Original Article

The effect of *PPP2CA* expression on the prognosis of patients with hepatocellular carcinoma and its molecular biological characteristics

Jingchang Liang[1#], Yu Huang[2#], Chenglei Yang[1], Shen Huang[1], Jinlong Xie[1], Xiang Nong[1], Jianyong Liu[1], Yumei Zhang[2*], Zhiming Zhang[1*^]

[1]Department of Hepatobiliary Surgery, Guangxi Medical University Cancer Hospital, Nanning, China; [2]Department of Medical Oncology, Guangxi Medical University Cancer Hospital, Nanning, China

Contributions: (I) Conception and design: Y Zhang, Z Zhang; (II) Administrative support: Y Zhang, Z Zhang; (III) Provision of study materials or patients: J Liang, Y Huang; (IV) Collection and assembly of data: C Yang, S Huang; (V) Data analysis and interpretation: J Xie, Y Huang, X Nong, J Liu; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

[#]These authors contributed equally to this work and should be considered as the co-first authors.

[*]These authors contributed equally to this work.

Correspondence to: Yumei Zhang. Department of Medical Oncology, Guangxi Medical University Cancer Hospital, 71 Hedi Rd, Nanning 530021, Guangxi, China. Email: zhzm05@163.com; Zhiming Zhang. Department of Hepatobiliary Surgery, Guangxi Medical University Cancer Hospital, 71 Hedi Rd, Nanning 530021, China. Email: z450211@yeah.net.

Background: To investigate the role of the *PPP2CA* gene in the prognosis of patients with hepatocellular carcinoma (HCC) and its molecular biological characteristics.

Methods: We performed comparison of the expression of *PPP2CA* in HCC and non-HCC tissues of HCC patients who underwent surgery for the first time in the Tumor Hospital of Guangxi Medical University from July 2017 to July 2019, and retrospectively analyzed the relevant clinical data and prognosis. The GSE76427 data set and bioinformatics and public databases were used to compare the expression of *PPP2CA* between HCC and non-cancer tissues. Gene Ontology (GO) analysis was performed of *PPP2CA* and its differential genes and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway. A protein-protein interaction (PPI) network of *PPP2CA* and its differentially expressed genes (DEGs) was constructed from the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database and visualized by Cytoscape software.

Results: The immunohistochemistry (IHC) of tissue sections confirmed that *PPP2CA* was highly expressed in most HCC tissues; the high expression of *PPP2CA* was significantly correlated with microvascular invasion (MVI) and portal vein tumor thrombi (P<0.05). Participants in the *PPP2CA* high expression group had worse overall survival (OS; P=0.04) and recurrence-free survival (RFS; P=0.019). The *PPP2CA* gene and 71 DEGs were mainly enriched in the nuclear division, organelle fission, nuclear chromosome separation, and chromatid separation process, and KEGG analysis revealed enrichment in drug metabolism-cytochrome metabolism of xenobiotics by P450 and cytochrome P450. Finally, through the PPI network, *CCNA2*, *AURKB*, *TOP2A*, *NCAPG*, *MCM2*, *CDC20*, *CCMB2*, *AURKA*, and *MGST1* were identified as the top 9 highly connected hub genes.

Conclusions: The *PPP2CA* gene is highly expressed in HCC tissues. The high expression of *PPP2CA* is significantly associated with poor prognosis. Through the analysis of DEGs, GO and KEGG pathway analysis, it was found that *PPP2CA* may act on liver cancer through multiple targets and multiple pathways, and *PPP2CA* plays a promoting role in HCC.

Keywords: Hepatocellular carcinoma (HCC); *PPP2CA*; prognosis; bioinformatics

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^ ORCID: 0000-0001-9823-4945.

Introduction

Hepatocellular carcinoma (HCC) is a malignant tumor with the highest cancer-related mortality in the world (1,2). The occurrence and invasion of HCC involve the activity and interaction of multiple genes. Protein phosphatase 2A (PP2A) is a serine/threonine phosphatase highly expressed in eukaryotes. It is composed of structural subunit A (PR65), catalytic subunit C (PP2Ac), and a complex composed of various variable regulatory subunits B. The *PPP2CA* gene encodes the α subtype of the PP2A catalytic subunit and regulates PP2A activity by selecting the PP2A regulatory subunit, thereby playing a vital role in the activity of PP2A (3-5). Depending on the setting, PP2A can act as a tumor suppressor or promoter (6,7), but whether PP2A plays an inhibitory or promoting role in HCC has remained controversial. A large number of studies have proved that PP2A is a tumor suppressor in HCC, which mainly includes the following three aspects: PP2A endogenous inhibitor; targeted inhibition of PP2A; PP2A expression of different subunits decrease (8). Kong *et al.* (9) found that jigging acid promotes the occurrence and invasion of HCC by inhibiting the activity of PP2A and promoting the process of epithelial-mesenchymal transition. This shows that PP2A plays an inhibitory role in HCC; however, Gong *et al.* (10) was found that the up-regulation of PP2Ac contributes to the aggressiveness of HCC. Therefore, whether PP2A is a tumor suppressor or a promoter in HCC is still controversial.

In this study, we will combine clinical, immunohistochemistry, and bioinformatics to explore the role of *PPP2CA* in HCC. We retrospectively analyzed 128 HCC patients who underwent surgery for the first time in the Department of Hepatobiliary Surgery, Cancer Hospital of Guangxi Medical University from July 2017 to July 2019, and compared the expression of *PPP2CA* in HCC tissues and non-HCC tissues. We also compared the clinical data and survival of patients with low or high *PPP2CA* expression using bioinformatics and public databases, the *PPP2CA* expression between HCC tissues and non-HCC tissues, and verified the survival rate of patients with low or high *PPP2CA* expression. Finally, we employed various bioinformatics programs to explore the possible pathways and targets of *PPP2CA*'s participation in HCC.

We present the following article in accordance with the REMARK reporting checklist (available at https://dx.doi.org/10.21037/jgo-21-720).

Methods

Data collection

Immunohistochemical (IHC) tests were performed on liver cancer tissues and matched non-liver cancer tissue samples of 128 HCC patients who were treated at the Department of Hepatobiliary Surgery, Cancer Hospital of Guangxi Medical University from July 2017 to July 2019. All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by board of Ethics Committee of Guangxi Medical University Cancer Hospital (No. LW2021097) and informed consent was taken from all the patients. Tissue samples were included for analysis based on the following participant inclusion criteria: (I) underwent radical hepatocarcinoma resection; (II) clearly diagnosed as HCC based on postoperative pathology; (III) did not receive any anti-cancer treatment before surgery; (IV) did not have any malignant tumor other than HCC.

Clinical data were collected including the patient's gender, age, family history of liver cancer, Child-Pugh grade, presence or absence of liver cirrhosis, alpha-fetoprotein (AFP), tumor size, tumor number, Barcelona clinic liver cancer (BCLC) stage, total bilirubin (TBil), aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin (ALB), prothrombin time (PT), surgical resection method, degree of tumor differentiation, whether the capsule was intact, whether there was microvascular invasion (MVI), liver capsule invasion, lymph node metastasis, portal cancer thrombus, bile duct cancer thrombus, P-53, Ki-67, and so on.

Follow-up

All participants were followed up as desired to their time of death, loss to follow-up, or until July 2021. Survival data was drawn from patient medical records and telephone follow-up. Recurrence-free survival (RFS) was defined as the time from surgery to recurrence or death. Overall survival (OS) was defined as the time from surgery to death. Both RFS and OS were defined as the time from the day of surgery to the discovery of tumor recurrence or the last follow-up, that is, July 2021.

IHC

The HCC specimens and matched non-HCC tissues

were fixed with 10% formalin solution, embedded in paraffin, and cut into slices about 4 μm thick. Sections were deparaffinized in xylene at 37 ℃, washed in a gradient ethanol series, incubated in 10 mmol/L citrate buffer (pH 6.0) for antigen retrieval, rinsed in phosphate-buffered saline (PBS), incubated in 0.3% H_2O_2 for 10 min at room temperature to inhibit endogenous peroxidase, and then rinsed again with PBS. The sections were incubated with anti-*PPP2CA* antibody (1:1,000) (ab106262; Abcam, Cambridge, UK) at 37 ℃ for 1 h, and then rinsed thoroughly in PBS. The sections were incubated with MaxVision™ (Shenzhen, China)/horseradish peroxidase for 30 min at room temperature, washed with PBS, and stained with diamino diphenyl and hematoxylin. The slices were dehydrated by a series of graded ethanol, dried, and sealed with neutral gum. At the same time, retinal tissue was treated as a positive control, while HCC tissue was treated with PBS instead of the primary antibody as a negative control.

The IHC staining used 2-component scoring for semi-quantification. First, the intensity of staining was assigned 0 points (uncolored), 1 point (light yellow), 2 points (yellow), or 3 points (dark yellow). Second, the percentage of the total number of observed cells that are stained was assigned as 1 point (≤25%), 2 points (26–50%), 3 points (51–75%), or 4 points (>75%). The 2 scores were multiplied to attain a total score of 0–12. A total score of 0–4 was considered "low expression", and a score of 5–12 was considered "high expression". All slides were evaluated by 2 senior pathologists in double-blind evaluation. If the results of the 2 pathologists were inconsistent, the review was repeated.

The clinical significance of PPP2CA in HCC

We stratified HCC patients according to *PPP2CA* expression and analyzed the relationship between *PPP2CA* expression and clinical outcome. We used the Kaplan-Meier plotter to compare the OS and RFS of patients with high or low *PPP2CA* expression.

Exploration of PPP2CA's potential routes of participation in HCC

Using the GSE76427 data set, we explored genes that may be related to *PPP2CA* and examined candidate differentially expressed genes (DEGs) in the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway and Gene Ontology (GO) databases in terms of biological processes, molecular functions, and cellular components. The GO and KEGG analyses were performed using the DAVID tool (https://david.ncifcrf.gov/) (11). The results of GO and KEGG analysis were depicted in bubble charts. The online Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database (https://string-db.org/) was used to construct a protein-protein interaction (PPI) network. The PPI network of DEGs was constructed from the STRING database and visualized by Cytoscape software (https://cytoscape.org/).

Statistical analysis

The statistical software SPSS 26.0 (IBM Corp., Armonk, NY, USA) was used to analyze participant data: count data was compared with the chi-square test; the Shapiro-Wilk test was used to determine the normality of measurement data, and independent sample *t*-test was used for data conforming to a normal distribution and homogeneous variance. The mean ± standard deviation ($\bar{x}\pm s$) was used for comparison; non-normally distributed data were compared using the nonparametric rank-sum test Mann-Whitney U test, and the results were expressed in median and quartile. A P value <0.05 indicated a statistically significant difference. The Kaplan-Meier method was used for survival analysis and comparison.

Results

The expression of PPP2CA in liver cancer tissues

The experimental results of IHC showed that *PPP2CA* was highly expressed in most HCC tissues, but low in most non-HCC tissues, and was mainly expressed in the cytoplasm (*Figure 1*).

The clinical application of PPP2CA in HCC

The general clinical data of the 2 groups of participants were compared (*Table 1*). Among them, the differences in Child-Pugh classification, AST, MVI, and portal cancer thrombus were statistically significant (all P<0.05). Gender, age, family history of liver cancer, Child-Pugh grade, liver cirrhosis, AFP, tumor size, tumor number, BCLC staging, TBil, ALT, ALB, PT, surgical resection method, tumor differentiation degree, whether the capsule was intact, whether there were no statistically significant differences in envelope invasion, lymph node metastasis, bile duct tumor

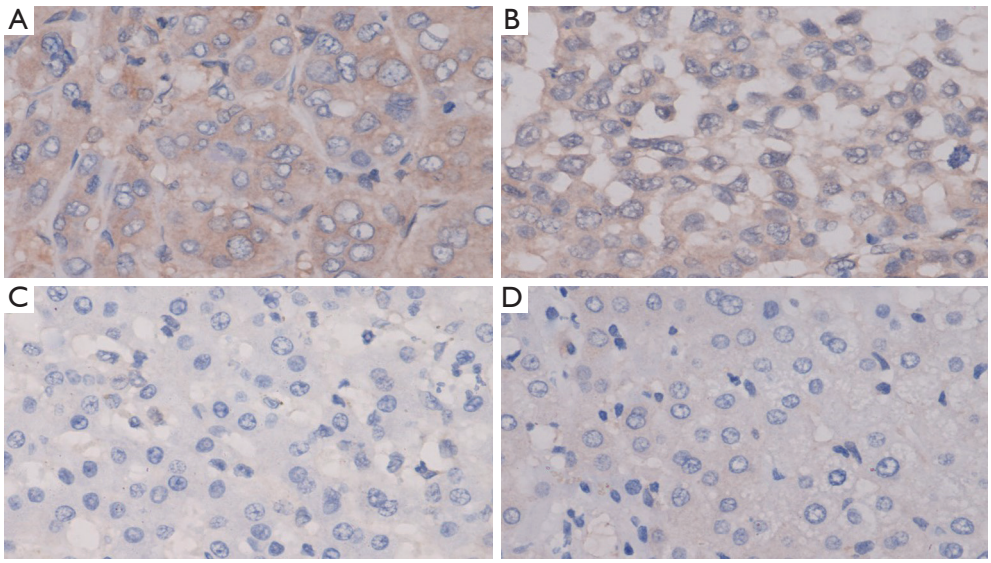

Figure 1 The expression of *PPP2CA* in HCC tissues and adjacent tissues of HCC. The protein expression of *PPP2CA* in HCC tissues is mainly located in the cytoplasm. (A,B) High expression in HCC tissues. The protein expression of *PPP2CA* in the adjacent tissues of HCC is mainly located in the cytoplasm. (C,D) Low expression in tissues adjacent to HCC. Magnification, ×400 (A-D). Stained with diaminobenzidene and hematoxylin. HCC, hepatocellular carcinoma.

thrombus, P-53, Ki-67, and so on were all also statistically significant (all P>0.05).

In addition, the Kaplan-Meier survival curve showed that the low expression group had better OS (P=0.04) and RFS (P=0.019) (*Figure 2*).

The expression of PPP2CA in HCC and non-HCC tissues in The Cancer Genome Atlas database

We used the GSE76427 data set to compare expression of the *PPP2CA* gene in HCC tissues and non-HCC tissues. The results showed that the expression level of *PPP2CA* in HCC tissues was higher than that in non-HCC tissues (*Figure 3*), and the difference was statistically significant (P=0.006).

The correlation between PPP2CA gene and HCC

We divided the groups by the median and third quartile of *PPP2CA* gene levels and compared the OS of the 2 participant groups, which revealed that there was no significant difference in the OS of the 2 groups (P>0.05) (*Figure 4*). Correlation analysis of clinical parameters showed that *PPP2CA* has little correlation with HCC patients' age and BCLC-stage (P>0.05), but it is significantly correlated with tumor-node-metastasis (TNM)-stage and gender (P<0.05) (*Figure 5*). Univariate Cox regression and multivariate COX regression analysis showed that age, BCLC-stage, TNM-stage, and *PPP2CA* are not factors that affect the survival of HCC patients (P>0.05) (*Table 2*, *Figure 6*).

PPP2CA and its DEGs

We analyzed HCC sample tissues and non-HCC control sample tissues from the GSE76427 data set. The *PPP2CA* gene and 71 DEGs were identified between HCC sample tissues and non-HCC control sample tissues, and they were drawn by heat map package and R software. The heat map (*Figure 7A*) and volcano map (*Figure 7B*) of the first 40 DEGs (the first 20 up-regulated DEGs and the first 20 down-regulated DEGs) were also analyzed, and the correlation between *PPP2CA* and DEGs was analyzed (*Figure 7C*).

GO analyses and KEGG pathways analyses

We performed enrichment analysis of the GO and KEGG pathways of *PPP2CA* and its DEGs. The GO analysis results showed that the main functions of biological processes are nuclear division, organelle fission, nuclear chromosome

Table 1 Relationship between clinicopathological parameters based on *PPP2CA* expression

Parameters	*PPP2CA* expression		P value	χ^2/Z
	High expression (n=88)	Low expression (n=40)		
Gender			0.114	2.495
Male	76	30		
Female	12	10		
Age, years			0.694	0.155
≤50	56	24		
>50	32	16		
Family history of HCC			0.255	1.298
No	67	34		
Yes	21	6		
Child-Pugh classification			0.041*	4.173
A	84	34		
B	4	6		
Liver cirrhosis			0.237	1.396
No	30	18		
Yes	58	22		
AFP, ng/mL			0.535	0.384
>400	41	21		
≤400	47	19		
Tumor size (cm)			0.623	0.242
≤5	28	11		
>5	60	29		
Number of tumors			0.537	0.381
Single	62	26		
Multiple	26	14		
BCLC-staging			0.564	1.144
A	39	14		
B	13	8		
C	36	18		
TBil	15 (15.8–30.7)	16.4 (15.7–21.3)	0.309	0.965
ALT	39.5 [44–65]	34.5 [33–60]	0.471	0.846
AST	47 [50–64]	37 [41–67]	0.018*	1.537
ALB	36.4 (35.6–37.2)	36.2 (35.7–37.9)	0.870	0.596
PT	12.2 (12.0–12.6)	12.3 (12.0–12.8)	0.977	0.477
Surgical resection method			0.787	0.073

Table 1 (*continued*)

Table 1 (*continued*)

Parameters	PPP2CA expression		P value	χ^2/Z
	High expression (n=88)	Low expression (n=40)		
≤2 liver segment	64	30		
>3 liver segment	24	10		
Tumor differentiation			0.848	0.037
Poor differentiation	38	18		
Well differentiated	50	22		
Envelope intact			0.365	0.821
Yes	43	17		
No	45	23		
MVI			0.001*	10.670
No	28	25		
Yes	60	15		
Liver capsule invasion			1.000	0.000
No	33	15		
Yes	55	25		
Lymph node metastasis			0.293	3.724
No	77	33		
Yes	9	6		
P-53			0.715	0.133
Negative	35	15		
Positive	53	25		
Ki-67			0.737	0.112
≤50	49	21		
>50	39	19		
Portal vein tumor thrombus			0.018*	5.623
No	63	20		
Yes	25	20		
Bile duct tumor thrombus			0.857	0.025
No	83	38		
Yes	5	2		

*, P<0.05. HCC, hepatocellular carcinoma; AFP, alpha fetoprotein; BCLC, Barcelona clinic liver cancer; Tbil, total bilirubin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALB, albumin; PT, prothrombin time.

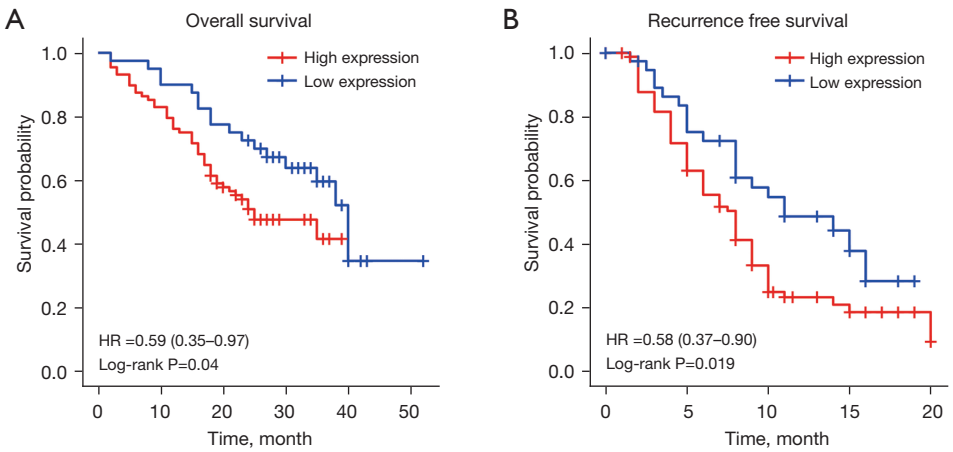

Figure 2 Kaplan-Meier survival curve of OS and RFS of two groups of HCC patients based on *PPP2CA* expression. OS, overall survival; RFS, recurrence-free survival; HCC, hepatocellular carcinoma.

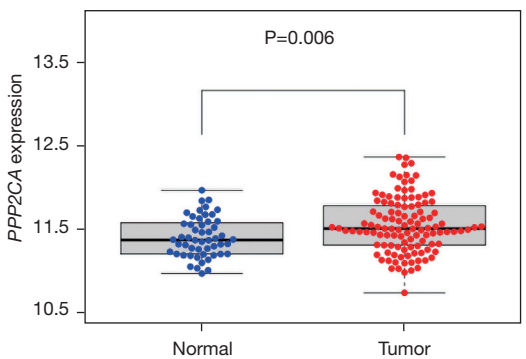

Figure 3 The expression of *PPP2CA* in HCC tissues and non-HCC tissues. HCC, hepatocellular carcinoma.

separation, and chromatid separation (*Figure 8A*). The most important of the 7 KEGG pathways identified was drug metabolism-cytochrome P450, followed by the metabolism of cytochrome P450 to xenobiotics (*Figure 8B*).

PPI network of PPP2CA and its DEGs and identification of key gene

We uploaded *PPP2CA* and its DEGs to STRING to construct a PPI network (*Figure 9A*). The PPI network was optimized using Cytoscape software to obtain a clearer PPI network (*Figure 9B*) and 2 sub-networks (*Figure 10*). The

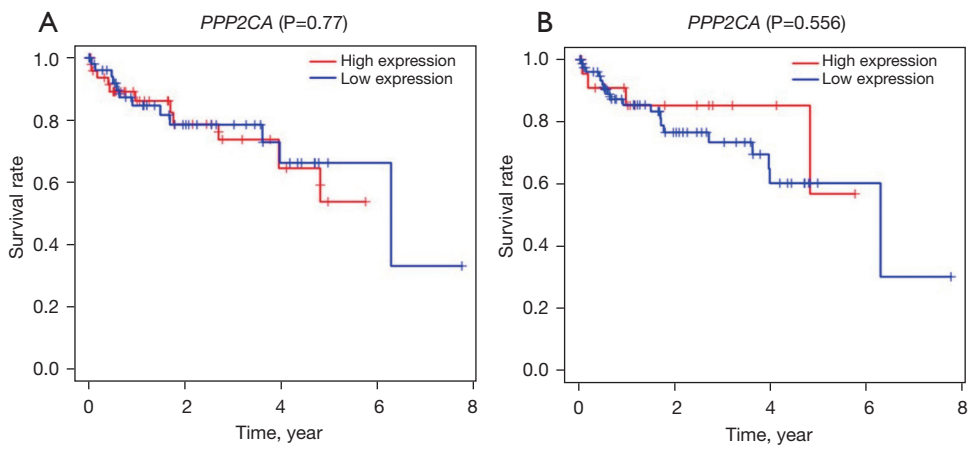

Figure 4 OS of HCC patients grouped based on the median (A) and third quartile (B) of the *PPP2CA* gene level. OS, overall survival; HCC, hepatocellular carcinoma.

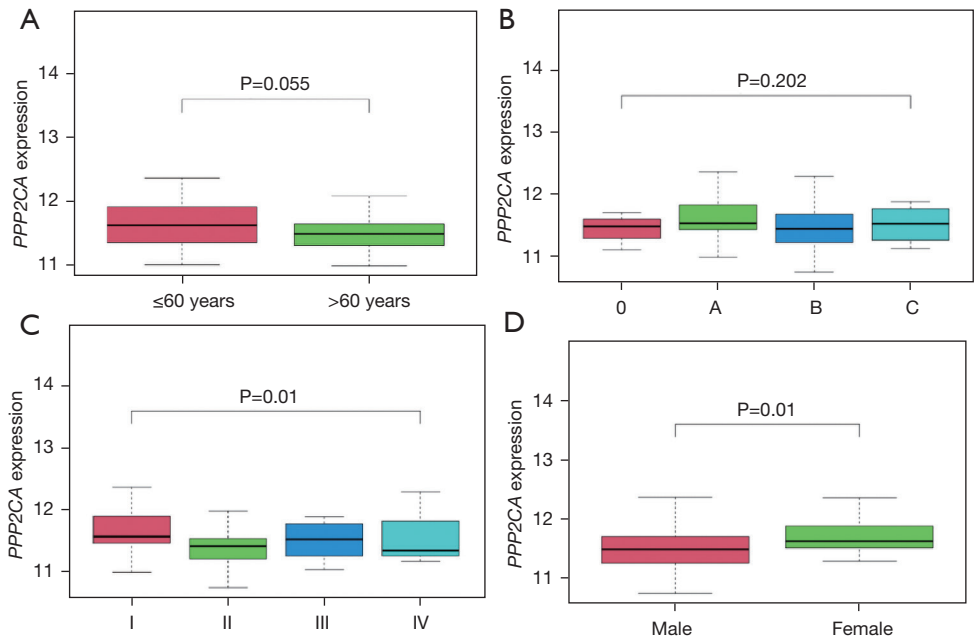

Figure 5 The correlation between *PPP2CA* and age (A), BCLC-staging (B), TNM-staging (C), and gender (D). BCLC, Barcelona clinic liver cancer; TNM, tumor-node-metastasis.

Table 2 Univariate and multivariate Cox regression analysis of OS in HCC patients

	Univariate COX regression				Multivariate cox regression			
	HR	HR.95CL	HR.95H	P value	HR	HR.95CL	HR.95H	P value
Age (years)	1.011052	0.976926	1.04637	0.530383	1.007871	0.972107	1.044951	0.670609
BCLC-staging	1.393437	0.732097	2.6522	0.312338	1.742795	0.550207	5.520351	0.345013
TNM-staging	1.163814	0.700212	1.93436	0.558405	0.82606	0.34265	1.991467	0.670387
PPP2CA	0.607936	0.16353	2.260051	0.457558	0.6164	0.153898	2.468829	0.494327

OS, overall survival; HCC, hepatocellular carcinoma; BCLC, Barcelona clinic liver cancer; TNM, tumor-node-metastasis; HR, hazard ratio.

results showed that *CCNA2*, *AURKB*, *TOP2A*, *NCAPG*, *MCM2*, *CDC20*, *CCMB2*, *AURKA*, and *MGST1* were the top 9 highly connected hub genes.

Discussion

The *PPP2CA* gene encodes the α subtype of the PP2A catalytic subunit, which regulates PP2A activity by selecting PP2A regulatory subunits (12). In previous studies, the up-regulation of *PPP2CA* was considered to be related to the poor prognosis of triple-negative breast cancer (13) and pancreatic cancer (14). High expression of *PPP2CA* is also related to glioma, and mir-130b-ceRNA can promote the epithelial-mesenchymal transition and invasion of glioma by targeting *PPP2CA* (15). In addition, the results of a phase I study showed that the anti-tumor drug LB-100 combined with existing anti-tumor drugs has better efficacy in the treatment of pancreatic cancer, glioma, and other diseases than single-agent efficacy, and supported that LB-100 is specific inhibit *PPP2CA* to improve efficacy (16). On the contrary, colorectal patients with low expression of *PPP2CA* have poor OS, and low expression of *PPP2CA* is significantly related to the later TNM stage (17). Some studies have shown that decreased expression of *PPP2CA* can increase the susceptibility of Chinese people to gastric cancer (18). The above studies showed that *PPP2CA* plays different roles in different cancer settings, and there may be multiple mechanisms and pathways that cause this phenomenon. The

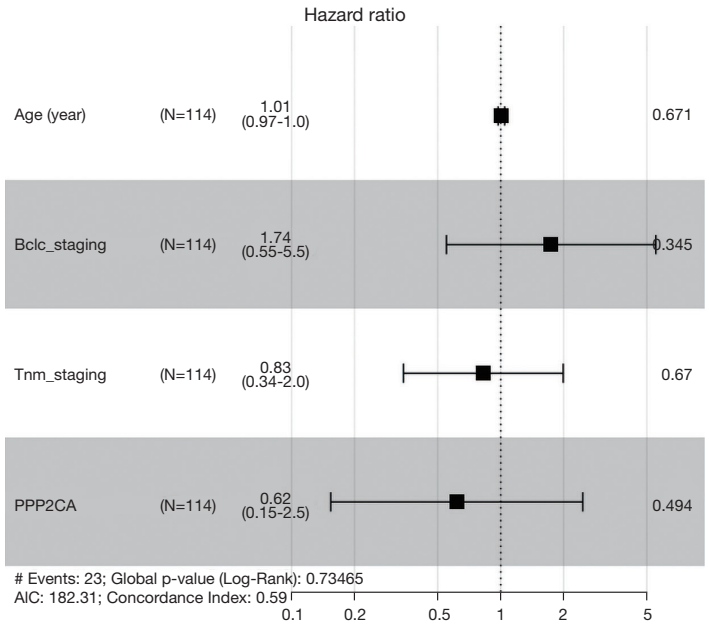

Figure 6 Forest plot of factors affecting the survival rate of HCC patients. HCC, hepatocellular carcinoma.

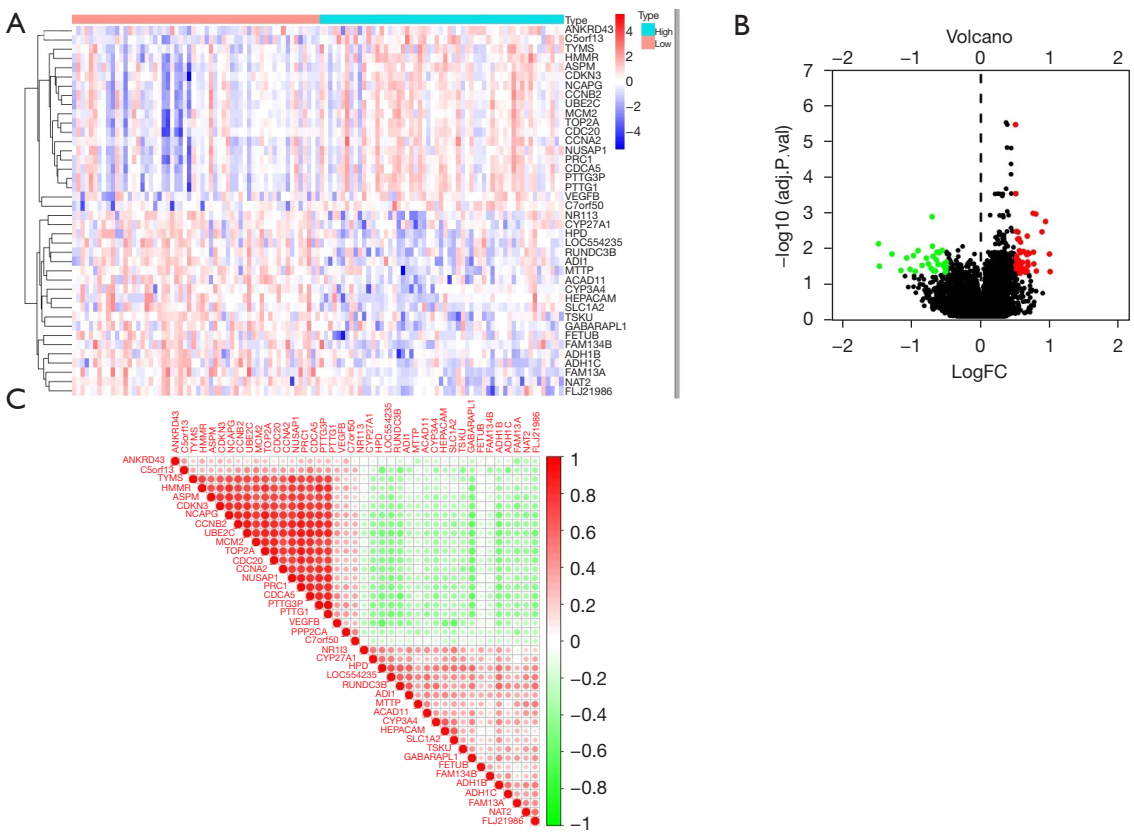

Figure 7 Heat maps of the top 20 up-regulated DEGs and the top 20 down-regulated DEGs. (A) Heat maps of the top 20 up-regulated DEGs and the top 20 down-regulated DEGs. Red means up-regulation; blue means down regulation. (B) Volcano map of *PPP2CA* and its DEGs. Green indicates down-regulation; red indicates up-regulation. (C) Correlation between *PPP2CA* and its significantly DEGs. Red means positive correlation, green means negative correlation. DEGs, differentially expressed genes.

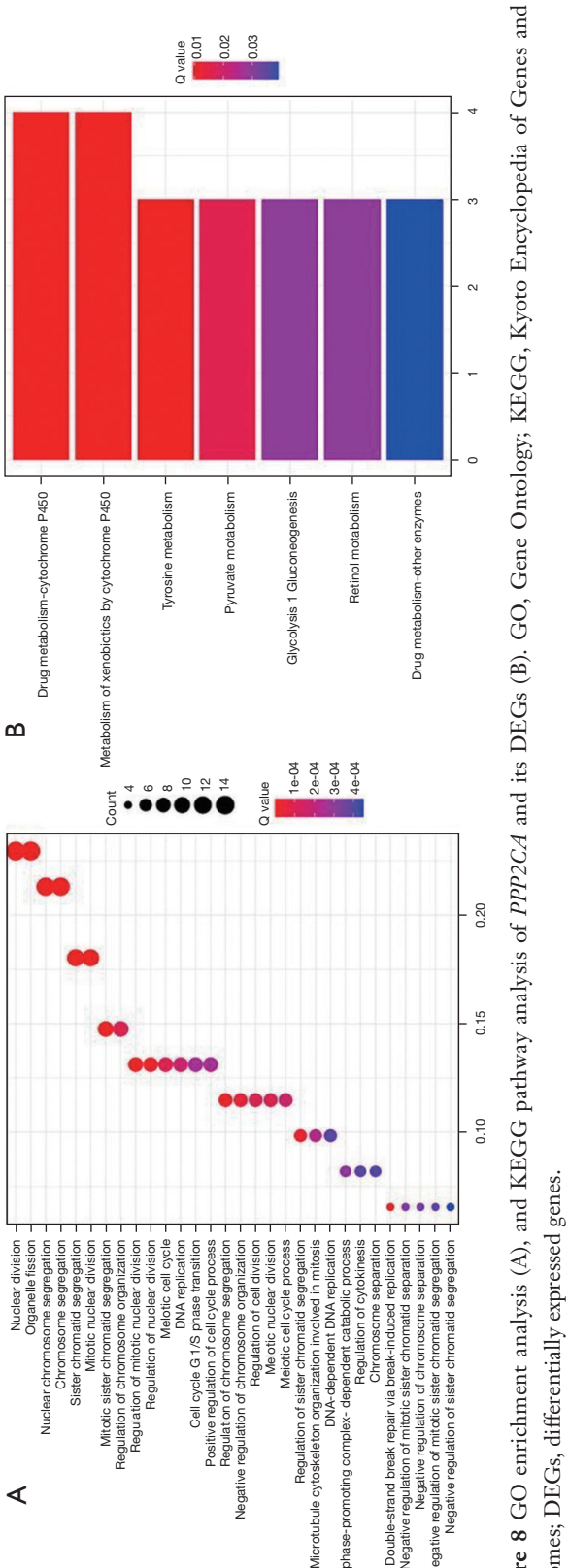

Figure 8 GO enrichment analysis (A), and KEGG pathway analysis of *PPP2CA* and its DEGs (B). GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; DEGs, differentially expressed genes.

role of PP2A in HCC is still controversial (9,19,20) and research of *PPP2CA* in the treatment of HCC is valuable.

We used IHC to detect the expression of *PPP2CA* in HCC tissues and non-HCC tissues. We found that *PPP2CA* is highly expressed in most HCC tissues, and its expression is mainly distributed in the cytoplasm. Duong *et al.* (21) found that the expression of PP2Ac in HCC tissues was significantly higher than that in non-HCC tissues from the same patient. We divided the expression of *PPP2CA* into a high expression group (medium positive and + strong positive) and a low expression group (negative and weak positive expression) according to the results of IHC detection. By comparing the clinical data of the 2 groups, we found that the high expression of *PPP2CA* and MVI is significantly related to portal vein tumor thrombus. In addition, participants in the *PPP2CA* high expression group had worse OS and RFS. These clinical data indicate that *PPP2CA* may promote the invasion and recurrence of HCC to a certain extent. In a WARD study, it was also found that PP2A promoted the invasion of HCC through extracellular remodeling (22), which is consistent with our speculation. In addition, some studies have shown that the high expression of *PPP2CA* has been associated with hepatitis B (HBV) or hepatitis C (HCV) viral infection (23). However, since most of this study is HBV-related HCC, verification would require much larger data and multi-center studies.

In addition, we used the GSE76427 dataset to analyze expression of the *PPP2CA* gene in HCC tissues and non-HCC tissues and found that the expression level of *PPP2CA* in HCC tissues was significantly higher than that in non-liver cancer tissues. Kaplan-Meier survival analysis showed that the expression of *PPP2CA* is not different from the OS of HCC patients, but the high expression of *PPP2CA* is significantly related to the TNM stage of HCC patients. Combined with the results of clinical studies, we can infer that *PPP2CA* plays an important role in the invasion and metastasis of HCC. However, in the end, multivariate regression analysis found that the *PPP2CA* gene may not be an independent risk factor for poor prognosis in HCC patients. In our clinical studies, participants in the *PPP2CA* high expression group had worse OS and RFS. At the same time, Gong *et al.* (10) showed that the up-regulation of PP2Ac expression has a negative impact on the OS of HCC patients, and the overexpression of PP2Ac is an independent risk factor for poor prognosis of HCC patients. Therefore, we have reason to believe that PP2A plays a role in promoting HCC. In addition, we found that the expression of *PPP2CA* is significantly related to gender

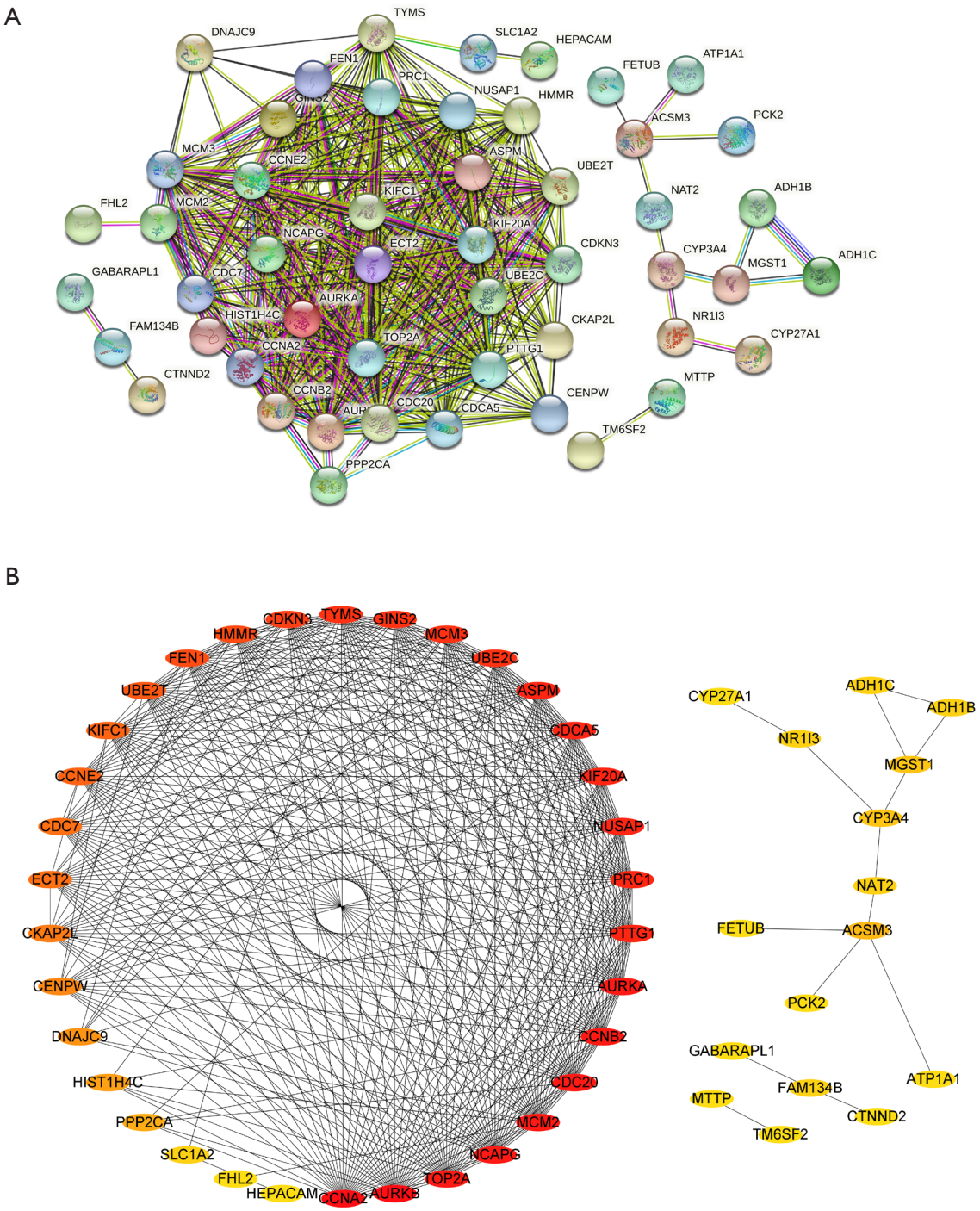

Figure 9 PPI network of *PPP2CA* and its DEGs. PPI, protein-protein interaction; DEGs, differentially expressed genes.

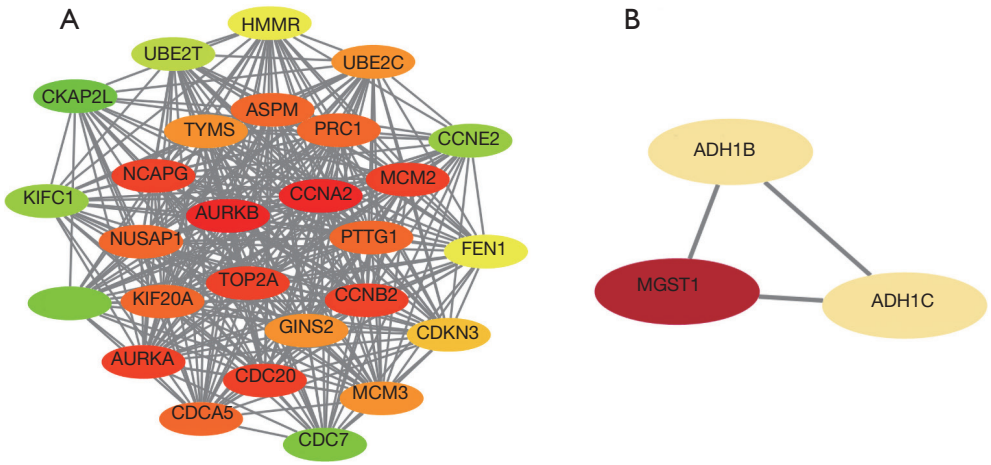

Figure 10 PPI sub-network analyzed by the molecular complex detection plug-in in Cytoscape software. PPI, protein-protein interaction.

in the analysis of bioinformatics research, but in clinical research, we did not find that *PPP2CA* is related to gender. This may be related to the fact that the number of cases we collected in the clinic was small, and more cases need to be collected and further analyzed. In addition, *PPP2CA* is highly expressed in HCC tissues. Our clinical studies have also shown that the expression of *PPP2CA* is significantly related to the RFS and OS of HCC patients. However, through the analysis of the GSE76427 data set, we found that the prognosis of HCC patients with different *PPP2CA* expressions is not different. Yes, this may be related to the insufficient number of cases in our data set, but in our previous research (24), we analyzed data from the cancer genome map database, gene expression comprehensive database, and our internal RNA-Seq database. The control data of 2,545 HCC patients and 1,993 non-HCC patients found that the expression of *PPP2CA* in HCC tissues was significantly higher than that in non-HCC tissues, and immunohistochemistry of tissue sections also confirmed that PPP2CA protein was up-regulated in HCC tissues; in addition, HCC patients shorter OS and DFS are all related to the high expression of *PPP2CA*.

We performed KEGG enrichment analysis on *PPP2CA* and its DEGs, revealing that the most important of the 7 KEGG pathways identified were drug metabolism-cytochrome P450, followed by the metabolism of cytochrome P450 to xenobiotics. These metabolic processes and the pathway provide evidence that pivot genes may be involved in the occurrence of HCC. The occurrence of HCC involves the up-regulation of drug metabolism-cytochrome P450 pathway and related genes (25), and is also related to poor OS. Therefore, we speculate that *PPP2CA* may affect HCC through the metabolism of cytochrome P450 and cytochrome P450 on xenobiotics. In addition, our PPI network further identified *CCNA2* as an up-regulated central gene of HCC, which is consistent with the study of Song *et al.* (26), that is, increased messenger RNA (mRNA) expression of the *CCNA2* gene is related to unfavorable RFS and OS in HCC patients. In addition, studies have also shown that *AURKB* has an important predictive value for the prognosis of HCC (27,28).

Our research also found that high expression of the *TOP2A*, *NCAPG*, *MCM2*, *CDC20*, *CCMB2*, *AURKA*, and *MGST1* genes has a negative impact on the prognosis of HCC. This requires further experimental research and the identification of targeted drugs for these hub genes to determine their clinical applications, explore their biological behaviors, such as invasion, metastasis, and proliferation capabilities, and verify the impact of potential drugs on their targeted genes.

In conclusion, our research showed that *PPP2CA* is highly expressed in liver cancer tissues and is significantly related to the gender and TNM staging of liver cancer patients. Through the analysis of differential genes, high *PPP2CA* expression is associated with poor prognosis of HCC patients, GO and KEGG pathway analysis, it was found that *PPP2CA* may act on liver cancer through multiple targets and multiple pathways, *PPP2CA* plays a role in promoting HCC. The study of *PPP2CA* may be a potential therapeutic target for liver cancer, and the study of the effect and mechanism of *PPP2CA* on liver cancer has great potential value for the research of new drugs for liver cancer.

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Footnote

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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. All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013) and were approved by the Ethics Committee of Guangxi Medical University Cancer Hospital (No. LW2021097), and all participants provided their written informed consent.

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