A nomogram combining PPARγ expression profiles and clinical factors predicts survival in patients with hepatocellular carcinoma

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Abstract. Hepatocellular carcinoma (HCC) is the most common primary liver cancer with poor prognosis. Peroxisome proliferator-activated receptor γ (PPARγ) is involved in the development of various tumor types. However, its role in hepatocellular carcinoma (HCC) remains unclear. Multiple databases including The Cancer Genome Atlas, Gene Expression Omnibus and Kaplan-Meier plotter were used for bioinformatics analysis of the *PPARγ* gene or protein. Immunohistochemical labeling of tumor and adjacent normal tissues obtained from 125 patients with HCC was performed to analyze the relationship between PPARγ expression and overall survival (OS) rate. *PPARγ* was evaluated using functional enrichment analyses and Lasso regression was used to conduct a dimensionality reduction analysis of 43 clinical factors for HCC. An OS prognostic nomogram was then established using seven independent risk factors screened via Lasso regression. PPARγ expression in HCC tumor tissues was higher compared with that in normal liver tissues, and its high expression was associated with poor prognosis, as indicated by bioinformatics analysis. However, opposite results were obtained using the clinical specimens. Functional enrichment analysis indicated that *PPARγ* was enriched in the 'fatty acid metabolism' pathway. Lasso regression identified seven clinical factors associated with prognosis, including Tumor-Node-Metastasis stage, grade, vascular invasion, α fetoprotein, carbohydrate antigen 199, γ-glutamyl transpeptidase and the PPARγ protein. These seven clinical factors were to construct an OS prognostic nomogram. Overall, PPARγ was highly expressed in the livers of patients with HCC and can be included in an OS prognostic nomogram. However, the factors underlying the differential association of PPARγ expression with HCC prognosis in different datasets should be further investigated.

Introduction

Hepatocellular carcinoma (HCC) is the most commonly occurring form of primary liver cancer and is one of the leading causes of cancer-associated death worldwide (1). HCC has poor prognosis and accounts for 75-85% of all primary liver cancer cases according to global cancer statistics in 2018 (2). Current therapies against HCC include ablation, liver transplantation and radical resection, which is the main treatment (3). However, HCC shows a high recurrence. Roayaie *et al* (4) reported that the recurrence rate of HCC is 60% at 5 years after surgery (4). Another study reported HCC recurrence, including true recurrence due to dissemination and *de novo* tumors within the oncogenic liver, makes 70% of cases worsen within 5 years after surgery (5). These have become the main problems that limit the 5-year survival rate of patients with liver cancer. This highlights the need to determine molecular mechanisms involved in the development of HCC and identify molecules that can be used in anti-HCC therapy. Developing new methods for predicting the prognosis of this disease is also important for

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improving the prognosis prediction of patients with HCC may help inform treatment choices.

Peroxisome proliferator activated receptor γ (PPARγ), a ligand-activated transcription factor, is a member of the nuclear receptor superfamily of PPAR proteins. PPARγ has two subtypes: PPARα And PPARβ (6). PPARα is involved in regulating various processes, from inflammation and immunity to nutrient metabolism and energy balance (7). PPARβ has been shown to be involved in metabolism, angiogenesis and inflammatory responses (8). PPARγ plays an important role in the occurrence and development of various diseases, including obesity, inflammation, diabetes, atherosclerosis and cancer (9). This protein is also a major regulator of fat cell formation. In adipocytes, PPARγ regulates the expression of genes controlling the uptake and storage of free fatty acids and mediates the endocrine function of adipose tissue (10). A previous study showed that PPARγ may be involved in the occurrence and development of liver cancer (11). However, the specific mechanisms involved are unclear. It is also undetermined whether PPARγ acts as an antitumor or tumor-promoting factor in liver cancer (12).

Patients with HCC usually have a background of chronic liver disease such as non-alcoholic fatty liver disease (NAFLD), and chronic hepatitis B and C (13). NAFLD, a disease closely associated with metabolic syndrome, includes chronic liver diseases ranging from simple steatosis to non-alcoholic steatohepatitis (NASH) (14). Increasing epidemiological evidence indicates that NAFLD is a major cause of HCC (15). Numerous studies have shown that PPARγ is involved in the pathogenesis of NAFLD (10,15-20). Elevated PPARγ levels can increase the expression of genes responsible for lipid metabolism in the liver, which induces liver steatosis and leads to NAFLD (21-23). Therefore, based on the previously identified role of PPARγ in liver cancer (24-26), the present study hypothesized that PPARγ may be involved in the development of HCC by affecting the metabolism of liver fat cells.

The present study examined the role of PPARγ in HCC development. Databases including The Cancer Genome Atlas (TCGA), Gene Expression Omnibus (GEO) and Kaplan-Meier plotter were used to predict the expression and function of PPARγ using bioinformatics. Then, the differences between the results obtained via bioinformatics analysis and those obtained by analysis of clinical data of 125 patients with HCC were compared. It was explored whether PPARγ participates in the development and progression of HCC by affecting lipid metabolism. Finally, PPARγ expression profiles and other selected prognostic clinical indicators were used to build a model for predicting the prognosis of patients with HCC.

Materials and methods

Differential analysis of PPARG mRNA expression in patients with HCC conducted using TCGA and GEO databases. The data on *PPARγ* expression were downloaded from TCGA database (https://genome-cancer.ucsc.edu/) and GEO database (https://www.ncbi.nlm.nih.gov/geo/), then analyzed using the limma package of R software (version 3.5.2.) (27). The Wilcoxon signed-rank test was used to evaluate *PPARG* expression in HCC tissues and normal adjacent tissues of patients with HCC.

Interactive gene expression profiling. Gene Expression Profiling Interactive Analysis (GEPIA) (http://gepia.cancer-pku.cn/) is an interactive web application based on 9,736 tumors and 8,587 samples of normal tissue from TCGA and Genotype-Tissue Expression databases. GEPIA can be used for the profiling of cancerous and normal gene expression, and for interactive analyses (28). This database was used to explore the relationship between *PPARγ* expression levels and prognosis in patients with HCC.

Kaplan-Meier plotter analysis. The Kaplan-Meier plotter (http://kmplot.com/analysis/) database contains gene expression and clinical data for 21 different types of cancer including liver, lung, ovarian, gastric and breast cancer (29). Kaplan-Meier plotter was used to evaluate the prognostic value of *PPARγ* in patients with HCC. The median *PPARγ* expression (score of 1; Table I) was used as the cut-off value, which was used to divide patients into high and low expression groups and the prognosis of the two groups was compared.

Analysis of the human protein atlas. The Human Protein Atlas (https://www.proteinatlas.org), an open-access resource, was used to map all the human proteins in cells, tissues and organs. This mapping was performed to explore the distribution of PPARγ protein in tumor and adjacent normal tissues of patients with HCC.

Analysis of kyoto encyclopedia of genes and genomes (KEGG) and gene set enrichment analysis (GSEA). GSEA (http://www.broadinstitute.org/gsea/index.jsp) is a powerful analytical method that can interpret genome-wide expression profiles (30). KEGG enrichment analysis (31) for *PPARγ* was performed using The Database for Annotation, Visualization and Integrated Discovery (https://david.ncifcrf.gov), and evaluated the *PPARγ* pathways using GSEA. These analyses were performed using the clusterProfiler package of R software (version 3.5.2 (32).

Clinical factors affecting HCC development and progression. The study was approved by The Ethics Committee of the Zhejiang Provincial People's Hospital (Hangzhou, China). Clinical data on 125 patients with HCC were collected from the Zhejiang Provincial People's Hospital. The diagnosis of HCC in all the patients included was confirmed using histopathological examination by independent pathologists. Paraffin samples of HCC were collected in March 2020. These patients underwent surgical operations at Zhejiang Provincial People's Hospital from January 2008 to December 2015, and patients provided written informed consent at the time of tissue collection. The patients had complete follow-up data from the day of surgical resection of the primary tumor to death or the last follow-up. The last follow-up date was March 2016. The Tumor-Node-Metastasis (TNM) stage was defined according to the criteria described in the 8th American Joint Committee on Cancer guidelines (33). The inclusion criteria were as follows: i) Patients who underwent surgery, ii) patients with the histological type hepatocellular carcinoma and iii) patients with complete follow-up information. The exclusion criteria included: i) patients with other malignant diseases and (II) those without detailed clinical information. In addition, 43 clinical factors, including PPARγ protein

Table I. Specific scoring criteria for staining intensity and proportion of stained cells.

Specific criteria	Points				
	0	1	2	3	4
Dyeing intensity	Absent	Weak positive	Moderately positive	Strong positive	Strong positive
Percentage range of positively stained cells, %	0	1-25	26-50	51-75	76-100

expression, were collected for all the patients (Table SI). Lasso regression was then performed on these clinical factors using the glmnet package (version 4.0-2.) in R software (http://www.jstatsoft.org/v33/i01/) (34). Univariate Cox regression analysis was also performed using R software on the selected clinical factors. Finally, the selected factors were used to establish a nomogram model using the rms, foreign and survival package in R software. The area under the curve (AUC) of receiver operating characteristic (ROC) and concordance index (C-index) were used to evaluate the accuracy of the model. Meanwhile, in order to validate that PPARγ did play a role in the established nomogram, other clinical factors besides PPARγ were used to establish another nomogram to obtain its C-index and AUC for comparison.

Tissue microarray and immunohistochemical (IHC) analyses. Specimens that included tumor tissue and corresponding adjacent normal tissues were collected from 125 patients with HCC. A tissue microarray was constructed using paired tumor tissue and adjacent normal tissue collected 0.5-1.0 cm from the margin of the tumor (35). Immunolabeling of the tissue microarray was performed using an anti-PPARγ polyclonal antibody (1:200; cat. no. ab209350; Abcam). Briefly, sections from the tissue microarray were baked at 70°C for 2 h and then deparaffinized using xylene (cat. no. 534596; Sigma-Aldrich; Merck KGaA). A gradient of ethanol concentrations (100, 95 and 80%) was used to rehydrate the sections, and antigen retrieval was performed by boiling the sections using a high-pressure cooker for 3 min in 1 mM Tris-EDTA buffer. The sections were blocked with 3% hydrogen peroxide at room temperature for 15 min to inhibit endogenous peroxidase activity. Sections were then incubated with 10% goat non-immune serum (cat. no. ZLI-9022; Zsbio Store) for 20 min to reduce non-specific staining. Consequently, the sections were incubated with the primary antibody at 4°C overnight, and then with the Biotin-SP-AffiniPure Goat Anti-Rabbit IgG (H+L) (cat. no. 111-065-144; 1:500; Jackson ImmunoResearch Europe, Ltd.) at room temperature for 15 min. Afterwards, the sections were incubated with HRP-conjugated streptavidin (cat. no. 3999s; CST Biological Reagents Co., Ltd.) at room temperature for another 15 min. Protein expression was visualized using a diaminobenzidine substrate kit (cat. no. ab64238; 1:50; Abcam) and hematoxylin was used for 5 min at room temperature to counterstain the sections.

Evaluation of IHC labeling. Two experienced pathologists, blinded to the patients' pathology reports, independently scored the results of IHC labeling based on intensity and proportion of positively stained cells by light microscopy (cat. no. NI-SS 933679; Nikon Corporation). Signal intensity was expressed as: 0 for absent, 1 for weak positive, 2 for moderately positive and 3 for strong positive. The proportion of positively stained cells was also quantified as: 0 for 0% positively stained cells, 1 for 1-25% positively stained cells, 2 for 26-50% positively stained cells, 3 for 51-75% positively stained cells and 4 for 76-100% positively stained cells. Specific evaluation criteria are shown in Table I. The final score, obtained by multiplying the intensity score by the percentage of positively labeled cells, was used to indicate the expression of the PPARγ protein. A score of ≤1 was defined as low PPARγ expression, while that of >1 was defined as high PPARγ expression.

Statistical analysis. Kaplan-Meier analysis was performed using log-rank tests. Lasso regression was used to assess the prognosis of patients with HCC and identify potential risk factors, and Lasso analysis sampled and analyzed the sample 1,000 times. Univariate Cox regression analysis was used to analyze the prognosis of clinical factors screened by Lasso analysis. The χ^2 test was used to examine the association between PPARγ and other clinical factors in categorical variables. When variables with ≥20% of the cells had a count of ≤5, the data were analyzed using Fisher's exact test. Additionally, the ROC curve was plotted and the AUC was calculated. The C-index and AUC were used to analyze the accuracy of the nomogram. All statistical analyses were performed using the R software package. Confidence interval (CI) was set to 95%. P<0.05 was considered to indicate a statistically significant difference.

Results

Bioinformatics analysis of PPARγ mRNA and protein expression. The results of the bioinformatics analysis showed that *PPARγ* mRNA expression in HCC tumor tissues was higher compared with that in normal tissues obtained from TCGA database (P=6.561x10^{-11}; Fig. 1A). High *PPARG* expression was indicative of poor prognosis in patients with HCC as assessed using GEPIA (P=0.00074; Fig. 1B). It was concluded that high *PPARγ* expression was detrimental to patient prognosis by using Kaplan-Meier plotter analysis (P=0.0014; Fig. 1C). Overexpression of PPARγ protein in HCC tissues was predictive of unfavorable prognosis in patients with HCC as assessed using analysis of the Human Protein Atlas (P<0.001; Fig. 1D). In summary, bioinformatics analysis reported that high *PPARγ* mRNA or protein level in tumor tissues compared with normal tissues indicated a poor prognosis.
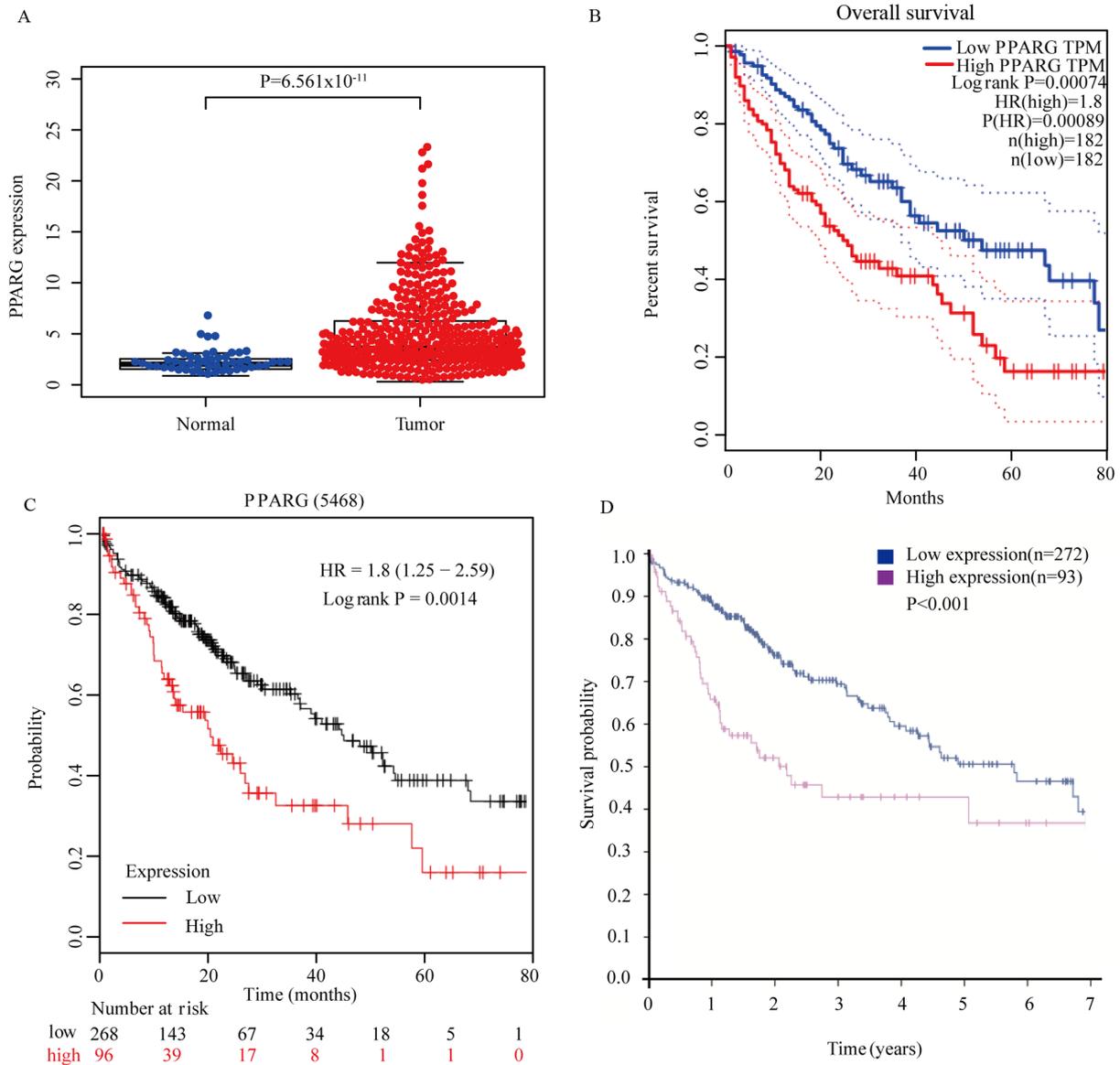
Figure 1. Bioinformatics analysis of *PPARγ* mRNA and protein expression. (A) mRNA expression of *PPARγ* in HCC tumor tissues was higher compared with that in adjacent normal tissues as assessed using The Cancer Genome Atlas database. (B) High *PPARγ* expression was associated with poor prognosis in patients with HCC as assessed using Gene Expression Profiling Interactive Analysis. (C) High *PPARγ* expression was associated with poor prognosis in patients with HCC as assessed using Kaplan-Meier plotter. (D) High expression of PPARγ protein in HCC tissues was unfavorable for prognosis in patients with HCC as assessed using analysis of the Human Protein Atlas. *PPARγ*, peroxisome proliferator-activated receptor γ; HCC, hepatocellular carcinoma; HR, hazard ratio.

Relationship between PPARγ expression in HCC tissues and prognosis of patients with HCC. PPARγ expression in tumor tissues was higher compared with that in normal liver tissues by analysis of PPARγ expression in 125 patients with HCC (P<0.001; Fig. 2A), and the high expression of PPARγ was beneficial to the prognosis of patients (P=0.015; Fig. 2B). IHC was used to evaluate PPARγ expression in tumor tissues and corresponding adjacent normal tissues. PPARγ expression in tissues was graded according to the criteria aforementioned.

Evaluation of IHC labeling. As shown in Fig. 3, PPARγ levels in tumor tissues (Fig. 3A and B) were higher compared with those in the adjacent normal tissues (Fig. 3C and D) of patients with HCC (P<0.001; Fig. 3A). Increased expression of PPARγ

in HCC tumor tissues was indicative of an improved prognosis in patients with HCC (P=0.015; Fig. 2B).

Association between PPARγ expression and clinical features in patients with HCC. The associations between PPARγ expression levels and key clinical characteristics of patients with HCC were examined (Table II). The results indicated that three clinical features including age (P=0.019), tumor diameter (P=0.041) and vascular invasion (P=0.026) were significantly associated with PPARγ expression. However, PPARγ expression was not significantly associated with factors such as hepatitis B, cirrhosis and metabolism-related indicators (Table II).

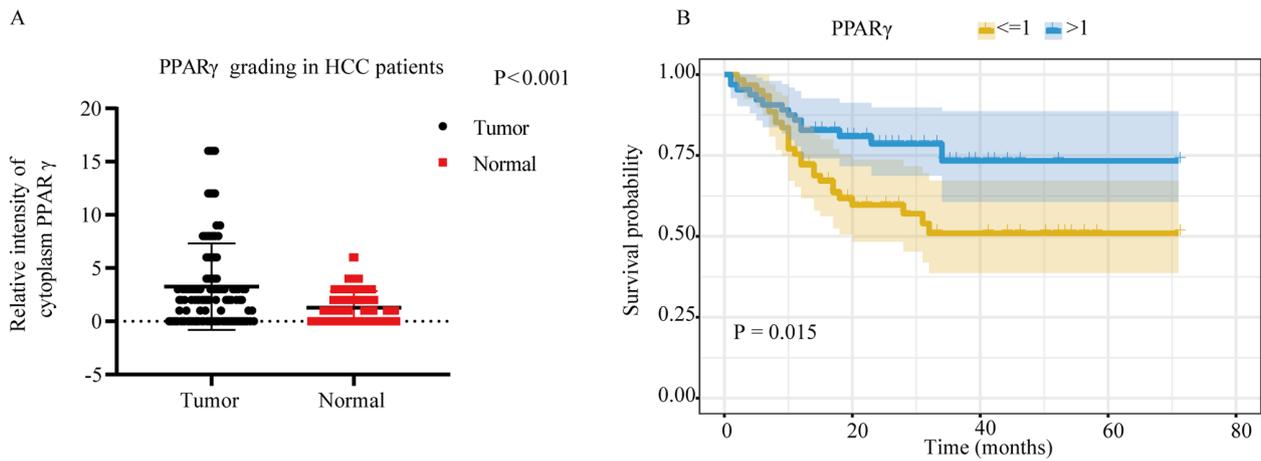*Functional enrichment analyses for PPARγ*. KEGG pathway enrichment was conducted through GSEA in high-expression

Figure 2. Relationship between PPARγ expression in HCC tissues and prognosis of patients with HCC. (A) PPARγ expression level in tumor tissues was higher compared with those in adjacent normal tissues of 125 patients with hepatocellular carcinoma. (B) High expression of PPARγ was associated with improved prognosis. PPARγ, peroxisome proliferator-activated receptor γ; HCC, hepatocellular carcinoma.
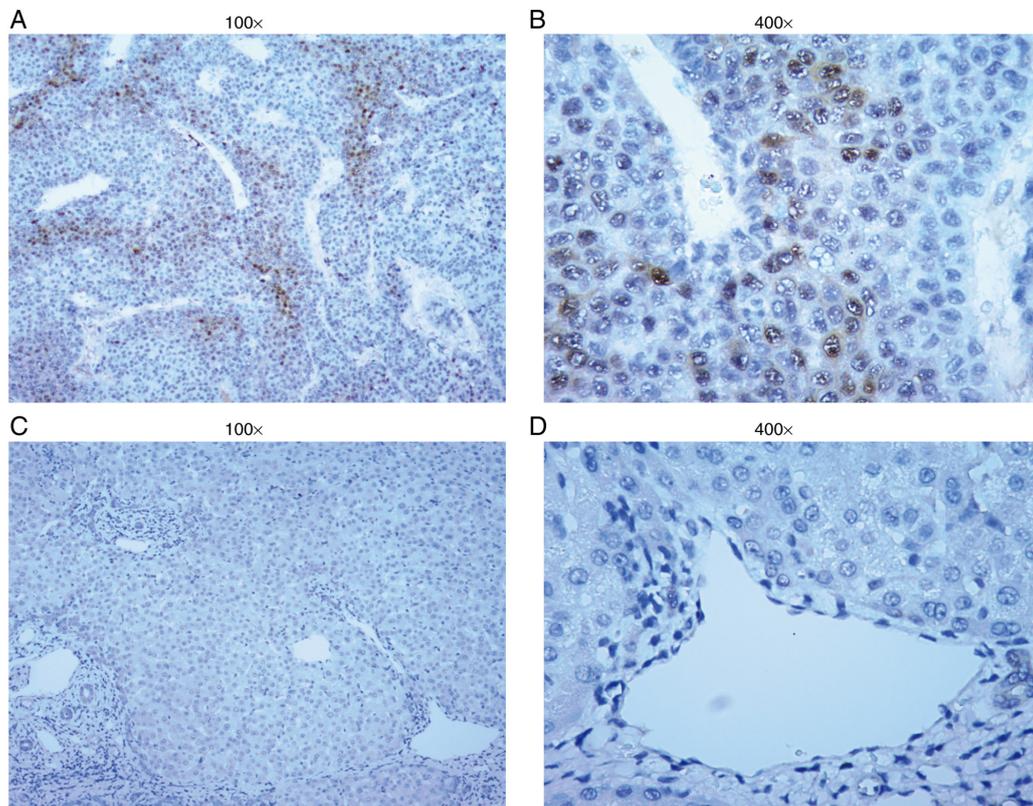

Figure 3. Peroxisome proliferator-activated receptor γ expression in tumor tissues at (A) 100x and (B) 400x magnification and in the adjacent normal tissues at (C) 100x and (D) 400x magnification of patients with hepatocellular carcinoma.

group in the entire set by R software (clusterProfiler package). It revealed that patients with low PPARγ expression were associated with some pathways, including 'mitotic spindle,' 'G$_2$M checkpoint,' 'E2F targets', 'spermatogenesis', 'mammalian target of rapamycin complex 1 signaling', 'fatty acid metabolism', 'TNFA signaling via NFκB' and 'xenobiotic metabolism' (Fig. 4A and B). Adjusted P<0.05 for a gene set were considered statistically significant. Then a GSEA pathway analysis was performed with respect to fatty acid metabolism pathways (Fig. 4C). This indicated that

the high expression of *PPARγ* gene in liver cancer is closely related to fatty acid metabolism.

Screening for clinical factors associated with prognosis. Lasso regression was used to analyze the 43 clinical factors aforementioned (Table SI), and identified the following seven clinical factors associated with prognosis: Tumor-Node-Metastasis (TNM) stage, type of differentiation, vascular invasion, α feto-protein (APF), carbohydrate antigen 199 (CA199), γ-glutamyl transpeptidase (γ-GT) and PPARγ protein expression (Fig. 5).

Table II. Association between PPARγ and the clinical characteristics.

Factors	PPARγ expression Low	High	Total, n	χ^2	P-value
Sex				0.629	0.428
Male	46	52	98		
Female	15	12	27		
Age, years				5.464	0.019
≤60	43	32	75		
>60	18	32	50		
Tumor diameter, cm				4.163	0.041
≤4	29	42	71		
>4	32	22	54		
Grade				2.132	0.144
I+II	45	54	99		
III	16	10	26		
TNM stage				0.642	0.663
IA+IB+II	56	61	117		
IIIA+IIIB+IVA+IVB	5	3	8		
AFP, μg/l				0.321	0.571
≤200	41	46	87		
>200	20	18	38		
Vascular invasion				4.979	0.026
Negative	31	45	76		
Positive	30	19	49		
Hepatitis B				0.261	0.609
Negative	12	15	27		
Positive	49	49	98		
Cirrhosis				0.913	0.339
Negative	21	17	38		
Positive	40	47	87		
TG, mmol/l				2.243	0.134
≤1	31	41	72		
>1	30	23	53		
HDL, mmol/l				0.001	0.990
≤1.04	42	44	86		
>1.04	19	20	39		
LDL, mmol/l				0.003	0.954
≤2.1	34	36	70		
>2.1	27	28	55		
TCHO, mmol/l				0.473	0.492
≤3.11	23	28	51		
>3.11	38	36	74		

AFP, α fetoprotein; TCHO, total cholesterol; TG, triglyceride; LDL, low density lipoprotein; HDL, high density lipoprotein; TNM, Tumor-Node-Metastasis.

Univariate analysis of the seven clinical factors associated with prognosis. Univariate analysis was performed on the seven clinical factors of TNM stage, grade, vascular invasion, APF, CA199, γ-GT and PPARγ protein expression that were screened previously using Lasso regression analysis. The results indicated that TNM stage (P=0.005; Table III), grade (P<0.001; Table III), vascular invasion (P<0.001; Table III), APF (P=0.035; Table III), CA199 (P=0.024;
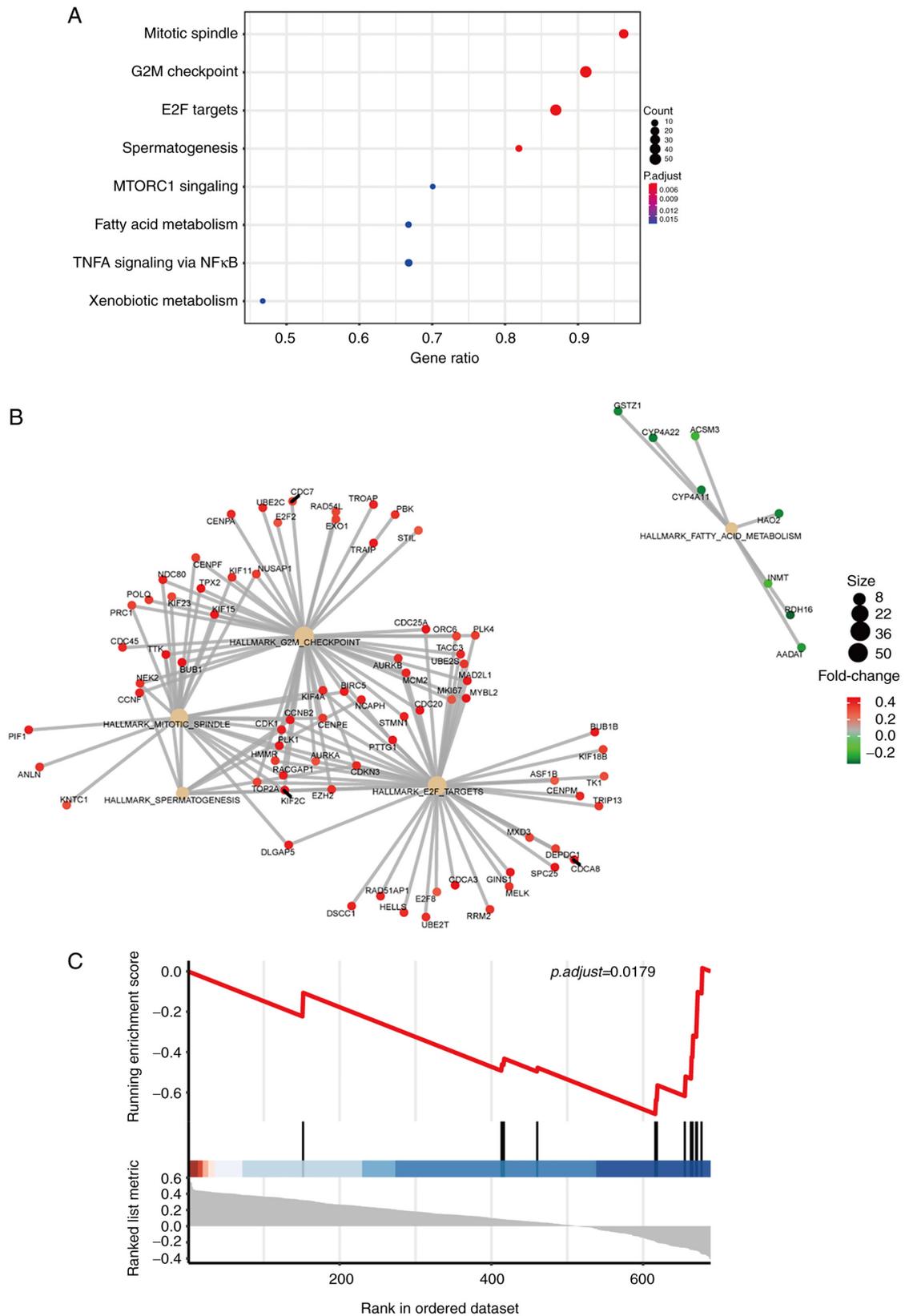
Figure 4. Functional enrichment analyses for *PPARγ*. (A) Pathway enrichment of *PPARγ*. (B) Genes associated with *PPARγ*-enriched pathways. (C) Gene Set Enrichment Analysis of fatty acid metabolism pathways. PPARγ, peroxisome proliferator-activated receptor γ; MTORC1, mammalian target of rapamycin complex 1.

Table III), γ-GT (P=0.002; Table III) and expression of PPARγ protein (P=0.015; Table III) were statistically significant factors affecting survival of patients with HCC

(Table III). These results indicated that that these seven factors were independent risk factors for the prognosis of patients with HCC.
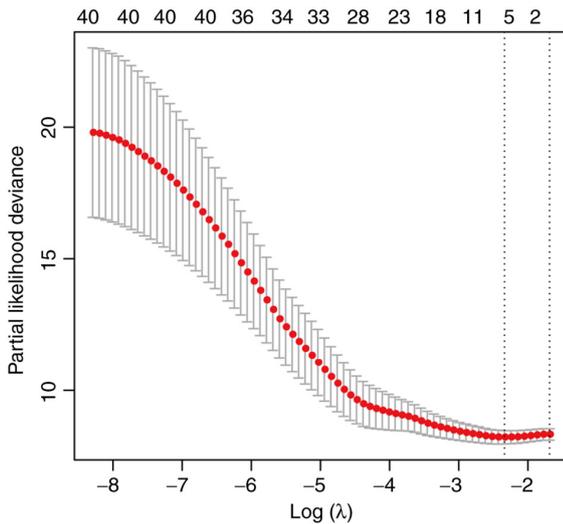
Figure 5. Lasso regression analysis of 43 clinical factors was used to screen out seven factors associated with the prognosis of patients with hepatocellular carcinoma. Seven clinical factors included: Tumor-Node-Metastasis stage, type of differentiation, vascular invasion, α fetoprotein, carbohydrate antigen 199, γ-glutamyl transpeptidase and peroxisome proliferator-activated receptor γ protein expression.

Figure 6. In total, seven clinical factors, including TNM stage, grade, vascular invasion, APF, CA199, γ-GT and PPARγ protein expression, were used to establish an overall survival prognostic nomogram. PPARγ, peroxisome proliferator-activated receptor γ; TNM, Tumor-Node-Metastasis; AFP, α fetoprotein; CA199, carbohydrate antigen 199; γ-GT, γ-glutamyl transpeptidase.

Construction of an OS prognostic nomogram. The aforementioned seven clinical factors were used to build an effective OS prognostic nomogram to predict the prognosis of patients with HCC (Fig. 6). The C-index used to evaluate the accuracy of the nomogram using these clinical factors was 0.755 (95% CI, 0.591 to 0.919; P<0.001; data not shown) and the AUC values predicting the 1-, 3- and 5-year OS of the nomogram were 0.744, 0.780 and 0.780, respectively (Fig. 7A-C). As for the nomogram established by clinical factors that did not include PPARγ, the C-index and AUC values were both lower. The C-index was 0.748 (95% CI, 0.584 to 0.912; P<0.001; data not shown). The AUC values predicting the 1-, 3- and 5-year OS of the nomogram were 0.706, 0.751 and 0.751, respectively (Fig. 7D-F).

Discussion

HCC is a major human health concern because of its high rates of recurrence and metastasis (36). Elucidating the molecular regulatory mechanisms involved in HCC is necessary for improving diagnostic methods and anti-HCC therapies. This requires detailed understanding of the molecular regulatory mechanisms involved in HCC (37). PPARγ is potentially involved in mediation of HCC-related mechanisms (38,39). However, the role of PPARγ expression in HCC remains controversial. Some studies have shown high expression of PPARγ protein in HCC tissues (40-42), while another report indicated that PPARγ protein expression is decreased in HCC (43). Presently, PPARγ is considered to exert an anti-tumor effect in HCC because *PPARγ* has been identified as a tumor suppressor gene (44). PPARγ plays a key role in the proliferation, migration, invasion and apoptosis of HCC cells. Cao *et al* (45) have demonstrated that PPARγ activation can inhibit the proliferation of liver hepatic cancer cells by downregulating septin 2 expression. Lee *et al* (46) reported
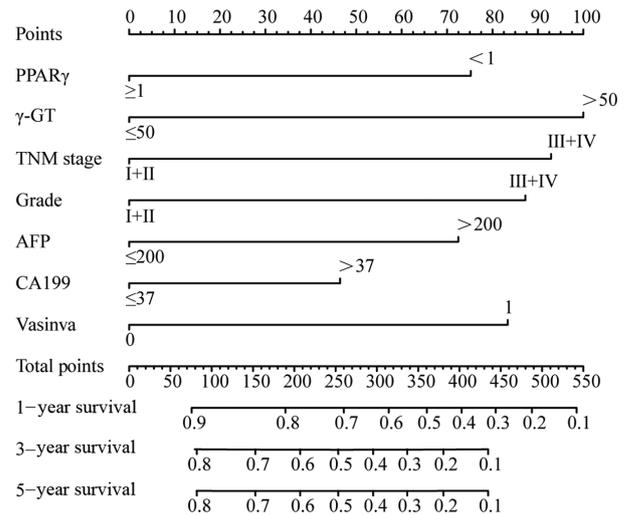
that PPARγ may be involved in modulating E-cadherin expression and motility of HepG2 cells. Another study demonstrated that PPARγ can directly inhibit the migration of HCC cells and is negatively correlated with macrovascular invasion observed in HCC (47). Han *et al* (48) showed that hispidulin inhibits the growth and metastasis of HCC via PPARγ activation, mediated by *AMPK* and *ERK* signaling. In summary, previous studies indicate that high expression of PPARγ in liver cancer exerts a robust tumor-suppressive effect.

The present study first employed bioinformatics analyses to establish that PPARγ expression in tumor tissues of patients with HCC was higher compared with that in adjacent normal liver tissues. PPARγ expression was examined in 125 clinical samples collected from patients with HCC by performing tissue microarray and IHC analyses and concluded that PPARγ was highly expressed in liver tumor tissues. These results showed that PPARγ can be used as a tumor biomarker in HCC.

The relationship between PPARγ expression levels and several key clinical characteristics in patients with HCC were also evaluated. The results indicated that PPARγ expression is associated with age, tumor diameter and vascular invasion. However, PPARγ expression was not significantly associated with factors such as hepatitis B, cirrhosis and metabolism-related indicators. These results concurred with the results of a previous study by Hsu *et al* (47).

Bioinformatics analyses using multiple databases was used to evaluate the relationship between PPARγ expression and prognosis of patients with HCC. The results showed that increased PPARγ protein or gene expression was indicative of a less favorable prognosis, which contradicted the results from previous studies (11,36,44). To explain this discrepancy, the relationship between expression levels of PPARγ and prognosis of 125 patients with HCC was evaluated. The results indicated that high expression of PPARγ was indicative of improved prognosis. The inconsistency between these results

Table III. Univariate analysis of survival in 125 patients with hepatocellular carcinoma.

Factors	Value, n	P-value	HR	P-value (HR)
PPARγ				
≤1	61	0.015	Reference	0.018
>1	64		0.459 (0.240-0.875)	
Grade				
I+II	99	<0.001	Reference	0.001
III	26		2.848 (1.519-5.341)	
TNM stage				
IA+IB+II	117	0.005	Reference	0.009
IIIA+IIIB+IVA+IVB	8		3.537 (1.372-9.116)	
Vascular invasion				
Negative	76	<0.001	Reference	0.001
Positive	49		2.748 (1.475-5.121)	
AFP, μg/l				
≤200	87	0.035	Reference	0.039
>200	38		1.928 (1.035-3.593)	
CA199, μg/ml				
≤37	65	0.024	Reference	0.027
>37	60		2.141 (1.09-4.025)	
γ-GT, U/l				
≤51	66	0.002	Reference	0.003
>51	59		2.707 (1.417-5.17)	

PPARγ, peroxisome proliferator activated receptor γ; AFP, α fetoprotein; γ-GT, γ-glutamyl transpeptidase; CA199, carbohydrate antigen 199; TNM, Tumor-Node-Metastasis; HR, hazard ratio.
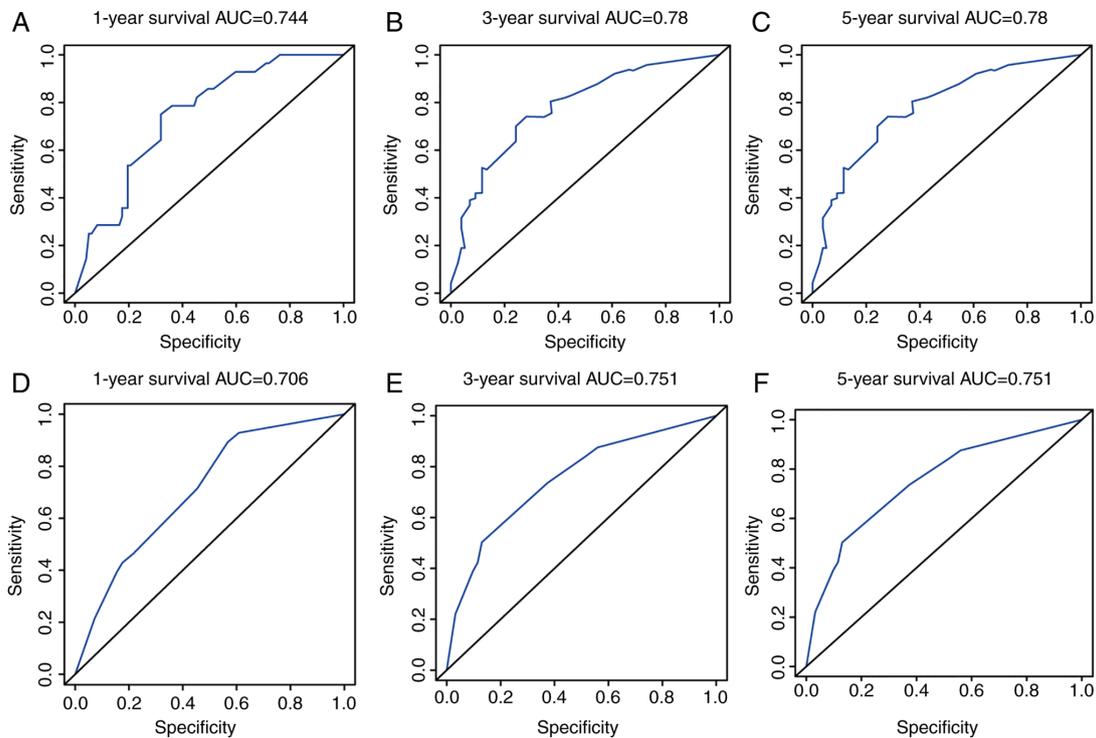

Figure 7. AUC values predict the 1-, 3- and 5-year OS of the nomogram that used seven clinical factors including PPARγ. ROC curve prediction for (A) 1-, (B) 3- and (C) 5-year OS. AUC values predicted the 1-, 3- and 5-year OS of the nomogram that used six clinical factors without PPARγ. ROC curve for prediction of (D) 1- (E) 3- and (F) 5-year OS. AUC, area under the curve; ROC, receiver operating characteristic; PPARγ, peroxisome proliferator-activated receptor γ.

and those obtained using bioinformatics analysis may be due to the following reasons. The data used for the bioinformatics analyses was collected on patients in Western countries, while the present patient cohort included patients residing in China, which may leads to bias in prognostic results. Second, China has a high incidence of hepatitis B, and most cases of liver cancer in China are caused by hepatitis B (49). However, in Western countries, most liver cancer cases are caused by over-consumption of alcohol (50). Therefore, the different pathogenesis of liver cancer may account for the differences in prognosis. Third, due to differences in eating habits, Westerners weigh more than East Asians (51). PPARγ expression may be affected by differences in body weights. These differences in eating habits and weight likely contribute to differences in prognosis. In addition, as the two sets of data to be analyzed have different sources, other factors (such as ethnicity, weight and treatment method) for the two sets of data cannot be controlled. At the same time, there is research showing that *PPARγ* mRNA and protein levels do not have the same expression level, which indicates that there may be post-transcriptional modifications in HCC (11).

Although the present study did not observe an association between PPARγ expression and related indices of lipid metabolism, it was reported that *PPARγ* was indeed enriched in the 'fatty acid metabolism' pathway. Changes in liver metabolism are critical to the development of liver disease. PPARγ is involved in mitochondrial oxidative phosphorylation, gluconeogenesis and fatty acid synthesis. Since modifications that affect mitochondria and lipid metabolism all contribute to the occurrence and/or development of liver steatosis, NAFLD, NASH and HCC, the function of PPARγ in lipid metabolism is closely related to the occurrence and development of liver cancer (25). This observation will be investigated further in our future study into the role of PPARγ in liver cancer pathogenesis.

To make the present study more comprehensive and accurate, data on a total of 43 clinical factors for patients with HCC were collected. First, Lasso regression was used to perform a dimensionality reduction analysis on these factors. Consequently, seven indicators were identified, including PPARγ expression, that were closely associated with the prognosis of patients with HCC. A univariate analysis was then performed on these indicators to verify the results obtained using Lasso analysis. The results of univariate analysis confirmed that these seven factors were indeed independent risk factors associated with the prognosis of patients with HCC. Finally, these prognosis-associated factors were used to establish an OS prognostic nomogram for rapidly and accurately predicting HCC prognosis. The C-index and AUC values of the nomogram established with PPARγ were higher compared with those of the nomogram established without PPARγ, which further demonstrated that the expression of PPARγ may play a role in predicting the prognosis of patients with HCC.

The present study used several innovative approaches. First, PPARγ protein and gene expression was assessed using bioinformatics analyses. Then Lasso analysis was used to identify clinical and comprehensive factors associated with patient prognosis. These data demonstrated that PPARγ was associated with prognosis in patients with HCC. As single

prognostic factors play limited roles in prognosis (52), a OS prognostic nomogram based on independent factors, such as PPARγ expression, was constructed to predict the OS rate in patients with HCC. This type of nomogram, which has been used previously as a prognostic prediction model, integrates all prognostic factors to achieve an individualized prediction (53). Additionally, this nomogram incorporates an enhanced visual interface and is straightforward to operate, which is advantageous in clinical practice (54). The limitations of the present study were as follows. First, semi-quantitative analysis was used to evaluate the results of PPARγ immuno-labeling assay, which may have led to statistical bias. Second, the insufficient number of samples collected could not rule out the possibility of sampling error. Additionally, single-center data were used to build the prognostic model and was not verified using external data. Lastly, the role of PPARγ in HCC pathogenesis needs to be explored in greater detail. PPARγ should be knocked down or overexpressed in cells to observe its effect on tumor progression and specific mechanisms in *in vivo* and *in vitro* experiments, which will be the subject of our future studies.

The present study reported that PPARγ was highly expressed in the tumor tissues of patients with HCC, and its expression level was associated with age, tumor diameter and vascular invasion. These results indicated that PPARγ expression can be used as a biomarker for predicting the prognosis of HCC. The OS prognostic nomogram, established using clinical factors and PPARγ expression levels, can be used to rapidly and accurately predict the prognosis of patients with HCC, leading to improved monitoring of the present patient population.

Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request. The additional datasets analyzed during the current study are available in The Cancer Genome Atlas database (https://genome-cancer.ucsc.edu/), Gene Expression Omnibus database (https://www.ncbi.nlm.nih.gov/geo/) and The Human Protein Atlas (https://www.proteinatlas.org).

Authors' contributions

XLZ designed the study. WP provided administrative support and made substantial contributions to the conception. XZ collected the data. ZD and TT performed data analysis. YC contributed to manuscript revisions and participated in data

collection and sorting. YW was involved in revising the manuscript critically for important intellectual content, and analysis and interpretation of data. XLZ, WP, YC and YW confirm the authenticity of all raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by The Ethics Committee of the Zhejiang Provincial People's Hospital (Hangzhou, China; approval no. 2020QT103).

Patient consent for publication

The study received an informed consent exemption approved by The Ethics Committee of the Zhejiang Provincial People's Hospital.

Competing interests

The authors declare that they have no competing interests.

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