Curcumin inhibits proliferation of hepatocellular carcinoma cells by blocking PTPN1 and PTPN11 expression

JINGRU ZHANG[1], YANG LIU[1], XIAOJIE WANG[2], ZHIYI WANG[1], ENJIA XING[1], JINGMIN LI[3] and DONG WANG[1]

[1]Department of Histology and Embryology, Binzhou Medical University; [2]Quality Department, Shandong Runzhong Pharmaceutical Co., Ltd.; [3]Department of Human Anatomy, Binzhou Medical University, Yantai, Shandong 264003, P.R. China

Received January 16, 2023; Accepted May 4, 2023

DOI: 10.3892/ol.2023.13893

Abstract. The antitumor mechanism of curcumin is unclear, especially in hepatocellular carcinoma (HCC) cells. To clarify the mechanism of action of curcumin in the effective treatment of HCC, the targets of curcumin were screened and validated. Candidate genes of curcumin for HCC were screened using the traditional Chinese medicine systems pharmacology (TCMSP) database and validated using The Cancer Genome Atlas (TCGA) database. The correlation of mRNA expression levels between key candidate genes was identified in the TCGA liver hepatocellular carcinoma (LIHC) dataset. The effects on prognosis were analyzed to identify the target gene of curcumin, which inhibits HCC cell proliferation. Based on the subcutaneous xenograft model of human HCC in nude mice, the expression levels of target proteins were observed using immunohistochemistry. The analysis results of the present study identified the target genes of curcumin, which were obtained by screening the TCSMP database. The protein tyrosine phosphatase non-receptor type 1 (PTPN1) was obtained from TCGA database analysis of the targeted genes. The expression levels of PTPN1 and its homologous sequence genes in TCGA LIHC project was analyzed to identify the potential target gene of curcumin, for use in HCC treatment. Next, xenograft experiments were performed to investigate the therapeutic effects of curcumin in an animal model. Curcumin was demonstrated to inhibit the growth of HCC xenograft tumors in mice. Immunohistochemistry results demonstrated that the protein expression levels of PTPN1 and PTPN11 in the curcumin group were significantly lower compared with those in the control group. In conclusion, these results demonstrated that curcumin inhibits the proliferation of HCC cells by inhibiting the expression of PTPN1 and PTPN11.

Correspondence to: Professor Dong Wang, Department of Histology and Embryology, Binzhou Medical University, 346 Guanhai Road, Yantai, Shandong 264003, P.R. China
E-mail: wangdby@bzmc.edu.cn

Miss Jingmin Li, Department of Human Anatomy, Binzhou Medical University, 346 Guanhai Road, Yantai, Shandong 264003, P.R. China
E-mail: lijingmin0535@163.com

Key words: curcumin, protein tyrosine phosphatase non-receptor type 11, hepatocellular carcinoma, cell proliferation

Introduction

The 5 year survival rate for liver cancer was 20% between 2010 and 2016 (1). Recent trends in liver cancer are promising because of the long-term rise in mortality slowed in female patients and stabilized among men in the United States (1). Treatment strategies for hepatocellular carcinoma (HCC), a prominent histological type of liver cancer include surgical resection, local radiofrequency ablation, liver transplantation and systemic therapy with different drugs (2,3). Currently, there are effective therapeutic options available for patients with HCC that significantly improve survival rates, but a significant number of patients are unresponsive to locoreginal or systemic therapy and ultimately succumb to their disease (2,3). Therefore, it is very significant to develop effective therapeutic regiments for patients with HCC patients.

Curcumin is a diphenolic compound derived from *curcuma longa*. Curcumin alone or in combination with other drugs (including metformin, N-n-butyl haloperidol iodide, resveratrol, and quercetin) has been reported to demonstrate anticancer activity in preclinical and clinical trials (4-7). As a potential chemo-preventive and therapeutic drug for liver cancer, curcumin has been reported to demonstrate good prospects in preclinical studies of liver cancer (5,8). The anticancer effects of curcumin are mainly manifested via activation of the apoptosis pathway and inhibition of cell proliferation for HCC cells and affects the tumor microenvironment, such as inhibiting inflammatory processes angiogenesis and metastasis (4,9). Previous studies have reported that curcumin can inhibit the proliferation of liver cancer cells by regulating certain genes and signaling pathways, such as hypoxia-inducible factor-1α, nuclear factor E2-related factor 2 (Nrf2), IL-6/STAT3 pathway, and so on (10,11).

The antitumor mechanism of curcumin is unclear, particularly in HCC cells. Therefore, the present study utilized the pharmacology database and analysis platform of the traditional Chinese medicine (TCM) system in combination with bioinformatics technology to screen potential target genes of curcumin, and subsequently verified these targets through an animal xenograft tumor model of human HCC treated by curcumin.

Materials and methods

Screening of candidate genes. Possible target genes of curcumin were searched using the term 'curcumin' through the TCM Systems Pharmacology Database and Analysis Platform (TCMSP; tcmspw.com/tcmsp.php). RNA-sequencing (RNA-seq) data from The Cancer Genome Atlas (TCGA; http://portal.gdc.cancer.gov) for liver hepatocellular carcinoma (LIHC) and other types of cancer (including bladder, thyroid, kidney, colon, lung, prostate, cervix uteri, bile duct, and breast) were used to analyze the expression levels and correlation among the candidate target genes (12). Kaplan-Meier plots were generated using the survminer (version 0.4.4) package in R (version 4.0.3, https://www.R-project.org/) according TCGA HCC gene expression data and clinical data (TCGA-LIHC). The enrichment of Gene Ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways was performed using the R packages. After ID conversion of the candidate target genes using the org.Hs.eg.db package (version 3.11.4), enrichment analysis was performed using clusterProfiler package (version 3.12.0) and bubble plots of GO terms and KEGG pathways were generated using the ggplot2 package (version 2.0.0) (13). Homologous sequences of candidate genes were summarized using the GeneCards database (genecards.org/). Specifically, 'PTPN1' was used as the search term, and then paralogs for the protein tyrosine phosphatase non-receptor type 1 (PTPN1) gene were examined. The protein interaction network was generated using the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database (cn.string-db.org), which is a protein-protein interaction prediction website, and the confidence interval for the minimum required interaction score was set to 0.7. The igrap package (version 1.2.6) and ggrap package (version 2.0.5) in R were used to analyze and visualize protein-protein interactions. Cytoscape (version 3.8.0; Cytoscape Consortium; www.cytoscape.org) was used for visualization of the protein network. The CytoHubba plugin (version 0.1, https://apps.cytoscape.org/apps/cytohubba) were used to screen the top 5 genes as hub genes by the maximal clique centrality (MCC) algorithm.

Establishment of human HCC xenograft model in mice. The human HCC cell line HuH7 (Procell Life Science & Technology Co., Ltd.) was cultured in Roswell Park Memorial Institute 1640 containing 10% fetal bovine serum (Gibco; Thermo Fisher Scientific, Inc.) in a 37˚C incubator with 5% CO_2. A total of 12 6-week-old male BALB/c-nu/nu mice (18±2 g) were purchased from Ji'nan Penyue Laboratory Animal Breeding Co., Ltd. Mice were examined every day for their health status and behavior. The food and water supply is inspected regularly to ensure that it is free from contamination and meets the nutritional requirements of mice before they were sacrificed. There were no dead mice during the experiment. Mice were maintained in standard conditions, with an ambient temperature of 21±2˚C, 50% humidity and a 12-h light/dark cycle. All animal experiments were approved by the Institutional Animal Care and Use Committee of the Binzhou Medical University (approval no. 2022-607).

Treatment of xenograft model with curcumin. After 1 week of adaptive feeding, each nude mouse was implanted with a subcutaneous injection of $1x10^6$ HuH7 cells in 100 μl phosphate buffered saline. When the tumor volume was 60-100 mm^3 (the 9th day after injection), the 12 nude mice were randomly divided into two groups (the control and curcumin groups). Animals in the curcumin group were intraperitoneally injected with curcumin (100 mg/kg/day dissolved in corn oil at 10 mg/ml) (14), and the animals in the control group were treated with corn oil (10 ml/kg/day). Curcumin was purchased from Dalian Meilun Biology Technology Co., Ltd. Corn oil was purchased from COFCO Corporation. The length and width of the tumors were measured with a vernier caliper every two days. The tumor volume was calculated using the following formula: Volume=0.5 x length x $width^2$. The day on which the treatment began was recorded as day 0. The relative tumor volume was calculated as follows: Relative tumor volume=(tumor volume on the day of measurement)/(tumor volume on day 0). Mice were sacrificed using cervical dislocation after 12 days of treatment. Following euthanasia by cervical dislocation, mice mortality was verified by the absence of a heartbeat, respiratory arrest and the lack of reaction to a painful stimulus. Tumors were then removed, and Image-Pro Plus software (IPP; version 6.0; Media Cybernetics, Inc.) was used to measure the length and width of each tumor to calculate the tumor volume.

Immunohistochemistry. Tumor tissues from each group were fixed using 4% paraformaldehyde for 24 h at room temperature and embedded in paraffin. Sections (4 μm thick) were obtained from the paraffin blocks, which were then dewaxed in xylene for 15 min and rehydrated in water. Antigen retrieval was performed using citrate buffer (pH 6.0) for 15 min at 95-100˚C. After blocking in 3% hydrogen peroxide for 10 min at 37˚C and 10% goat serum (SL038, Solarbio, China) for 10 min at 37˚C successively, sections were incubated with the following primary antibodies (all purchased from Proteintech Group, Inc.); proliferating cell nuclear antigen (PCNA; 1:400; cat. no. 24036-1AP), BAX (1:500; cat. no. 50599-2Ig), PTPN1 (1:200; cat. no. 11334-1AP) and PTPN11 (1:400; 24570-1AP) overnight at 4˚C. Sections were then incubated with the enzyme labeled sheep anti mouse/rabbit IgG polymer in the GTVision Detection System/Mo&Rb (GK600505, Gene Tech Biotechnology Co., Ltd.) at room temperature for 30 min and visualized using the DAB solution (GK600505, Gene Tech Biotechnology Co., Ltd.). Sections were counterstained with Harris hematoxylin for 2 min at room temperature. The sections were dehydrated in graded alcohol, cleared in xylene and mounted. Images were acquired using an AJ-VERT station (Chongqing Optec Instrument Co., Ltd.) with a light microscope. IPP (version 6.0; Media Cybernetics, Inc.) software was used for semi-quantitative analysis by randomly selecting three high-power fields from each section. The integrated optical density (IOD) of BAX, PTPN1 and PTPN11 expression levels and the positive cell numbers of PCNA expression levels in tumors were quantified.

Statistical analysis. All data are expressed as mean ± standard error. For the statistical comparisons of the protein expression levels and tumor volume in control and curcumin groups, the normal distribution and homogeneity of variances were tested using the Shapiro-Wilk test and F test. To evaluate differences between study groups, unpaired and paired Student's t-tests

were used for the data with normal distribution, and homogeneity of variance and Mann-Whitney U tests were used for the data with non-normal distribution and inhomogeneity of variance from unpaired samples and Wilcoxon signed rank tests were used for the data from paired samples. Statistical analyses were performed using GraphPad Prism 8 (GraphPad Software; Dotmatics). Survival curves of candidate target genes to HCC were plotted using the survminer package in R, and data were analyzed using a log-rank test (Mantel-Cox). Patients were grouped according to the target genes expression levels relative to the median of the group (high > median and low ≤ median). The two-stage test using the TSHRC package (version 0.1.6) was used when there was an intersection in the survival curve. Correlations between PTPN1 and its paralogous genes were assessed using Spearman's rank correlation test in R. $P<0.05$ was considered to indicate a statistically significant difference.

Results

PTPN1, heat shock protein 90 α-family class a member 1 (HSP90AA1) and potassium voltage-gated channel subfamily h member 2 (KCNH2) are overexpressed in HCC. The following target genes of curcumin were identified using TCMSP: PTPN1, HSP90AA1, coagulation factor X (F10), prostaglandin-endoperoxide synthase 2 (PTGS2) and KCNH2. To understand the relationships among the target proteins, protein-protein interaction analysis was performed using the STRING search tool. With a confidence cut-off of 0.4, a network was generated. The results showed interactions among these target proteins (Fig. S1A). Enrichment analysis of target genes indicated that the most enriched GO terms and pathways, included the 'IL-17 signaling pathway', 'protein tyrosine kinase binding', 'ubiquitin protein ligase binding', 'scaffold protein binding', 'endoplasmic reticulum lumen', 'cytoplasmic side of endoplasmic reticulum membrane', and positive regulation of reactive oxygen species processes (Fig. S1B).

To assess the expression levels of these target genes in HCC, the TCGA database was used to compare and analyze the expression levels of the aforementioned target genes in HCC. mRNA expression levels of PTPN1, HSP90AA1 and KCNH2 were significantly higher in tumor tissues compared with in normal liver tissues (Fig. S1C). The expression levels of PTGS2 in tumor tissues was significantly lower compared with that in normal tissues and the expression levels of F10 were markedly increased compared with those in normal tissue (Fig. S1C). Additionally, the correlation of the five target genes was analyzed, and the results demonstrated that the correlation coefficients between these genes were between -0.5 and 0.5 which suggested that any correlations between these genes was weak (Fig. S1D).

Patients with HCC and high PTPN1 expression levels have a poor prognosis. RNA-seq data from the TCGA LIHC dataset were divided into high and low expression groups according to the median expression level of each target gene. Survival data were analyzed using Cox regression analysis. The results demonstrated that HCC patients with high PTPN1 and HSP90AA1 expression levels had shorter overall survival compared with those with low expression levels (Fig. S2A and B). Differences in the median overall survival between the high expression level group of PTGS2, F10 and KCNH2 and the low expression level group were not statistically significant (Fig. S2C-E). The median disease specific survival rates in the high expression level group for PTPN1, HSP90AA1 and KCNH2 were shorter compared with those in the low expression group (Fig. S2F, G and I). However, the expression levels of PTGS2, F10 and were not associated with median disease specific survival (Fig. S2H and J). The progress free interval in patients with high PTPN1 expression levels was significantly shorter compared with that in patients with low PTPN1 expression levels (Fig. S2K). The expression levels of HSP90AA1, PTGS2, F10 and KCNH2 were not associated with the progress free interval (Fig. S2L-O). These results suggested that patients with HCC and high PTPN1 expression levels had a worse prognosis. Furthermore, the expression level of PTPN1 in different types of cancer was analyzed using RNA-seq data from the TCGA project. mRNA expression levels of PTPN1 in tumor tissues were significantly higher compared with those in normal tissues from certain types of cancer, including breast carcinoma, cholangiocarcinoma, colon adenocarcinoma, esophageal adenocarcinoma, head and neck squamous cell carcinoma, kidney renal clear cell carcinoma, kidney renal papillary cell carcinoma, HCC, rectum adenocarcinoma and stomach adenocarcinoma (Fig. S3). However, expression levels of PTPN1 were significantly lower in tumor tissues compared with normal tissues from lung adenocarcinoma, lung squamous cell carcinoma and thyroid carcinoma (Fig. S3).

Curcumin inhibits the expression of PTPN1 in HCC xenograft tumor. To verify whether curcumin could inhibit expression of PTPN1, a human HCC xenograft model was established by subcutaneously injecting HuH7 cells into nude mice. Intraperitoneal injection of curcumin was then administered for 12 days. The results demonstrated that the tumor growth rate of the curcumin group was significantly reduced compared with that of the control group on day 6, 8, 10 and 12 after treatment (Fig. 1A), and the tumor volume of the curcumin group was significantly smaller compared with that of the control group (Fig. 1B). Immunohistochemistry indicated that the protein expression levels of PCNA in the tumor tissue of the curcumin group were significantly reduced compared with those of the control group (Fig. 1C). The protein expression levels of BAX in the tumor tissue of the curcumin group were significantly increased compared with those of the control group. These results suggested that curcumin inhibited cell proliferation and promoted apoptosis in HCC. Next, the expression levels of PTPN1 in tumor tissues were assessed. The results demonstrated that the IOD values for PTPN1 protein expression levels in tumor tissues of the curcumin group were significantly reduced compared with those in the control group (Fig. 1C), which suggested that curcumin could inhibit PTPN1 protein expression.

Differential expression of classical PTP family genes in HCC. PTPN1 belongs to the classical PTP family. Using the data from TCGA LIHC dataset, unpaired sample statistical analysis was performed on the mRNA expression levels of classical PTPs,

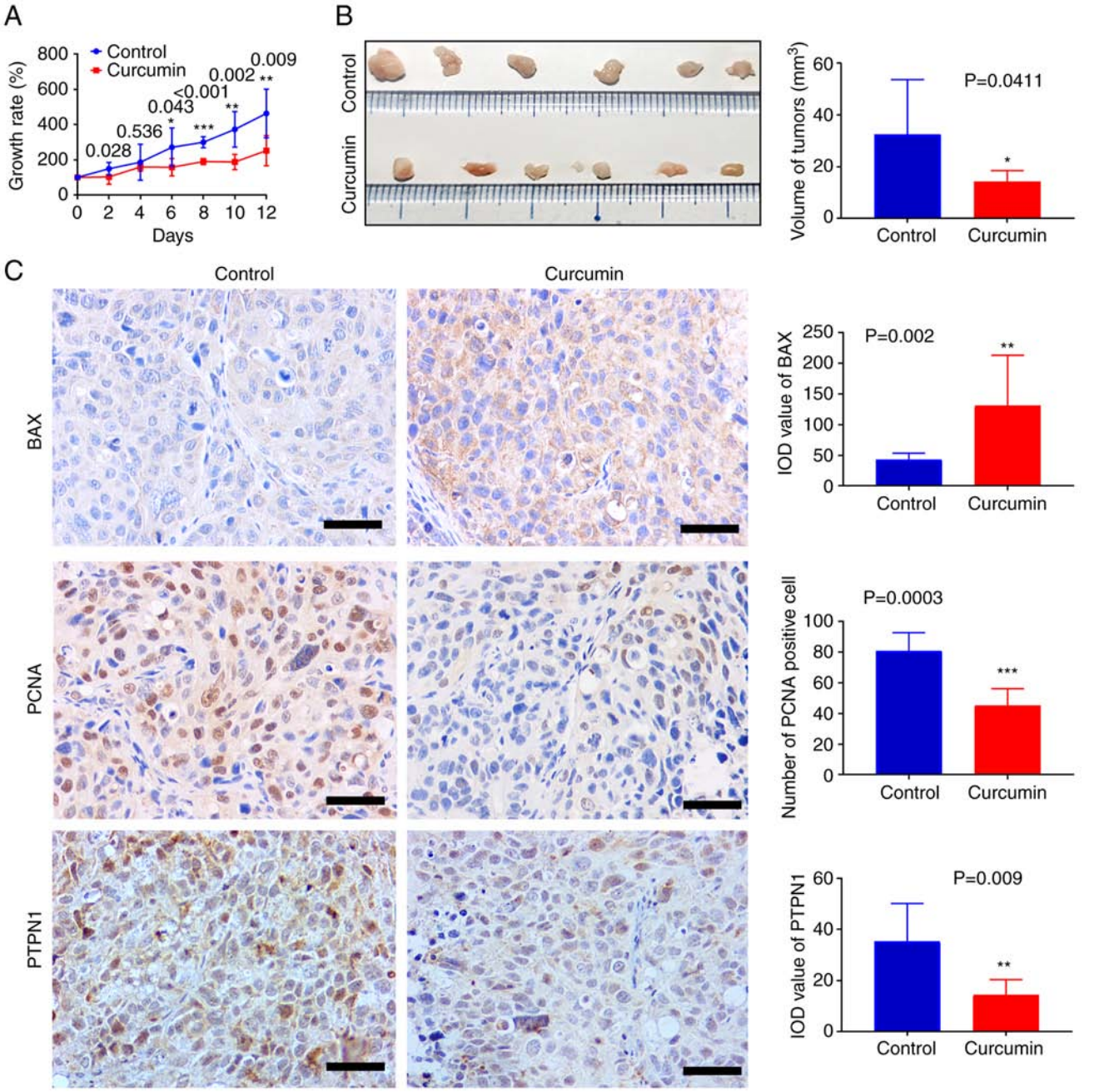

Figure 1. Curcumin inhibited the growth of HCC tumor and PTPN1 expression in the HCC xenograft model. (A) The growth rate of tumors in xenograft mice treated with or without curcumin. Tumor growth rate was calculated as follows: Tumor growth rate (%)=(tumor volume on the day of measurement)/(tumor volume on day 0) x100. The value indicated at each time point is the P-value. (B) Imaged xenograft tumors treated with vehicle (control) or curcumin (n=6). The right panel indicates the average tumor volume. (C) Representative images of BAX, PCNA and PTPN1 staining by immunohistochemistry in xenograft tissues. *P≤0.05, **P≤0.01 and ***P≤0.001. Scale bar, 50 μm. HCC, hepatocellular carcinoma; PTPN1, protein tyrosine phosphatase non-receptor type 1; PCNA, proliferating cell nuclear antigen; IOD, integrated optical density.

the results demonstrated that certain PTP family genes were highly expressed in HCC tumor tissues, including PTPN1, PTPN2, PTPN4-7, PTPN9, PTPN11, PTPN12, PTPN14, PTPN18, PTPN23, PTPRA, PTPRF, PTPRG, PTPRH, PTPRJ, PTPRK, PTPRM, PTPRN, PTPRR and PTPRU. PTPs with reduced expression levels in HCC tumor tissue included PTPN3, PTPN13, PTPRB, PTPRC, PTPRD, PTPRN2, PTPRS and PTPRT (Fig. 2). Statistical analysis of paired samples demonstrated that PTPs with significantly increased expression levels in HCC tumor tissues included PTPN1, PTPN2, PTPN4-7, PTPN9, PTPN11, PTPN12, PTPN14, PTPN18, PTPN23, PTPRA, PTPRF, PTPRG, PTPRJ, PTPRK, PTPRM, PTPRN, PTPRR and PTPRU. PTPs with significantly reduced expression levels in HCC tumor tissues included PTPN3, PTPN13, PTPRB, PTPRC, PTPRD and PTPRS (Fig. 3). Using the STRING online tool, a protein interaction network diagram for classical PTP family proteins was constructed (Fig. 4A). The GO and KEGG pathways enrichment of high- and low-expression genes indicated that these genes were mainly enriched in 'peptidyl-tyrosine dephosphorylation', 'protein dephosphorylation', 'dephosphorylation', 'cytoplasmic side of membrane', 'cytoplasmic side of plasma membrane',

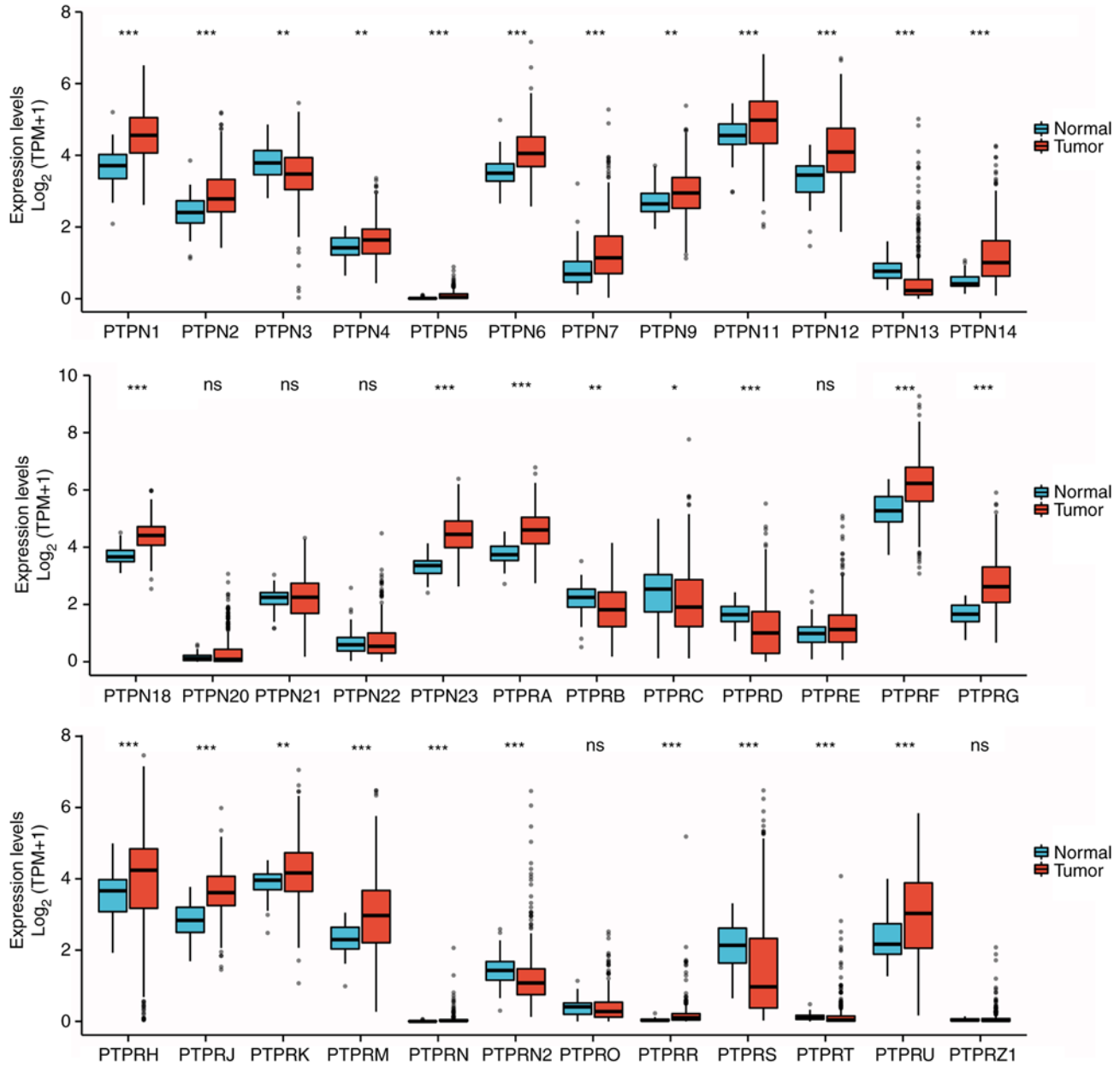

Figure 2. Expression levels of PTPs in unpaired hepatocellular carcinoma samples. The RNA sequencing data (data release 35.0) in the TCGA-LIHC cohort (http://portal.gdc.cancer.gov) was used. The normality test results indicated that the samples did not meet the normality test ($P<0.05$), so the Mann-Whitney U test was used. $^{*}P\leq0.05$, $^{**}P\leq0.01$ and $^{***}P\leq0.001$. PTP, protein tyrosine phosphatase; PTPN, PTP non-receptor type; PTPR, PTP receptor-type; ns, not significant; TPM, transcripts per kilobase million.

'secretory granule membrane', protein tyrosine entries such as 'protein tyrosine phosphatase activity', 'phosphoprotein phosphatase activity', 'phosphatase activity', 'adherens junction', 'cell adhesion molecules' and 'MAPK signaling' (Fig. 4B).

Expression levels of PTPN11 are correlated with PTPN1 in HCC. RNA-seq data from the TCGA LICH dataset was used to analyze the correlation between the expression levels of the classical PTP family and PTPN1 in HCC. The results demonstrated that there were 14 genes whose correlation coefficients with PTPN1 were >0.5 and which were statistically significant (Fig. 4C). The cytohubba plug-in for Cytoscape, was used to screen five core genes (PTPN11, PTPN7, PTPN14, PTPN5 and PTPN2) according to the score of the MCC and Degree algorithms (Fig. 4D). Finally, PTPN11 was screened by intersecting the upregulated differentially expressed genes in HCC, genes strongly correlated with PTPN1 and Hub genes (Fig. 4E). Together, these results suggested that both PTPN1 and PTPN11 may be the targets of curcumin.

Curcumin inhibits the expression of PTPN11 in HCC xenograft tumor tissue. The effect of curcumin on expression levels of PTPN11 was assessed using immunohistochemistry. The results demonstrated that the integrated optical density of PTPN11 protein expression in the tumor tissue of the curcumin group was significantly lower compared with that of the control group (Fig. 5), which indicated that curcumin may inhibit the growth of HCC tumors via downregulation of PTPN11 expression.

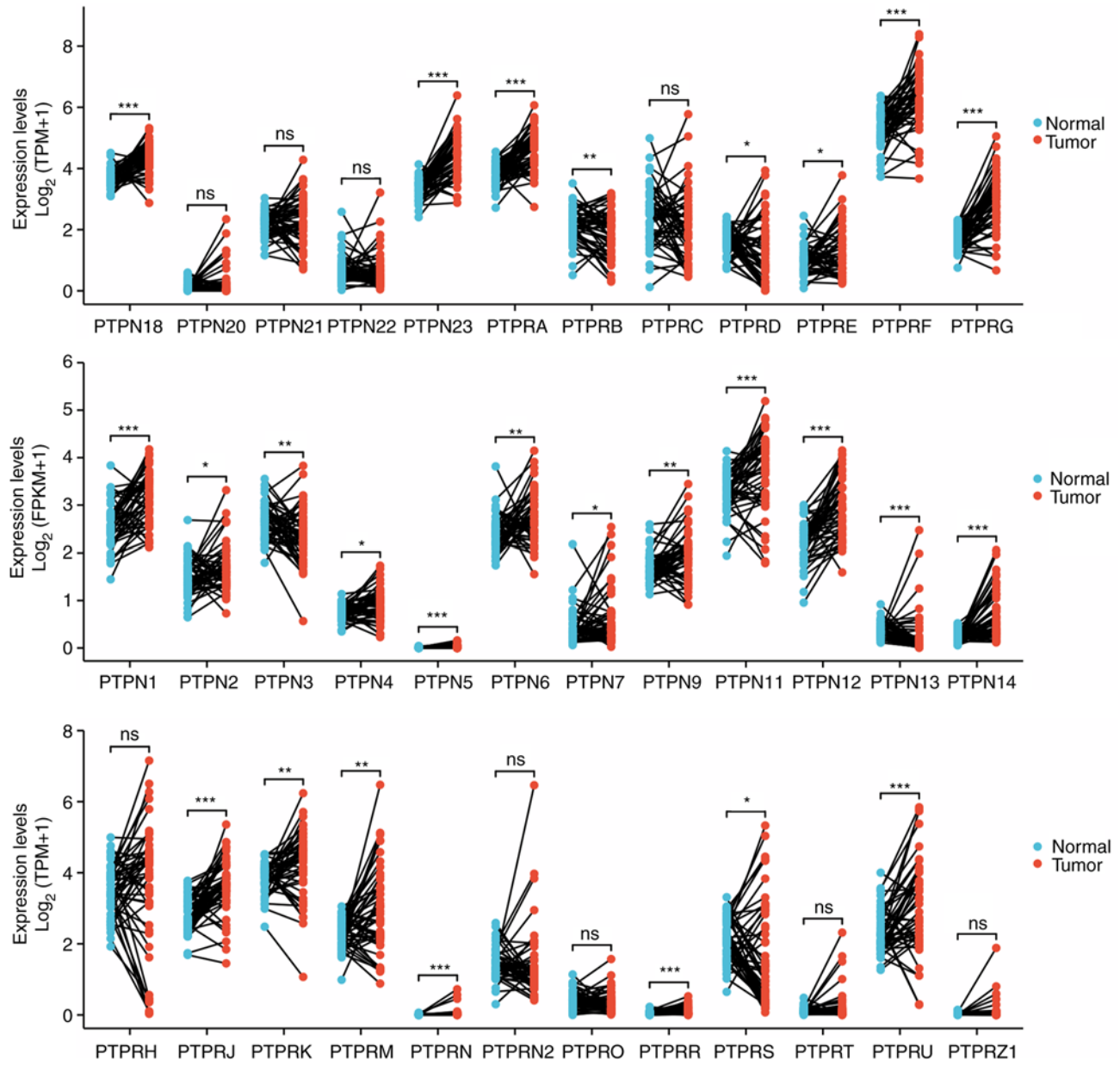

Figure 3. Expression levels of PTPs in paired hepatocellular carcinoma samples. The RNA sequencing data (data release 35.0) in the TCGA-LIHC cohort (http://portal.gdc.cancer.gov) was used. The normality test results indicated that the samples did not meet the normality test ($P<0.05$), so the Wilcoxon signed rank test was selected. $^{*}P\leq0.05$, $^{**}P\leq0.01$ and $^{***}P\leq0.001$. PTP, protein tyrosine phosphatase; PTPN, PTP non-receptor type; PTPR, PTP receptor-type; ns, not significant; TPM, transcripts per kilobase million.

Discussion

A previous clinical trial reported that curcumin has considerable potential for treating patients with cancer (15). In the present study, curcumin was demonstrated to promote apoptosis, inhibit cell proliferation and inhibit HCC tumor growth, which was consistent with the experimental results reported in the literature (16). Curcumin is a phenolic compound extracted from turmeric, which has antioxidant, anti-angiogenic and anti-inflammatory effects (17,18). The effectiveness of curcumin has been previously reported by detailed *in vitro*, *in vivo* and clinical trials (15,19). Curcumin can modulate certain signaling pathways, including growth factors, cytokines, transcription factors, and genes which modulate cellular proliferation and apoptosis in cancer cells, which can lead to mortality or inhibition of cancer cell proliferation (11,16).

In the current study, TCMSP database combined with the RNA-seq data from the TCGA LIHC dataset were used to screen the target gene, PTPN1, of curcumin in HCC. PTPN1 is a member of the classical PTP family (20,21). PTPs remove phosphate groups from proteins involved in cell signal transduction and regulate cell growth, differentiation, metabolism, gene transcription and immune responses (21). Aberrant tyrosine phosphorylation levels, which result from PTPN1 dysfunction are associated with the progression of numerous diseases including cancer (21,22), diabetes (23,24) and obesity (24). Recent studies have reported that PTPN1 inhibition may be an option for the treatment of such diseases (21,23). In the present study, the expression levels of

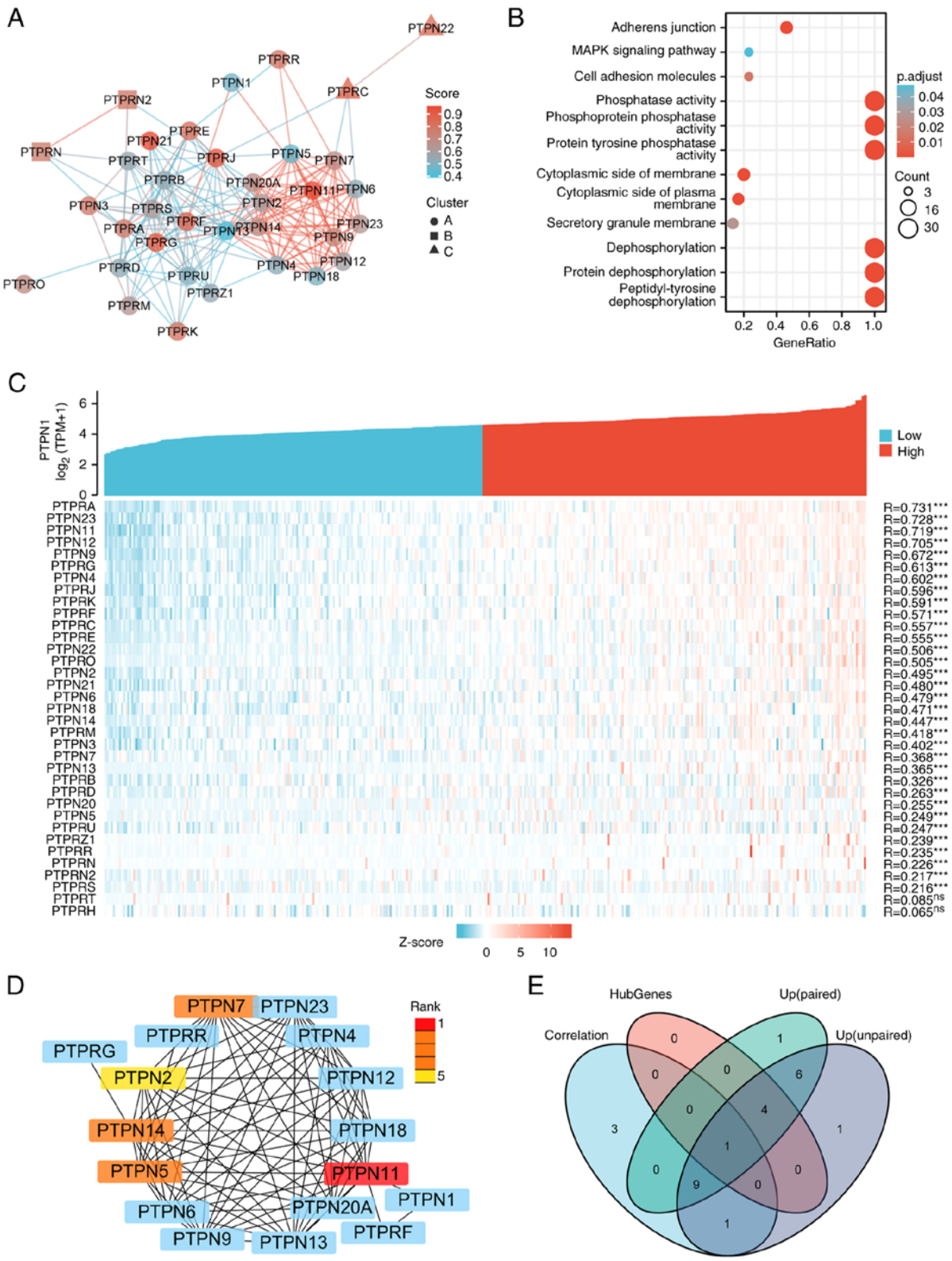

Figure 4. Screening the key genes in the PTP gene family in HCC. (A) Interactive relationship of the PTP gene family. The Search Tool for the Retrieval of Interacting Genes/Proteins online tool (https://cn.string-db.org/cgi/input.pl) was utilized to analyze the relationship of the target genes. (B) Gene Ontology (https://bioconductor.org/packages/release/data/annotation/html/GO.db.html) and Kyoto Encyclopedia of Genes and Genomes (bioconductor.org/packages//2.7/data/annotation/html/KEGG.db.html) enrichment of the PTP gene family. ID conversion of the candidate target genes using the org.Hs.eg.db package (version 3.11.4), enrichment analysis was performed using clusterProfiler package (version 3.12.0) and bubble plots were generated using the ggplot2 package (version 2.0.0). (C) The correlation between PTPN1 and its paralogous genes in HCC. Correlation coefficients were calculated with a Spearman's rank correlation coefficient test. (D) Hub genes of PTP family genes were screened from the protein-protein interaction network using the cytoHubba plugin (version 0.1, https://apps.cytoscape.org/apps/cytohubba) in Cytoscape (version 3.8.0; Cytoscape Consortium; www.cytoscape.org). The maximal clique centrality algorithm was used to calculate the score of each protein node and to identify the top 5 hub genes. (E) Venn diagrams indicating the overlap between Correlation (the correlation coefficient with PTPN1 is >0.5), HubGenes (Hub genes of PTP family genes), Up (paired) (Up regulated genes in paired samples of HCC), and Up (unpaired) (Up regulated genes in unpaired samples of HCC). Venn diagrams were generated using the venn Diagram package (version 1.7.3 in R. HCC, hepatocellular carcinoma; PTP, protein tyrosine phosphatase; PTPN, PTP non-receptor type; PTPR, PTP receptor-type; TPM, transcripts per kilobase million.

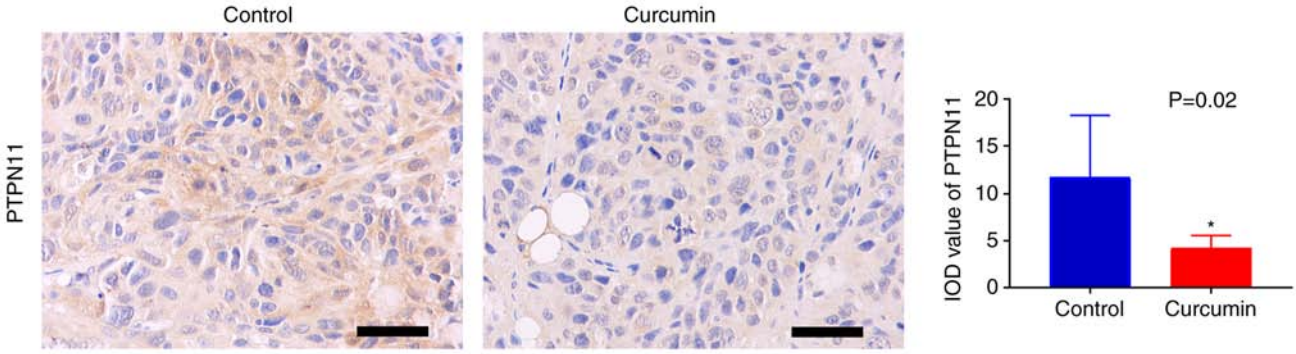

Figure 5. Curcumin inhibited PTPN11 protein expression in hepatocellular carcinoma xenograft tissue. Representative images of PTPN11 staining by immunohistochemistry. Scale bar=50 μm. *P≤0.05. PTPN11, protein tyrosine phosphatase non-receptor type 11; IOD, integrated optical density.

PTPN1 in 18 cancer types, from TCGA data, were analyzed, and the results indicated that 10 types of cancer had significantly higher expression levels of PTPN1 and 3 types of cancer had significantly lower expression levels of PTPN1 compared with normal tissues. Therefore, modulation of PTPN1 phosphatase activity with inhibitors may contribute to innovative targeted pharmacotherapy for certain cancer types. Previous studies have reported that curcumin, and its derivatives with a cytotoxic effect against breast cancer cells, exhibited an inhibitory effect against PTPN1 phosphatase (25,26). Another study reported that curcumin was an activator of PTPN1 and could reduce cell motility in colon cancer through dephosphorylation of cortactin (27). Curcumin inhibits PTPN1 phosphatase activity in breast cancer and activates it in colon cancer cells, so the effect of curcumin on PTPN1 in HCC cells needs to be verified.

Furthermore, the differential expression of typical tyrosine-specific PTPs in HCC tissues and their correlation with PTPN1 expression levels was analyzed. The results demonstrated that the mRNA expression levels of PTPN1 in HCC were significantly correlated with that of PTPN11. The SH2 domain-containing phosphatase 2 (SHP2) encoded by PTPN11 is involved in transcriptional regulation, cytokine signaling, cell differentiation, and tumor cell proliferation and migration (28,29). Mutations in PTPN11 cause certain types of cancer, such as lung, neuroblastoma, breast, skin and cervical cancer.

Previous studies have reported that SHP2 is overexpressed in the majority of HCC and is more highly expressed during metastasis (30,31). SHP2 promotes the growth of liver cancer cells through the Ras/Raf/MEK/ERK cascade and the growth of liver cancer cells through the PI3K/Akt/mTOR signaling pathway enhances the potential malignancy of liver cancer (30,32). SHP2 can promote the self-renewal ability of liver cancer stem cells by activating the β-catenin signaling pathway (33). Therefore, high SHP2 expression levels often indicate a poor prognosis in patients with liver cancer. The results of the present study suggest that curcumin inhibits PTPN11 protein expression in HCC cells. Further experiments are needed to elucidate the mechanism by which curcumin inhibits the expression of PTPN11. It is also necessary to clarify whether the reduction in protein expression levels of SHP2 is the consequence or the cause of curcumin-mediated inhibition of HCC cell proliferation.

The present study had certain limitations. First, bioinformatic techniques were used to identify potential targets of curcumin for HCC and *in vivo* experiments were conducted to validate the findings; however, an in-depth investigation of its specific mechanism was not performed. Second, it was difficult to obtain sufficient tumor samples for western blotting analysis to accurately measure the candidate protein. While western blotting is considered the benchmark method for protein expression quantification in tissues, it is imperative to also observe the localization of candidate proteins within tumor cells. Therefore, the present study prioritized the preparation of specimens for immunohistochemical techniques.

In summary, the results of the present study suggest that curcumin can inhibit the proliferation of HCC cells and promote apoptosis. Furthermore, curcumin inhibits PTPN1 and PTPN11 expression. Curcumin may inhibit the proliferation of HCC cells by inhibiting the expression of PTPN1 and PTPN11 genes. However, this conclusion must be verified experimentally. Future experiments are required to elucidate the mechanism of curcumin inhibition of PTPN1 and PTPN11 expression in HCC cells.

Acknowledgements

Not applicable.

Funding

The present study was funded by the Provincial Medicine and Health Science Technology Development Program Shandong (grant no. 2017WS822) and the Innovation and Entrepreneurship Training Program for College Students (grant no. S202010440077).

Availability of data and materials

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

Authors' contributions

JZ, YL, XW, ZW, EX, JL and DW contributed to the study conception and design. Material preparation and data collection were performed by XW and DW. Data collection and analysis

were performed by JZ, YL, XW, ZW and EX. The first draft of the manuscript was written by JZ, JL and DW. All authors commented on previous versions of the manuscript. All authors read and approved the final version of the manuscript. JZ and DW confirm the authenticity of all the raw data.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Siegel RL, Miller KD, Fuchs HE and Jemal A: Cancer statistics, 2021. CA Cancer J Clin 71: 7-33, 2021.
2. Forner A, Reig M and Bruix J: Hepatocellular carcinoma. Lancet 391: 1301-1314, 2018.
3. Luo XY, Wu KM and He XX: Advances in drug development for hepatocellular carcinoma: Clinical trials and potential therapeutic targets. J Exp Clin Cancer Res 40: 172, 2021.
4. Zhang HH, Zhang Y, Cheng YN, Gong FL, Cao ZQ, Yu LG and Guo XL: Metformin incombination with curcumin inhibits the growth, metastasis, and angiogenesis of hepatocellular carcinoma in vitro and in vivo. Mol Carcinog 57: 44-56, 2018.
5. Baby J, Devan AR, Kumar AR, Gorantla JN, Nair B, Aishwarya TS and Nath LR: Cogent role of flavonoids as key orchestrators of chemoprevention of hepatocellular carcinoma: A review. J Food Biochem 45: e13761, 2021.
6. Ghobadi N and Asoodeh A: Co-administration of curcumin with other phytochemicals improves anticancer activity by regulating multiple molecular targets. Phytother Res 37: 1688-1702, 2023.
7. Khan H, Ni Z, Feng H, Xing Y, Wu X, Huang D, Chen L, Niu Y and Shi G: Combination of curcumin with N-n-butyl haloperidol iodide inhibits hepatocellular carcinoma malignant proliferation by downregulating enhancer of zeste homolog 2 (EZH2)-lncRNA H19 to silence Wnt/β-catenin signaling. Phytomedicine 91: 153706, 2021.
8. Bai C, Zhao J, Su J, Chen J, Cui X, Sun M and Zhang X: Curcumin induces mitochondrial apoptosis in human hepatoma cells through BCLAF1-mediated modulation of PI3K/AKT/GSK-3β signaling. Life Sci 306: 120804, 2022.
9. Khan H, Ullah H and Nabavi SM: Mechanistic insights of hepatoprotective effects of curcumin: Therapeutic updates and future prospects. Food Chem Toxicol 124: 182-191, 2019.
10. Xu J, Lin H, Wu G, Zhu M and Li M: IL-6/STAT3 is a promising therapeutic target for hepatocellular carcinoma. Front Oncol 11: 760971, 2021.
11. Shao S, Duan W, Xu Q, Li X, Han L, Li W, Zhang D, Wang Z and Lei J: Curcumin suppresses hepatic stellate cell-induced hepatocarcinoma angiogenesis and invasion through downregulating CTGF. Oxid Med Cell Longev 2019: 8148510, 2019.
12. Liu J, Lichtenberg T, Hoadley KA, Poisson LM, Lazar AJ, Cherniack AD, Kovatich AJ, Benz CC, Levine DA, Lee AV, *et al*: An integrated TCGA pan-cancer clinical data resource to drive high-quality survival outcome analytics. Cell 173: 400-416.e11, 2018.
13. Yu G, Wang LG, Han Y and He QY: clusterProfiler: An R package for comparing biological themes among gene clusters. OMICS 16: 284-287, 2012.
14. Sreepriya M and Bali G: Chemopreventive effects of embelin and curcumin against N-nitrosodiethylamine/phenobarbital-induced hepatocarcinogenesis in Wistar rats. Fitoterapia 76: 549-555, 2005.
15. Karaboga Arslan A, Uzunhisarcıklı E, Yerer M and Bishayee A: The golden spice curcumin in cancer: A perspective on finalized clinical trials during the last 10 years. J Cancer Res Ther 18: 19-26, 2022.
16. Wang L, Zhu Z, Han L, Zhao L, Weng J, Yang H, Wu S, Chen K, Wu L and Chen T: A curcumin derivative, WZ35, suppresses hepatocellular cancer cell growth via downregulating YAP-mediated autophagy. Food Funct 10: 3748-3757, 2019.
17. Giordano A and Tommonaro G: Curcumin and cancer. Nutrients 11: 2376, 2019.
18. Elkhamesy A, Refaat M, Gouida MSO, Alrdahe SS and Youssef MM: Diminished CCl_4-induced hepatocellular carcinoma, oxidative stress, and apoptosis by co-administration of curcumin or selenium in mice. J Food Biochem 46: e13845, 2022.
19. Salehi B, Stojanović-Radić Z, Matejić J, Sharifi-Rad M, Anil Kumar NV, Martins N and Sharifi-Rad J: The therapeutic potential of curcumin: A review of clinical trials. Eur J Med Chem 163: 527-545, 2019.
20. Tonks NK, Diltz CD and Fischer EH: Purification of the major protein-tyrosine-phosphatases of human placenta. J Biol Chem 263: 6722-6730, 1988.
21. Chen PJ and Zhang YT: Protein tyrosine phosphatase 1B (PTP1B): Insights into its new implications in tumorigenesis. Curr Cancer Drug Targets 22: 181-194, 2022.
22. Sivaganesh V, Sivaganesh V, Scanlon C, Iskander A, Maher S, Lê T and Peethambaran B: Protein tyrosine phosphatases: Mechanisms in cancer. Int J Mol Sci 22: 12865, 2021.
23. Teimouri M, Hosseini H, ArabSadeghabadi Z, Babaei-Khorzoughi R, Gorgani-Firuzjaee S and Meshkani R: The role of protein tyrosine phosphatase 1B (PTP1B) in the pathogenesis of type 2 diabetes mellitus and its complications. J Physiol Biochem 78: 307-322, 2022.
24. Maheshwari N, Karthikeyan C, Trivedi P and Moorthy NSHN: Recent advances in protein tyrosine phosphatase 1B targeted drug discovery for type II diabetes and obesity. Curr Drug Targets 19: 551-575, 2018.
25. Kostrzewa T, Przychodzen P, Gorska-Ponikowska M and Kuban-Jankowska A: Curcumin and Cinnamaldehyde as PTP1B inhibitors with antidiabetic and anticancer potential. Anticancer Res 39: 745-749, 2019.
26. Kostrzewa T, Wołosewicz K, Jamrozik M, Drzeżdżon J, Siemińska J, Jacewicz D, Górska-Ponikowska M, Kołaczkowski M, Łaźny R and Kuban-Jankowska A: Curcumin and its new derivatives: Correlation between cytotoxicity against breast cancer cell lines, degradation of PTP1B phosphatase and ROS generation. Int J Mol Sci 22: 10368, 2021.
27. Radhakrishnan VM, Kojs P, Young G, Ramalingam R, Jagadish B, Mash EA, Martinez JD, Ghishan FK and Kiela PR: pTyr421 cortactin is overexpressed in colon cancer and is dephosphorylated by curcumin: Involvement of non-receptor type 1 protein tyrosine phosphatase (PTPN1). PLoS One 9: e85796, 2014.
28. Chen C, Cao M, Zhu S, Wang C, Liang F, Yan L and Luo D: Discovery of a novel inhibitor of the protein tyrosine phosphatase Shp2. Sci Rep 5: 17626, 2015.
29. Asmamaw MD, Shi XJ, Zhang LR and Liu HM: A comprehensive review of SHP2 and its role in cancer. Cell Oncol (Dordr) 45: 729-753, 2022.
30. Liu JJ, Li Y, Chen WS, Liang Y, Wang G, Zong M, Kaneko K, Xu R, Karin M and Feng GS: Shp2 deletion in hepatocytes suppresses hepatocarcinogenesis driven by oncogenic β-catenin, PIK3CA and MET. J Hepatol 69: 79-88, 2018.
31. Han T, Xiang DM, Sun W, Liu N, Sun HL, Wen W, Shen WF, Wang RY, Chen C, Wang X, *et al*: PTPN11/Shp2 overexpression enhances liver cancer progression and predicts poor prognosis of patients. J Hepatol 63: 651-660, 2015.
32. Yue X, Han T, Hao W, Wang M and Fu Y: SHP2 knockdown ameliorates liver insulin resistance by activating IRS-2 phosphorylation through the AKT and ERK1/2 signaling pathways. FEBS Open Bio 10: 2578-2587, 2020.
33. Xiang D, Cheng Z, Liu H, Wang X, Han T, Sun W, Li X, Yang W, Chen C, Xia M, *et al*: Shp2 promotes liver cancer stem cell expansion by augmenting β-catenin signaling and predicts chemotherapeutic response of patients. Hepatology 65: 1566-1580, 2017.