The legend on the right showed significance and quantity. The redder the color, the more significant it is. (D) GO terminology analysis revealed related functions of PTPN1 upregulated genes in liver cancer. (E) KEGG pathway analysis revealed related functions of PTPN1 down-regulated genes in liver cancer. (F) GO terminology analysis showed related functions of PTPN1 down-regulated genes in liver cancer.

the change of PTPN1 expression on the disease-free survival (DFS) of liver cancer patients. DFS refers to the time from randomization to disease recurrence or death from any cause. Patients with liver cancer were divided into PTPN1 high expression group (N = 185) and PTPN1 low expression group (N = 185). We further analyzed the HR based on DFS and found that PTPN1, PTPN4, PTPN12, PTPN18, and PTPN23 were the risk factors for DFS in patients with liver cancer (Fig. 3E). The results showed that the higher the expression of PTPN1, the worse the DFS of patients (Fig. 3E and F). We further analyzed the HR based on DFS and found that PTPN1, PTPN4, PTPN12, PTPN18, and PTPN23 were the risk factors for DFS in patients with liver cancer (Fig. 3F). Finally, we analyzed the influence of the change of PTPN1 expression on the disease-specific survival (DSS) of patients with liver cancer, which refers to the time of death caused by a specific disease. HR analysis showed that PTPN1, PTPN2, PTPN4, PTPN6, PTPN9, PTPN11, PTPN12, PTPN13, PTPN14, PTPN18, PTPN20, PTPN21, and PTPN23 were all risk factors for DSS in patients with liver cancer (Fig. 3G). The experimental results showed that the higher the expression of PTPN1, the worse the DSS of patients (Fig. 3G and H). Based on the HR analysis of patients' OS, PFS, DFS, and DSS, the results showed that PTPN1 was a risk factor affecting patients' OS, PFS, DFS, and DSS, and it was statistically significant ($P < 0.05$). To sum up, the higher the expression of PTPN1, the worse the prognosis of patients.

To further explore the conditions affecting the development of liver cancer, we conducted univariate and multivariate cox analyses. Univariate analysis showed that the expression of PTPN1, pT-stage, and pTNM-stage had significant effects on liver cancer, and they were all risk factors for liver cancer. Multivariate analysis showed that the expression of PTPN1 and pT-stage significantly impacted the occurrence of liver cancer, and they were all risk factors for liver cancer. Based on these findings, we configured a nomogram (Fig. 4C), which predicted the 1-,2-,3-and 5-year survival rates of patients with liver cancer. Fig. 4D also predicted the 1-, 2-, 3- and 5- year survival rates. The figure shows the p-value, HR, and confidence intervalof gene expression and clinical features (Fig. 4A–B).

3.3. Expression and prognosis of PTPN1 were analyzed based on the ICGC database

To further substantiate the impact of PTPN1 expression on liver cancer prognosis, an analysis was conducted to compare the levels of PTPN1 expression between 240 liver cancer patients and 202 healthy individuals in the ICGC database. The findings revealed a significant elevation in PTPN1 expression among liver cancer patients compared to the healthy control group (Fig. 5A), which aligns with the results obtained from the TCGA database. Subsequently, the 240 liver cancer patients were categorized into two groups based on PTPN1 expression levels: a high expression group (N = 120) and a low expression group (N = 120). The findings of this study indicate a positive correlation between the expression level of PTPN1 and the overall survival (OS) in liver cancer patients. Furthermore, the change in PTPN1 expression level significantly influences the occurrence and progression of liver cancer, as demonstrated in Fig. 5B. Similarly, liver cancer patients were categorized into high and low expression groups for PTPN4 (N = 120) and PTPN22 (N = 120) (Fig. 5C and D). The results revealed a negative association between the expression levels of PTPN4 and PTPN22 and the OS of patients, as depicted in Fig. 5C and D. Furthermore, the Hazard Ratio (HR) of patients with PAAD was analyzed based on their OS. The results indicated that PTPN1, PTPN4, and PTPN22 were identified as risk factors influencing the development of liver cancer ($P < 0.05$, Fig. 5E). Lasso cox regression models of PTPN1, PTPN4, and PTPN22 are also shown in the figure (Fig. 6A–B). Fig. 6C shows detailed prognostic analyses of PTPN1, PTPN4, and PTPN22 associated with prognosis. The risk score, survival status, and gene expression changes are all shown in Fig. 6C. In the ICGC database, patients diagnosed with liver cancer were categorized into two groups based on their expression levels of PTPN1: a high expression group and a low expression group. The findings indicate that higher expression of PTPN1, along with lower levels of PTPN4 and PTPN22, are associated with a poorer prognosis for patients. Fig. 6D provides the hazard ratio (HR) and confidence interval for PTPN1, PTPN4, and PTPN22, with PTPN1 exhibiting an HR of 3.628, indicating it as a risk factor for liver cancer. The median time of survival for patients is 3.7 years, as depicted in Fig. 6D. Additionally, Fig. 6E presents the results of a time-dependent ROC analysis for PTPN1, PTPN4, and PTPN22. Receiver operating characteristic (ROC) analysis was conducted for durations of 1, 2, 3, 4, and 5 years (Fig. 6E). It was observed that a higher area under the curve (AUC) value corresponded to a stronger predictive ability. Notably, the graphical representation indicated that PTPN1, PTPN4, and PTPN22 exhibited the most robust predictive power specifically for the fourth year.

3.4. Functional analysis of PTPN1 in ICGC database

Patients with liver cancer were divided into groups with high expression of PTPN1 and low expression of PTPN1. Differential gene analysis ($FC > 2$, $P < 0.05$) showed differentially expressed genes regulated by the PTPN1 gene in liver cancer in the volcano map (Fig. 7A). Fig. 7B Heat map showing the top50 up-regulated down-regulated genes regulated by the PTPN1 gene. Through functional analysis of the above prominent differential genes, it was found that among the up-regulated genes, KEGG analysis showed that the cell cycle enrichment rate was large and significant, which had a great influence on HCC (Fig. 7C). The cell cycle drives the cell to divide and produce two new daughter cells. And most cellular events, such as DNA replication, transcription, protein translation, and post-translational modification, occur in a cell cycle-dependent manner [18]. In HCC, PTPN1 upregulates the cell cycle function, which

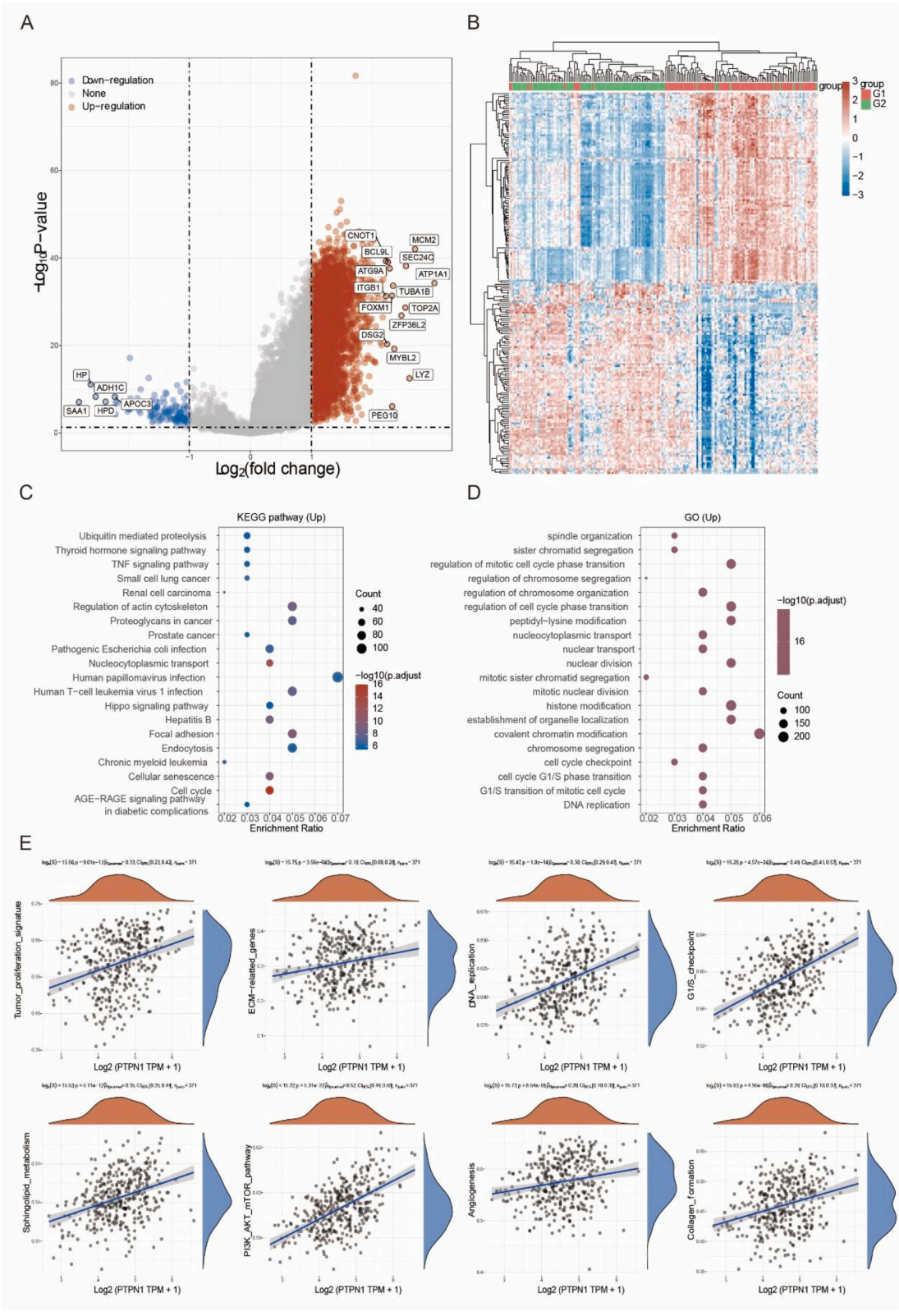

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Fig. 8. Functional analysis of PTPN1 up-regulation in the TCGA database. (A) Differential expression of genes. Blue dots represent down-regulated genes, red dots represent up-regulated genes, and gray dots represent genes with no change in expression. (B) Heat map of differential expression of top50 genes in liver cancer. (C) KEGG pathway analysis revealed related functions of PTPN1 up-regulated genes in liver cancer. (D) GO terminology analysis revealed related functions of PTPN1 upregulated genes in liver cancer. (E) The up-regulated function of PTPN1 overexpression. The x-coordinate represents the expression quantity of PTPN1, the y-coordinate represents the function name, and the density curve on the right represents the function distribution trend. The upper density curve showed the distribution trend of gene expression. The top number represents the correlation p value, correlation coefficient and correlation calculation method.

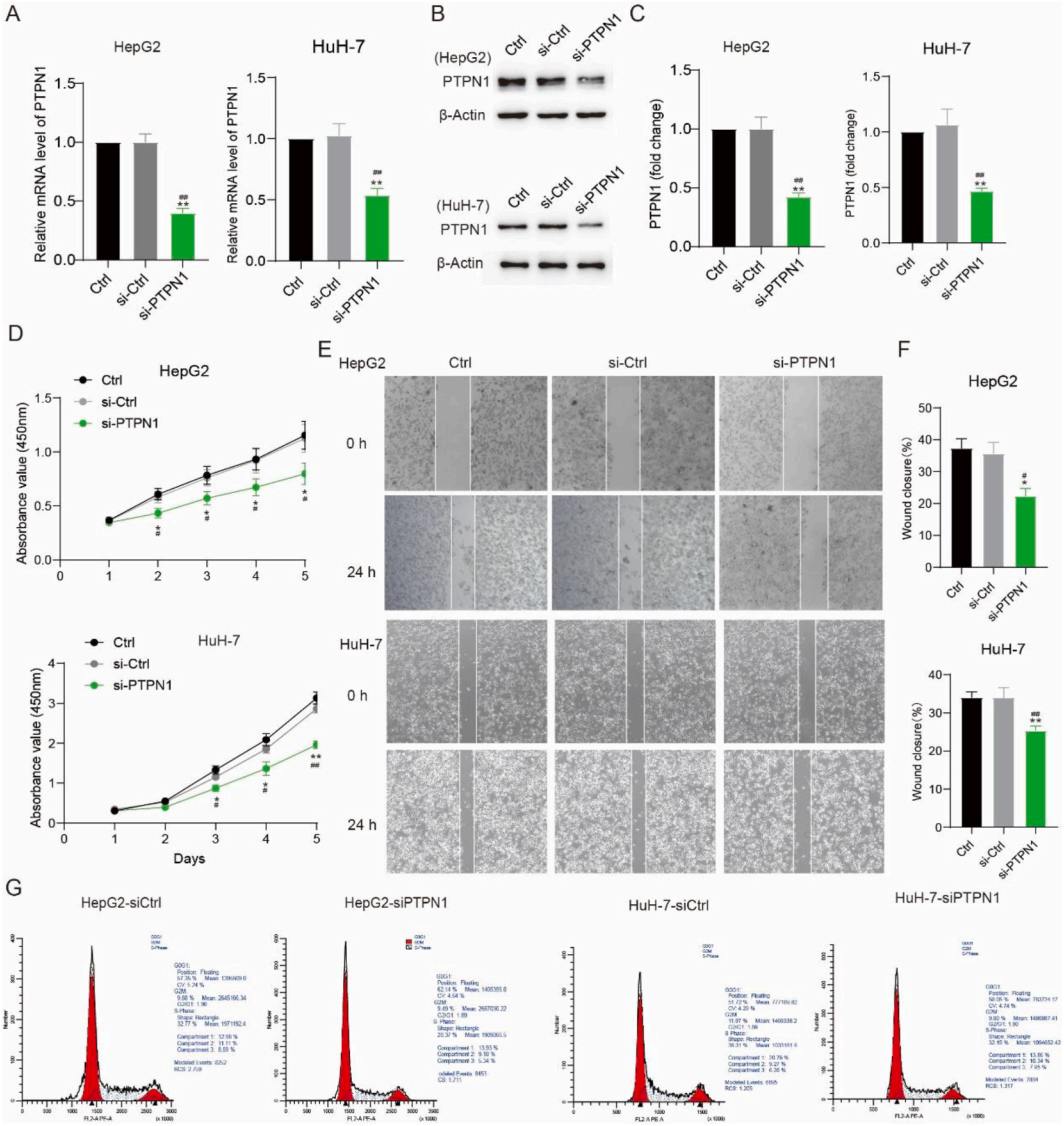

Fig. 9. Functional verification of PTPN1. (A) The expression level of PTPN1 in each group (Ctrl, si-Ctrl, si-PTPN1) of HepG2 and HuH-7 cells was detected by qPCR. (B–C) Western blot assay was used to detect the expression of PTPN1 in liver cancer cells of each group (Ctrl, si-Ctrl, si-PTPN1). (D) CCK-8 was used to detect the proliferation of HCC cells in each group (Ctrl, si-Ctrl, si-PTPN1) at day 1, day 2, day 3, day 4 and day 5. Silencing PTPN1 significantly reduced the proliferation of hepatoma cells. (E–F) The migration ability of liver cancer cells (Ctrl, si-Ctrl, si-PTPN1) was detected by scratch assay. Silencing PTPN1 significantly reduced the migration ability of HCC cells. (G) The cell cycle was measured by flow cytometry. The Kruskal-Wallis test was used for the significance of more than two groups of samples. $p < 0.05$ indicated statistical significance. (*$p < 0.05$, **$p < 0.01$ compared to Ctrl; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared to si-Ctrl).

suggests that PTPN1 may promote the proliferation of liver cancer cells by upregulating the process related to the cell cycle. GO analysis showed that the related functions of genes up-regulated by PTPN1 in HCC mainly concentrated in regulation of cell-cell adhesion, nuclear division, immune response, and so on (Fig. 7D). The above results show that in HCC, high expression of PTPN1 may promote the proliferation of HCC cells by up-regulating the pathway related to the cell cycle. In addition, we analyzed the related functions of genes down-regulated by PTPN1 in liver cancer by KEGG pathway and GO analysis (Fig. 7E–F). The results showed that completion and coalescence cascades and small molecule catabolic process had the most incredible relationship with the occurrence and development of liver cancer. In addition, PTPN1 with high expression down-regulated fatty acid degradation and tyrosine metabolism. In the GO enrichment pathway of down-regulated genes, it was also found that highly expressed PTPN1 mainly affected the occurrence and development of HCC by down-regulating a series of metabolic processes, especially the fatty acid metabolic process.

3.5. Function analysis of PTPN1 in TCGA database

Additionally, utilizing the TCGA database, we have successfully validated the specific regulation of differential genes by highly expressed PTPN1. As depicted in Fig. 8A, a total of 1482 genes were observed to be up-regulated, while 725 genes were down-regulated within the TCGA database. Notably, down-regulated genes are represented by blue dots, up-regulated genes by red dots, and unchanged genes by gray dots (FC $>$ 2, P $<$ 0.05). The TOP20 differentially expressed genes were identified and marked, revealing a significant association with energy metabolism. Notably, genes such as SEC24 homolog C (SEC24C), ATPase Na+/K+ transporting subunit alpha 1 (ATP1A1), Integrin subunit beta 1 (ITGB1), Apolipoprotein C3 (APOC3), Alcohol dehydrogenase 1C (ADH1C), and 4-hydroxyphenylpyruvate dioxygenase (HPD) were found to be predominantly linked to energy metabolism. SEC24C can promote the endoplasmic reticulum to form vesicles and select substances to transport to the Golgi apparatus, which has an important influence on life activities [19]. ATP1A1, the main component of active enzyme, can promote the exchange of sodium ions and potassium ions on the plasma membrane, catalyze ATP hydrolysis and provide energy for the active transportation of various nutrients [20]. ITGB1 can regulate cell adhesion and participate in the up-regulation of the kinase (such as PKC) activity and may drive the progress of HCC through PXN/YWHAZ/AKT pathway [21]. APOC3 promotes the assembly and secretion of liver very low-density lipoprotein and may play an important role in lipid metabolism [18]. ADH1C has high activity for ethanol oxidation and plays an important role in ethanol catabolism [22]. HPD is involved in the catabolism of tyrosine by catalyzing the conversion of 4-hydroxyphenylpyruvic acid to

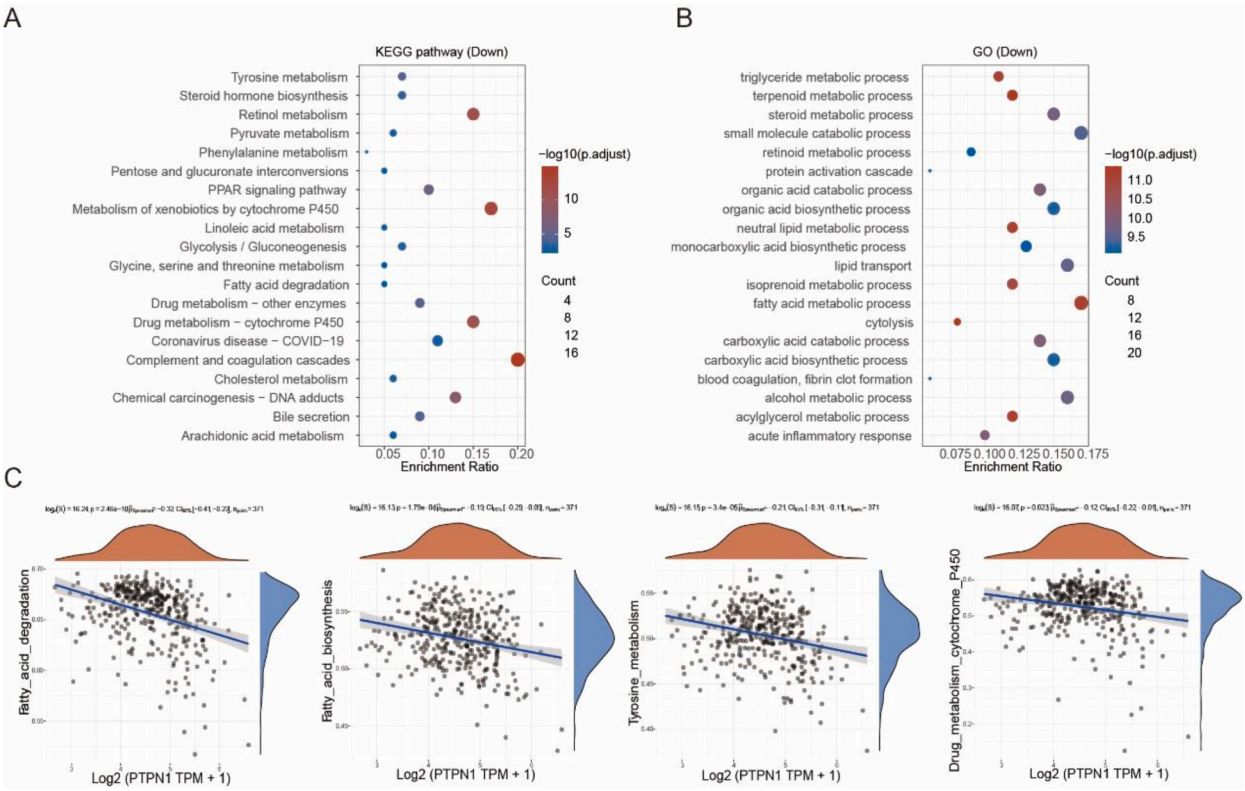

Fig. 10. Functional analysis of PTPN1 downregulation in the TCGA database. (A) KEGG pathway analysis showed the related functions of genes down-regulated by PTPN1. (B) GO terminology analysis showed related functions of genes down-regulated by PTPN1. (C) Analysis of down-regulated function after overexpression of PTPN1.

homogentisic acid [23]. In summary, the aforementioned genes have a significant impact on the body's energy metabolism, thereby suggesting the involvement of PTPN1 in liver cancer's energy metabolism through the regulation of gene expression. This finding holds great importance for the study of liver cancer. Additionally, Fig. 8B illustrates the contrasting expression of the top 50 genes in the TCGA database. Furthermore, the functional analysis of these differentially expressed genes reveals a notable up-regulation of the KEGG pathway associated with the cell cycle, as depicted in Fig. 8C. In the TCGA database, the analysis of gene ontology revealed the functional associations of genes that were upregulated by PTPN1 in HCC. The accompanying figure demonstrated a substantial enrichment ratio for covalent chromatin modification (Fig. 8D). Subsequently, we conducted further analysis to determine the specific functions in liver cancer that were enhanced by the increased expression of PTPN1. The over-expression of PTPN1 significantly upregulated various functions, including Tumor proliferation signature, Extracellular matrix (ECM)-related genes, DNA replication, G1/S checkpoint, sphingolipid metabolism, PI3K AKT mTOR pathway, Angiogenesis, and Collagen formation (Fig. 8E). Tumor proliferation signature, DNA replication, and Angiogenesis are related to the proliferation and development of tumor cells. The up-regulation of DNA replication and angiogenesis suggests that we may have excessive cell proliferation. Therefore, overexpression of PTPN1 may promote the proliferation of HCC cells by up-regulating Tumor proliferation signature, DNA replication, and Angiogenesis, thus promoting the development of HCC. ECM is a dynamic multifunctional structure that provides physical support and biological activity signals for cells in the microenvironment or niche, such as cytokines and growth factors [24]. The interaction between ECM and cells is very important for the proliferation and apoptosis of tumor cells [25]. PTPN1 may promote the growth of tumor cells by up-regulating ECM-related genes. The G1 phase is the prophase of DNA synthesis, and the S phase is the DNA synthesis phase. Studies have shown that a natural polyphenol flavonoid can promote G1/S transformation by activating dynamin-related protein 1(Drp1), thus significantly promoting cell proliferation [26]. Overexpression of PTPN1 may up-regulate the transition from G1 phase to S phase and promote the proliferation of HCC cells. PI3K AKT mTOR pathway is a common signal pathway in tumors, which is involved in the occurrence and development of tumors. PTPN1 may promote the development of liver cancer by activating PI3K signal pathway. Collagen is the most important structural protein and the main component of ECM, which plays a role in tumor progression. Collagen is the main component of the tumor microenvironment, which participates in the fibrosis of cancer and affects the behavior of tumor cells through tyrosine kinase receptors and some signal pathways [11]. In addition, the increase of collagen content in cancer cells leads to drug resistance. To reduce this drug resistance, collagen content is usually adjusted to interfere with the signal pathway of cancer cells and achieve the purpose of treatment [27]. To summarize, Tumor proliferation signature, ECM-related genes, DNA replication, G1/S checkpoint, sphingolipid metabolism, PI3K AKT mTOR pathway, Angiogenesis, and Collagen formation are some functions that promote cancer development. PTPN1 may accelerate the progress of HCC by up-regulating these functions in HCC. We constructed HepG2 and HuH-7 cell lines that interfered with PTPN1. q-PCR and Western blot showed that PTPN1 had obvious interference effect (Fig. 9A–C). In this study, we conducted a comprehensive analysis to investigate the impact of PTPN1 silencing on the proliferation capacity of HCC cells. The proliferation ability of human HCC cells was assessed at various time points (1, 2, 3, 4, and 5 days) using the CCK-8 experiment. Our findings revealed a significant reduction in the proliferation ability of HCC cells following PTPN1 silencing, as depicted in Fig. 9D. Additionally, we employed the scratch test to evaluate alterations in the migration ability of liver cancer cells. The results showed that the migration ability of liver cancer cells was significantly reduced after silencing PTPN1 compared with the control group (Fig. 9E and F). In summary, silencing PTPN1 significantly reduced the proliferation and migration of human HCC cells. Through bioinformatics analysis, we found that PTPN1 significantly affected the cell cycle of HCC cells, so we detected the cell cycle changes of human HCC cells by flow cytometry. The results showed that compared with the control group, the number of cells in the G1 phase increased, and the number of cells in the S phase decreased after silencing PTPN1 (Fig. 9G). This suggests that PTPN1 silencing will block cells from the G1 phase to the S phase and thus reduce the proliferation ability of hepatoma cells.

In addition, we also analyzed the down-regulated function in HCC. KEGG pathway showed that the enrichment ratio of completion and coalescing cascades was large and significant (Fig. 10A). GO analysis showed that the significance of the fatty acid metabolic process was strong (Fig. 10B). We further analyzed which functions were down-regulated by the overexpression of PTPN1. The results showed that PTPN1 down-regulated the functions of fatty acid degradation, fatty acid biosynthesis, tyrosine metabolism, and drug metabolism cytochrome P450 (Fig. 10C). The liver is the main organ of human metabolism. The results of bioinformatics experiments indicate that PTPN1 may promote the occurrence and development of liver cancer by disrupting lipid metabolism in the liver.

4. Discussion

PTPN1 affects the body's metabolic response, which is related to the regulation of insulin and leptin. In addition, the role of PTPN1 in cancer was also evaluated. According to the different environments of cell growth, PTPN1 is a tumor suppressor and a tumor promoter, which plays an important role in cancer [6]. Overexpression of PTPN1 activates src and ERK1/2 signaling pathways, which promotes the proliferation and metastasis of non-small cell lung cancer (NSCLC) and significantly affects the prognosis of patients [28]. In addition, PTPN1 can promote melanoma cells' metastasis and promote melanoma development by interacting with Ser [18]. On the contrary, PTPN1 also acts as a tumor suppressor in esophageal cancer and lymphoma [18,29]. In our study, PTPN1 was found to be a promoter of HCC.

Worldwide, the incidence of liver cancer is very high, and the prognosis of patients is very poor. It is necessary to find new molecular targets to provide the basis for the research of liver cancer. Many studies have shown that abnormal lipid and amino acid metabolism is the basis of tumorigenesis, which is significant in the study of HCC [30,31]. For lipid metabolism, hepatocytes are responsible for mobilizing lipids and storing excess lipids in the form of lipid droplets [32]. Lipid droplets play an important role. Lipid droplets are the providers of inner membrane components and can also provide energy for the proliferation of tumor cells [33]. The metabolic disorder of lipid droplets is related to fatty liver, obesity, and other metabolic diseases. At present, studies also show that

lipid droplets can provide energy pool for invasive cancer and have become a new symbol of cancer [34]. In liver cancer, steatosis leads to the accumulation of lipid droplets, and the number of lipid droplets will also change with the progression of the cancer [35]. In liver cancer, knock-down tumor suppressor gene PTEN and overexpression of Neuroblastoma RAS viral oncogene homolog (NRAS) cooperate to cause metabolic disorders and promote the increase of lipid droplets, thus promoting the development of cancer [36]. In addition, lipid metabolic disorder has also been studied in breast cancer, glioblastoma, prostate cancer, and other cancers [34]. These experiments in vitro showed that lipid droplets were very important for developing liver cancer. Our bioinformatics experiments also showed that PTPN1 participated in the progress of liver cancer by influencing energy metabolism-related genes such as SEC24C, ATP1A1, ITGB1, APOC3, ADH1C, and HPD and interfering with many lipid metabolism reactions (Figs. 7 and 8). It was further verified that the change of PTPN1 expression level in bioinformatics affected the metabolism of liver cancer cells and also indicated that the overexpression of PTPN1 promoted the development of liver cancer by regulating lipid metabolism (Fig. 10). For amino acid metabolism, methionine restriction, a dietary regimen that protects against metabolic diseases and aging, represses cancer growth and improves cancer therapy, especially in hepatocellular carcinoma [37]. Avlant Nilsson et al. found that partial inhibition of glutamate excretion reduced cell growth in liver cancer [38]. The branched-chain amino acid leucine metabolism was proved that BCAT1 decreased cisplatin sensitivity in liver cancer cells by inducing mTOR-mediated autophagy via it [39]. Inhibition of branched-chain amino acid and glutamine metabolism could further retard HCC growth [40]. Higher expression of PTPN1 is associated with drug metabolism cytochrome P450 in hepatocellular carcinoma. Meanwhile, dysregulated Cytochrome P450 expression and activities were reported in hepatocellular carcinoma [41], which further indicates that PTPN1 might play important role in hepatocellular carcinoma potentially through drug metabolism.

This study demonstrated that PTPN1 exhibited elevated expression levels in liver cancer and exerted a substantial impact on the prognosis of patients with hepatocellular carcinoma (HCC). Analysis of data from the TCGA and ICGC databases revealed that PTPN1 served as a risk factor influencing the prognosis of HCC, with higher PTPN1 expression levels correlating with poorer patient outcomes (Figs. 3, Fig. 5, Fig. 6). Additionally, Zhu et al. provided evidence suggesting that the enrichment of PTPN1 within the T cell population may be associated with its exhaustion within the liver cancer microenvironment [15]. In addition, the expression of PTPN1 was also significantly correlated with the clinical staging of HCC (Fig. 2). The limited quantity of patient samples in Stage IV resulted in a significant margin of error during the analysis of expression, consequently yielding a certain level of homogeneity in the expressions (Fig. 2). Compared with the control group, experimental results showed that PTPN1 silencing significantly reduced the proliferation and migration capacity of human hepatoma cells HepG2 (Fig. 9D–F). Through the functional analysis of PTPN1, we found that PTPN1 significantly affected the cell cycle (Fig. 7C). Cell cycle changes are of great significance to the proliferation of cancer cells. Many molecular targets affect the proliferation of liver cancer cells by interfering with cell cycle, such as deoxythymidylate kinase (DTYMK), MicroRNA-455-3p, etc., all affect the development of liver cancer by interfering with the cell cycle of liver cancer cells. Therefore, it can be a potential molecular target for liver cancer research and diagnosis [27,42]. Our results showed that when PTPN1 was silenced, the number of cells in the G1 phase increased significantly, while the number of cells in S phase decreased. The S phase is the period of DNA replication. Silencing PTPN1 causes more cells to undergo G1/S phase arrest and more cells to stay in the G1 phase, thus inhibiting cell proliferation.

In conclusion, this study combined bioinformatics with experiments in vitro to analyze and verify the function of PTPN1 in HCC. PTPN1 is overexpressed in liver cancer, and the higher the expression level, the worse the prognosis of patients. By exploring the function of PTPN1 in liver cancer, we know that PTPN1 may participate in the occurrence and development of liver cancer by affecting lipid metabolism and the cell cycle. Our research is expected to provide a new molecular target for the study and diagnosis of liver cancer and provide a new idea for the development of clinical drugs for the treatment of liver cancer.

Author contribution statement

Zehnan Wu: Conceived and designed the experiments.
Hongmei Wang: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.
Siyun Bu: Performed the experiments.
Liping Xie: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.
Wenxiu Tian: Performed the experiments.
Huimin Qi: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Data availability statement

Data included in article/supp. material/referenced in article.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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