The combination of serum lncRNA PTTG3P and mRNA PTTG1 serves as a diagnostic and prognostic marker for hepatocellular carcinoma

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Abstract. Long noncoding RNA (lncRNA) PTTG3P has been demonstrated to participate in the development of hepatocellular carcinoma (HCC) by targeting the mRNA PTTG1. The present study aimed to investigate the diagnostic efficacy of serum lncRNA PTTG3P, mRNA PTTG1 and their combination for the diagnosis and prognosis of HCC. A total of 373 participants were enrolled in the present study, including 73 patients with HCC, 100 patients with chronic hepatitis B (CHB), 100 patients with liver cirrhosis (LC) and 100 healthy controls (HCs). The expression levels of serum RNAs were quantified by reverse transcription-quantitative PCR. The association between serum lncRNA PTTG3P and clinical characteristics was further analyzed. Receiver operating characteristic (ROC) curve and area under curve (AUC) analyses were performed to estimate the diagnostic ability of serum lncRNA PTTG3P, PTTG1 and their combinations with other biomarkers for HCC. The results revealed that the expression levels of lncRNA PTTG3P and mRNA PTTG1 were markedly increased in the serum of patients with HCC and CHB compared with in the serum of HCs. Additionally, the postoperative levels of lncRNA PTTG3P and mRNA PTTG1 were significantly lower than the preoperative concentrations in 36 paired patients with HCC. Spearman's correlation coefficient analysis showed that serum lncRNA PTTG3P was correlated with aspartate transaminase (AST). ROC analysis showed that both lncRNA PTTG3P and mRNA PTTG1 had a significant predictive value for HCC. The AUC values of lncRNA PTTG3P and mRNA PTTG1 alone were 0.636 and 0.634, respectively. Furthermore, combining lncRNA PTTG3P, mRNA PTTG1, α-fetoprotein (AFP), alanine aminotransferase (ALT), AST, γ-glutamyl transpeptidase (GGT) and alkaline phosphatase (ALP) significantly increased the AUC value. The best performance was the combination of PTTG3P, PTTG1, AFP, ALT, AST, GGT and ALP with an AUC of 0.959, a sensitivity of 90.4% and a specificity of 98.0%. In conclusion, the combination of serum lncRNA PTTG3P, mRNA PTTG1 and AFP appeared to be a noninvasive biomarker with comparatively high specificity and sensitivity for the diagnosis of HCC.

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Introduction

Hepatocellular carcinoma (HCC) is a highly aggressive malignancy associated with high rates of mortality and morbidity. It is predicted that Asia accounts for 72% of the cases (with China alone contributing >50%), while Europe has accounts for 10%, Africa for 7.8%, North America for 5.1%, Latin America for 4.6% and Oceania for 0.5% (1). Due to the lack of symptoms, the majority of patients with HCC are diagnosed at advanced stages, resulting in a poor prognosis (2). At present, the methods used for diagnosing HCC in the clinic include ultrasound, computed tomography, detection of α-fetoprotein (AFP) levels and

pathological biopsy. Pathological biopsy has been recognized as the criterion for accurate diagnosis of HCC (3,4). Since pathological biopsy is an invasive examination that can cause bleeding and tissue damage, and imaging examinations lack specificity and sensitivity, serum AFP is currently the most frequently used screening biomarker for HCC in clinical practice (5). However, its diagnostic performance is relatively limited, particularly for patients with early stage HCC (6). Therefore, it is important to identify noninvasive biomarkers to improve the specificity and sensitivity of HCC diagnosis and prognosis.

Long noncoding RNAs (lncRNAs) are defined as noncoding RNA molecules >200 nucleotides in length, which were once regarded as transcriptional noise (7). Increasing evidence has demonstrated that lncRNAs serve crucial role in diverse biological processes, such as cell migration, metastasis and angiogenesis (8-10). Moreover, lncRNAs have been verified to participate in the occurrence and development of various tumors, including HCC (11). For example, the lncRNAs HOTTIP, PVT1 and HOTAIR have been confirmed to be closely associated with hepatocarcinogenesis (12-14). Additionally, it has been shown that lncRNAs are stably present in body fluids (15). Serum lncRNA LINC01535 has been suggested to be a novel biomarker of diagnosis, prognosis and disease progression in breast cancer (16). Additional lncRNAs have been verified as promising biomarkers for HCC diagnosis and prognosis, including MALAT1, UBE2CP3 and NETA-1; however, the diagnostic performance of previously reported lncRNAs for HCC varies considerably among different studies (17-19), and the majority of serum lncRNAs associated with HCC need further investigation.

The lncRNA PTTG3P is a processed pseudogene located at chromosome 8q13.1, which is involved in the development of different types of cancer, such as colorectal cancer, pancreatic cancer, osteosarcoma and non-small cell lung cancer (20-23). Moreover, our previous study confirmed that lncRNA PTTG3P acts as an oncogene in HCC; through elevating mRNA PTTG1 and activating PI3K/AKT signaling, lncRNA PTTG3P was shown to promote tumor growth and metastasis in HCC (24). Currently, there are few studies concerning the application of serum lncRNA PTTG3P and mRNA PTTG1 as diagnostic markers for HCC (25,26). Thus, to investigate the diagnostic efficacy of serum lncRNA PTTG3P, mRNA PTTG1 and their combinations for the diagnosis of HCC, the present study aimed to evaluate the serum expression of lncRNA PTTG3P and mRNA PTTG1 in patients with HCC, chronic hepatitis B (CHB) and liver cirrhosis (LC), and in healthy controls (HCs).

Materials and methods

Patients. In the present study, 373 participants were enrolled, including 73 patients with HCC, 100 patients with CHB, 100 patients with LC and 100 HCs. The participants were recruited from The Second Affiliated Hospital of Guangzhou University of Chinese Medicine (Guangzhou, China) between July 2022 and March 2023. The patients with HCC were diagnosed for the first time by histological examination and did not receive any treatment, whereas those with LC were diagnosed according to the American Association for the Study of Liver Diseases Practice Guidelines (27), and the patients with CHB were diagnosed according to the 2017 Clinical Practice Guidelines on the management of Hepatitis B virus infection of the European Association for the Study of the Liver (28). The 100 HCs were recruited during routine medical examinations at the same hospital during the aforementioned time period. Moreover, patient clinical data were collected from the hospital medical records for further study, including sex, age and HBV surface antigen (HBsAg). The present study was performed according to the principles of The Declaration of Helsinki. Each subject provided written informed consent and the research protocol was approved by the Ethics Committee of The Second Affiliated Hospital of Guangzhou University of Chinese Medicine (approval no. BE2020-211-01).

Sample collection. Peripheral blood samples were collected in separate vacuum tubes from patients prior to surgery, chemotherapy or pharmacological intervention. Paired preoperative and postoperative plasma samples were obtained from 36 patients with HCC, with postoperative samples collected 10 days after surgery. All serum samples were stored at -80˚C for further analysis.

RNA isolation and reverse transcription-quantitative PCR (RT-qPCR). Serum RNA was extracted from serum samples using a HiPure Liquid RNA Kit (cat. no. R416303; Magen Biotechnology Co., Ltd.) according to the manufacturer's instructions. DNase On Column Kit B (cat. no. R4911B; Magen Biotechnology Co., Ltd.) was used to remove DNA. The quantity and purity of RNA were verified using a NanoDrop 2000c Spectrophotometer (NanoDrop; Thermo Fisher Scientific, Inc.). Subsequently, cDNA was generated using Evo M-MVL RT Premix for qPCR (cat. no. AG11706; Hunan Accurate Bio-Medical Technology Co., Ltd.) according to manufacturer's protocol. RNA expression levels were assessed by qPCR using SYBR® Green Premix Pro Taq HS qPCR Kit (cat. no. AG11701 Hunan Accurate Bio-Medical Technology Co., Ltd.), which was performed on an LC480 Real Time PCR system (serial no. 28833; Roche Diagnostics GmbH). qPCR was performed under the following conditions: 95˚C for 30 sec, followed by 40 cycles at 95˚C for 5 sec and 60˚C for 30 sec. U6 and β-actin were used as internal controls. All results are presented as the mean ± standard deviation (SD) of ≥3 independent experiments. Comparative quantification was performed using the $2^{-\Delta\Delta Cq}$ method (29). The primer sequences used in the present study are shown in Table I.

Detection of liver function and serum AFP. Liver function-related indicators were detected on a Roche Cobas 8000 c702 chemistry analyzer (serial no. 16K9-07; Roche Diagnostics GmbH) according to the manufacturer's protocol, including g-glutamyl transpeptidase (GGT; cat. no. 05168775190), aspartate transaminase (AST; cat. no. 05850819190), alanine aminotransferase (ALT; cat. no. 05850797190), alkaline phosphatase (ALP; cat. no. 05166888190), total protein (TP; cat. no. 05171385190), albumin (ALB; cat. no. 05166861190), total bilirubin (TBIL; cat. no. 05795419190) and direct bilirubin (DBIL; cat. no. 05975921190) (all from Roche Diagnostics GmbH). Serum AFP (cat. no. 04481798190; Roche Diagnostics GmbH) was detected using the Roche Cobas 8000 e602 electrochemiluminescence immunoanalyzer (serial no. 16U3-11; Roche Diagnostics GmbH) according to the manufacturer's instructions.

Table I. Primers used for reverse transcription-quantitative PCR in the present study.

Gene symbol	Sequences
U6	Sense CTCGCTTCGGCAGCACA
	Antisense AACGCTTCACGAATTTGCGT
PTTG3P	Sense GGGGTCTGGACCTTCAATCAA
	Antisense GCTTTAGGTAAGGATGTGGGA
PTTG1	Sense ACCCGTGTGGTTGCTAAGG
	Antisense ACGTGGTGTTGAAACTTGAGAT
β-actin	Sense TGGCACCCAGCACAATGAA
	Antisense CTAAGTCATAGTCCGCCTAGAA GCA

Data analysis based on The Cancer Genome Atlas (TCGA)-liver hepatocellular carcinoma. RNA-sequencing expression (level 3) profiles and corresponding clinical information of 370 patients with HCC were downloaded from TCGA database (https://portal.gdc.cancer.gov/). Heatmap of PTTG1 was plotted using 'pheatmap' R package with zero-mean normalization (30). The time ROC (v 0.4) analysis (https://cran.r-project.org/web/packages/timeROC/index.html) (31) was used to compare the predictive accuracy of PTTG1 mRNA. For Kaplan-Meier curves, P-values and hazard ratio (HR) with 95% confidence interval (CI) were generated by log-rank tests and univariate Cox proportional hazards regression. All of the analysis methods and R packages were implemented by R (Foundation for Statistical Computing 2020) version 4.0.3 (32).

Statistical analysis. SPSS 17.0 software (SPSS, Inc.) was used to perform statistical analysis. The Kolmogorov-Smirnov test was used to evaluate the normality of the data distribution. Data are presented as the mean ± SD, and were analyzed by unpaired two-tailed Student's t-test if the variables were normally distributed. Comparisons between more than two groups were made by one-way ANOVA followed by the Dunnett T3 post hoc test. For nonparametric data, the Mann-Whitney U test was applied for comparisons between groups. For non-normally distributed variables, the Spearman's correlation coefficient test was performed. The χ^2 test or Fisher's exact test was used to evaluate the association between lncRNA PTTG3P expression levels and clinical characteristics. Wilcoxon signed-rank test was used to compare the preoperative and postoperative levels of lncRNA PTTG3P and mRNA PTTG1. Receiver operating characteristic (ROC) curve and area under the curve (AUC) analyses were used to assess the diagnostic performance of lncRNA PTTG3P, mRNA PTTG1, AFP, ALT, AST, GGT, ALP and their combinations. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Patient characteristics. A total of 373 participants were enrolled in the present study, including 73 patients with HCC, 100 patients with CHB, 100 patients with LC and 100 HCs. Detailed demographic information of all participants is presented in Table II. The mean age of patients with HCC, CHB and LC, and of HCs was 55 years (range, 29-83 years), 44 years (range, 21-72 years), 53 years (range, 23-80 years) and 42 years (23-84 years), respectively. There were 62 men and 11 women in the HCC group, 70 men and 30 women in the LC group, 64 men and 36 women in the CHB group, and 48 men and 52 women in the HC group. Additionally, the clinical features of the HCC, LC and CHB groups were compared with those of HC group. Compared with in the HC group, the HCC group exhibited significant differences in all parameters, whereas the LC group showed significant differences compared with the HC group in all parameters with the exception of AFP and TP. By contrast, only ALT, AST, ALP and ALB were significantly different between the CHB and HC groups.

Serum expression levels of lncRNA PTTG3P and mRNA PTTG1 in patients with HCC, LC and CHB, and in HCs. In the present study, blood samples obtained from patients with HCC, LC and CHB, and HCs were evaluated by RT-qPCR to screen for the presence of serum lncRNA PTTG3P and mRNA PTTG1. The results showed that lncRNA PTTG3P levels were markedly increased in patients with HCC and CHB compared with those in HCs (Fig. 1A). Since mRNA PTTG1 is a target of lncRNA PTTG3P (24), its expression was detected in the same cohort. The results indicated consistency with lncRNA PTTG3P; namely, mRNA PTTG1 was highly expressed in patients with HCC and CHB compared with in HCs (Fig. 1B).

Serum expression levels of lncRNA PTTG3P and mRNA PTTG1 in patients with HCC before and after surgery. To verify whether the combination of lncRNA PTTG3P and mRNA PTTG1 may be a potential biomarker for monitoring the prognosis of patients with HCC, the preoperative and postoperative expression levels of lncRNA PTTG3P and mRNA PTTG1 were compared in 36 patients with HCC. Among them, the group consisted of 34 men and 2 women, aged between 29 and 83 years (mean age, 53 years). The postoperative levels of lncRNA PTTG3P and mRNA PTTG1 were both significantly lower than the corresponding preoperative levels (Fig. 1C and D).

Survival analysis of PTTG1 in patients with HCC from TCGA dataset. In order to investigate the prognostic impact of PTTG1 on HCC, the present study used TCGA database. The median expression level of PTTG1 was used as the cutoff value; 185 patients were included in the low expression PTTG1 group and 185 patients were included in the high expression PTTG1 group. The results showed that in the high expression group a higher proportion of patients were dead than in the low expression group (Fig. S1A). In addition, the HR of the low-expression group relative to the high-expression group was 2.048 (95% CI: 1.435-2.923), and the median survival time of the high expression group was shorter than that of the low expression group (Fig. S1B). The heatmap results indicated that PTTG1 expression was elevated in the majority of patients with HCC (Fig. S1C). Time ROC analysis results showed that the 1-year AUC, 3-year AUC and 5-year AUC were 0.716 (95% CI: 0.657-0.775), 0.670 (95% CI: 0.605-0.735) and 0.634 (95% CI: 0.550-0.718), respectively (Fig. S1D), suggesting that PTTG1 had predictive ability regarding 1-, 3- and 5-year

Table II. Demographics of HCs, and patients with LC, CHB and HCC.

Feature	HCC	P-value[a]	LC	P-value[a]	CHB	P-value[a]	HCs
Number	73	/	100	/	100	/	100
Mean age, years (range)	55 (29-83)	/	53 (23-80)	/	44 (21-72)	/	42 (23-84)
Male/Female	62/11	/	70/30	/	64/36	/	48/52
Mean AFP, ng/ml (range)	8,129.42 (1.02-60,501.00)	<0.001[b]	4.16 (0.61-48.49)	0.810	3.61 (0.63-49.82)	0.794	2.84 (0.61-9.45)
Mean GGT, U/l (range)	129.89 (9.00-623.00)	<0.001[b]	33.64 (7.00-123.00)	<0.001[b]	25.46 (7.00-154.00)	0.779	28.77 (9.00-178.00)
Mean ALT, U/l (range)	49.26 (8.00-266.00)	<0.001[b]	26.93 (6.00-75.00)	<0.001[b]	29.87 (8.00-483.00)	0.046[b]	23.39 (5.00-182.00)
Mean AST, U/l (range)	64.83 (12.00-452.00)	<0.001[b]	27.03 (3.00-78.00)	<0.001[b]	26.16 (14.00-194.00)	0.014[b]	21.85 (10.00-72.00)
Mean ALP, U/l (range)	119.81 (50.00-606.00)	<0.001[b]	83.74 (39.00-260.00)	<0.001[b]	72.93 (31.00-128.00)	0.018[b]	68.61 (27.00-160.00)
Mean TP, g/l (range)	70.20 (31.30-95.00)	<0.001[b]	74.15 (56.80-86.10)	0.127	75.42 (42.00-87.70)	0.288	75.07 (63.20-85.60)
Mean ALB, g/l (range)	41.00 (26.60-191.00)	<0.001[b]	45.12 (24.70-52.20)	<0.001[b]	45.94 (28.00-52.90)	0.032[b]	46.92 (25.80-53.10)
Mean TBIL, μmol/l (range)	22.11 (4.10-588.00)	0.009[b]	14.69 (6.00-54.70)	<0.001[b]	10.89 (4.80-21.30)	0.934	11.15 (3.50-27.10)
Mean DBIL, μmol/l (range)	12.31 (1.50-403.90)	0.001[b]	6.41 (1.60-45.00)	<0.001[b]	4.18 (0.60-7.50)	0.886	4.31 (1.50-10.60)

[a]Compared with the HC group; [b]P<0.05. HCC, hepatocellular carcinoma; HCs, healthy controls; LC, liver cirrhosis; CHB, chronic hepatitis B; AFP, α-fetoprotein; GGT: γ-glutamyl transpeptidase; ALT, alanine aminotransferase; AST, aspartate transaminase; ALP, alkaline phosphatase; TP, total protein; ALB, albumin; TBIL, total bilirubin; DBIL, direct bilirubin.

survival. These findings indicated that higher expression of PTTG1 may be a risk factor for patients with HCC.

Association between serum lncRNA PTTG3P and clinical characteristics. In the current study, the associations between the serum expression levels of lncRNA PTTG3P and clinical parameters in patients with HCC were explored. The median expression level of lncRNA PTTG3P was used as the cutoff value; 36 patients were included in the low lncRNA PTTG3P expression group and 37 patients were included in the high lncRNA PTTG3P expression group. The results indicated that serum lncRNA PTTG3P expression was weakly associated with AST (Spearman's correlation test r=0.252, P=0.031; Table III; Fig. 2A and B). Furthermore, patients with HCC aged >55 years were confirmed to have an elevated level of lncRNA PTTG3P (Table III; Fig. 2C). However, there were no associations between serum lncRNA PTTG3P expression and sex, HBsAg, AFP level, cirrhosis, tumor size, tumor number, Edmondson grade (33), lymph node metastasis, portal vein invasion, ALT, GGT, ALB, ALP, TP, TBIL or DBIL (Table III). In addition, the clinical features of 36 patients with HCC before and after surgery were explored. As shown in Table IV and Fig. 2D, the AFP level post-surgery was significantly lower than that in pre-surgery, while other parameters showed no significant differences, including GGT, ALT, AST, ALP, TP, ALB, TBIL and DBIL.

Diagnostic efficiency of serum lncRNA PTTG3P and mRNA PTTG1 expression in patients with HCC. ROC curves were applied to evaluate the diagnostic efficacy of lncRNA PTTG3P and mRNA PTTG1 expression. When comparing patients with HCC with HCs, the AUC was 0.636 (95% CI: 0.547-0.724) for PTTG3P, 0.634 (95% CI: 0.547-0.722) for mRNA PTTG1, 0.891 (95% CI: 0.836-0.946) for AFP, 0.762 (95% CI: 0.692-0.833) for ALT, 0.879 (95% CI: 0.827-0.931) for AST, 0.886 (95% CI: 0.837-0.936) for GGT and 0.840 (95% CI: 0.782-0.899) for ALP (Table V; Fig. 3A-G). The diagnostic value of PTTG3P exhibited 37.0% sensitivity and 97.0% specificity, whereas that of mRNA PTTG1 showed 77% specificity and 50.7% sensitivity (Table V; Fig. 3A). These results suggested that PTTG3P and PTTG1 have certain diagnostic efficacy in assisting the diagnosis of HCC.

Moreover, whether lncRNA PTTG3P and mRNA PTTG1 expression could distinguish patients with HCC from others (CHB, LC and HCs) was investigated. The ROC curve analysis results indicated that AFP, PTTG3P, ALT, AST, GGT and ALP exhibited diagnostic efficacy for distinguishing patients with HCC from others (CHB, LC, and HCs) (Table V; Fig. 3H and J-N). However, mRNA PTTG1 [AUC: 0.573; 95% CI: 0.487-0.660] showed no diagnostic efficacy for discriminating patients with HCC from others (CHB, LC, and HCs) (P>0.05; Table V; Fig. 3I). The diagnostic efficacy of lncRNA PTTG3P displayed 97.3% specificity and 35.6% sensitivity (Table V; Fig. 3H).

AFP is the most commonly used biomarker for HCC screening and diagnosis in the clinic. Additionally, ALT, AST, GGT and ALP have been utilized as biomarkers for HCC. Therefore, the combinations of lncRNA PTTG3P and mRNA PTTG1 with the aforementioned five biomarkers were estimated for HCC diagnosis. Table V shows the diagnostic efficacy parameters for the

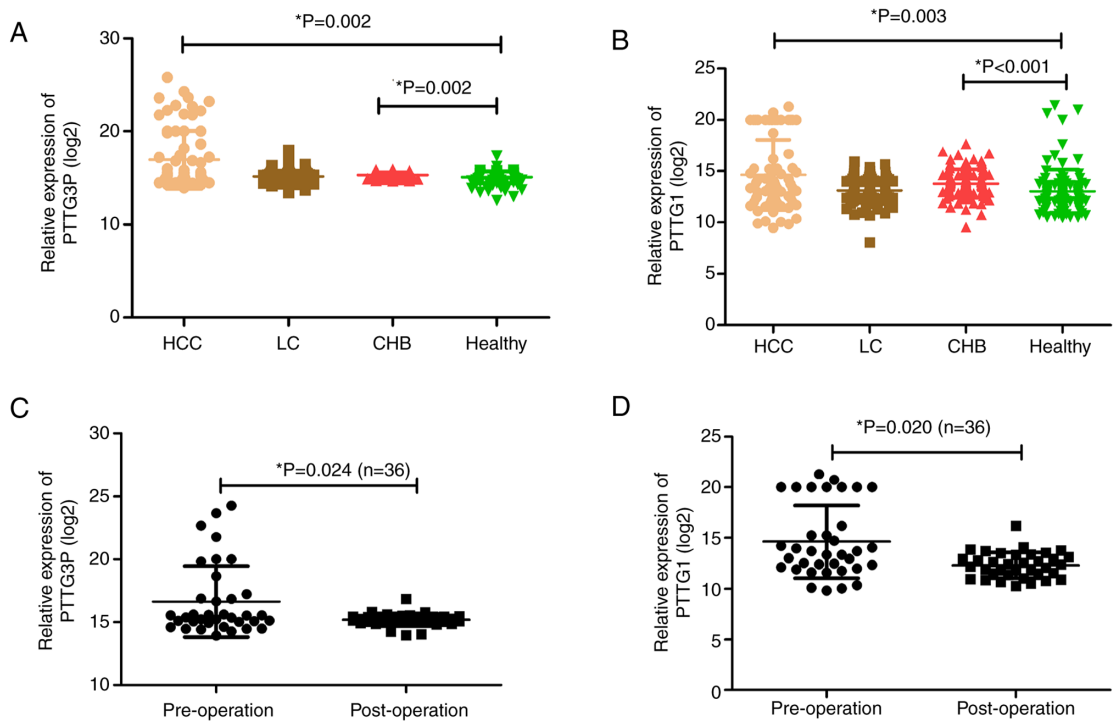

Figure 1. Expression of lncRNA PTTG3P and mRNA PTTG1 in patients with HCC, LC and CHB, and in HCs. The relative expression levels of (A) lncRNA PTTG3P and (B) mRNA PTTG1 in the serum of patients with HCC (n=73), LC (n=100) and CHB (n=100), and HCs (n=100) were evaluated by reverse transcription-quantitative PCR. Relative expression of (C) lncRNA PTTG3P and (D) mRNA PTTG1 in 36 pairs of patients with HCC before and after surgery. The expression levels of lncRNA PTTG3P were normalized to those of U6 and the expression levels of mRNA PTTG1 were normalized to those of β-actin. *P<0.05. lncRNA, long noncoding RNA; HCC, hepatocellular carcinoma; CHB, chronic hepatitis B; LC, liver cirrhosis; HC, healthy control.

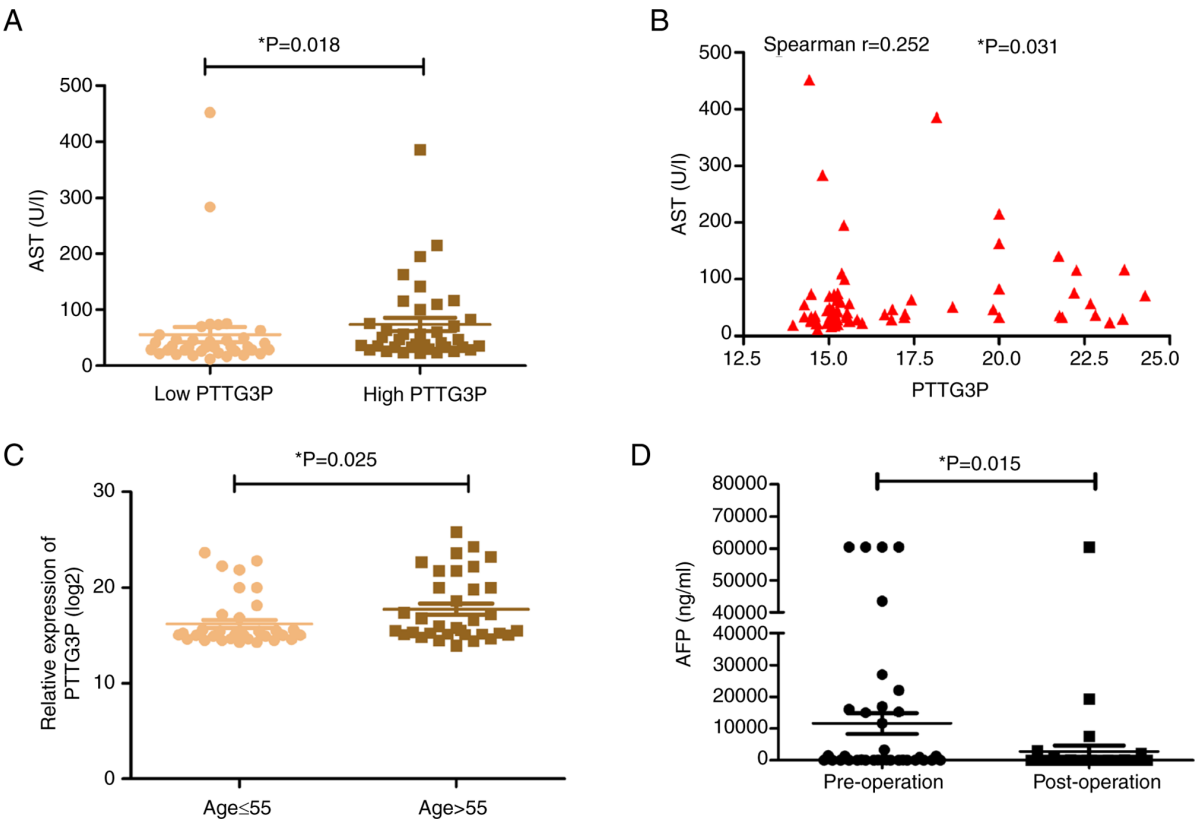

Figure 2. Association between serum lncRNA PTTG3P and clinical characteristics. (A) AST levels were significantly lower in patients with low levels of PTTG3P (n=36) than those in patients with high levels of PTTG3P (n=37). (B) Relative expression levels of serum lncRNA PTTG3P were correlated with AST in 73 patients with hepatocellular carcinoma. The correlation was estimated by Spearman correlation analysis. (C) Patients >55 years old had increased expression of PTTG3P. (D) AFP levels after surgery were significantly lower than those before surgery. *P<0.05. lncRNA, long noncoding RNA; AST, aspartate transaminase; AFP, α-fetoprotein.

Table III. Association between serum expression levels of PTTG3P and clinicopathological characteristics in 73 patients with HCC.

Feature	PTTG3P expression		P-value
	Low (n=36)	High (n=37)	
Sex			0.515[a]
Male	32	30	
Female	4	7	
Age			0.025[b]
≤55 years	24	15	
>55 years	12	22	
HBsAg			0.955
Positive	29	30	
Negative	7	7	
AFP level			0.279
≤20 ng/ml	13	18	
>20 ng/ml	23	19	
Cirrhosis			0.955
With	29	30	
Without	7	7	
Tumor size			0.741
≤5 cm	13	12	
>5 cm	23	25	
Tumor number			>0.999[a]
Single	5	6	
Multiple	31	31	
Edmondson grade			0.345
I-II	9	13	
III-IV	27	24	
Lymph node metastasis			0.281
With	22	27	
Without	14	10	
Portal vein invasion			0.384
With	24	21	
Without	12	16	
Mean GGT, U/l (range)	129.19 (9.00-623.00)	130.57 (15.00-340.00)	0.244
Mean ALT, U/l (range)	40.63 (8.00-179.00)	57.65 (12.00-266.00)	0.141
Mean AST, U/l (range)	55.75 (12.00-452.00)	73.68 (23.00-386.00)	0.018[b]
Mean ALP, U/l (range)	123.17 (50.00-606.00)	116.54 (56.00-227.00)	0.529
Mean TP, g/l (range)	72.24 (50.80-95.00)	68.21 (31.30-85.30)	0.055
Mean ALB, g/l (range)	43.98 (26.60-191.00)	38.10 (27.80-45.50)	0.153
Mean TBIL, μmol/l (range)	14.02 (4.10-40.90)	29.98 (4.90-588.00)	0.570
Mean DBIL, μmol/l (range)	6.44 (2.10-26.00)	18.02 (1.50-403.90)	0.069

[a]Fisher's exact test was performed; [b]P<0.05. AFP, α-fetoprotein; GGT, γ-glutamyl transpeptidase; ALT, alanine aminotransferase; AST, aspartate transaminase; ALP, alkaline phosphatase; TP, total protein; ALB, albumin; TBIL, total bilirubin; DBIL, direct bilirubin.

combinations of lncRNA PTTG3P, mRNA PTTG1, and AFP, ALT, AST, GGT and ALP to distinguish patients with HCC from HCs and from others (CHB, LC, and HCs). For the discrimination of patients with HCC from HCs, compared with AFP alone, the AUC values of the combinations of lncRNA PTTG3P and mRNA PTTG1 with AFP to predict HCC were elevated (Table V; Fig. 4A, B and D). Moreover, the combination of lncRNA PTTG3P, mRNA PTTG1, AFP, ALT, AST, GGT and ALP presented the highest accuracy, with an AUC of 0.959 (95% CI: 0.925-0.993), 90.4% sensitivity and 98.0% specificity (Table V; Fig. 4E). Furthermore,

Table IV. Demographics of patients with HCC pre-operation and post-operation (n=36).

Feature	Pre-operation	Post-operation	P-value
Mean AFP, ng/ml (range)	11,624.14 (1.33-60,501.00)	2,781.92 (1.16-60,501.00)	0.015[a]
Mean GGT, U/l (range)	130.42 (15.00-340.00)	286.94 (11.00-4,412.00)	0.706
Mean ALT, U/l (range)	52.47 (15.00-256.00)	35.85 (9.00-141.00)	0.086
Mean AST, U/l (range)	63.69 (17.00-452.00)	46.47 (18.00-129.00)	0.289
Mean ALP, U/l (range)	113.69 (63.00-210.00)	151.55 (55.00-881.00)	0.285
Mean TP, g/l (range)	70.22 (50.80-85.30)	68.70 (56.40-82.90)	0.292
Mean ALB, g/l (range)	38.93 (26.60-53.70)	36.51 (26.80-44.50)	0.065
Mean TBIL, μmol/l (range)	12.70 (4.10-25.70)	21.60 (4.60-185.70)	0.338
Mean DBIL, μmol/l (range)	5.70 (1.50-16.10)	12.42 (1.40-135.80)	0.235

[a]P<0.05. AFP, α-fetoprotein; GGT, γ-glutamyl transpeptidase; ALT, alanine aminotransferase; AST, aspartate transaminase; ALP, alkaline phosphatase; TP, total protein; ALB, albumin; TBIL, total bilirubin; DBIL, direct bilirubin.

Table V. Performance of AFP, ALT, AST, GGT, ALP, PTTG3P, PTTG1 and the combinations in HCs and patients with LC, CHB, and HCC.

	HCC vs. HCs				HCC vs. others[a]			
Method	AUC (95% CI)	SEN, %	SPE, %	P-value	AUC (95% CI)	SEN, %	SPE, %	P-value
AFP	0.891 (0.836-0.946)	0.822	0.860	<0.001[b]	0.881 (0.828-0.933)	0.849	0.790	<0.001[b]
ALT	0.762 (0.692-0.833)	0.699	0.720	<0.001[b]	0.721 (0.655-0.787)	0.699	0.663	<0.001[b]
AST	0.879 (0.827-0.931)	0.685	0.920	<0.001[b]	0.837 (0.783-0.891)	0.685	0.873	<0.001[b]
GGT	0.886 (0.837-0.936)	0.918	0.700	<0.001[b]	0.884 (0.838-0.930)	0.904	0.707	<0.001[b]
ALP	0.840 (0.782-0.899)	0.795	0.750	<0.001[b]	0.794 (0.734-0.855)	0.661	0.853	<0.001[b]
PTTG3P	0.636 (0.547-0.724)	0.370	0.970	0.003[b]	0.609 (0.522-0.695)	0.356	0.973	0.004[b]
PTTG1	0.634 (0.547-0.722)	0.507	0.770	0.030[b]	0.573 (0.487-0.660)	0.274	0.963	0.052
AFP + PTTG3P	0.908 (0.856-0.960)	0.795	0.970	<0.001[b]	0.861 (0.795-0.927)	0.767	0.973	<0.001[b]
AFP + PTTG1	0.907 (0.856-0.957)	0.822	0.900	<0.001[b]	0.853 (0.790-0.916)	0.726	0.933	<0.001[b]
PTTG1 + PTTG3P	0.748 (0.671-0.824)	0.452	0.990	<0.001[b]	0.693 (0.612-0.774)	0.452	0.983	<0.001[b]
AFP + PTTG3P + PTTG1	0.927 (0.881-0.973)	0.822	0.960	<0.001[b]	0.884 (0.825-0.943)	0.781	0.980	<0.001[b]
AFP + ALT + AST + GGT +ALP + PTTG3P + PTTG1	0.959 (0.925-0.993)	0.904	0.980	<0.001[b]	0.948 (0.911-0.985)	0.890	0.950	<0.001[b]

[a]Others include HCs, and patients with LC and CHB; [b]P<0.05. HCC, hepatocellular carcinoma; HCs, healthy controls; LC, liver cirrhosis; CHB, chronic hepatitis B; AFP, α-fetoprotein; GGT, γ-glutamyl transpeptidase; ALT, alanine aminotransferase; AST, aspartate transaminase; ALP, alkaline phosphatase; AUC, area under curve; CI, confidence interval; SEN, sensitivity; SPE, specificity.

combining lncRNA PTTG3P and mRNA PTTG1 showed a higher AUC than that of lncRNA PTT3P or mRNA PTTG1 alone (Table V; Fig. 4C and H). However, the AUC values of lncRNA PTTG3P and AFP, or mRNA PTTG1 and AFP were lower than that of AFP alone when used to distinguish patients with HCC from others (CHB, LC and HCs) (Table V; Fig. 4F and G).

Discussion

HCC is one of the most aggressive tumors worldwide and it is associated with a poor prognosis; therefore, the identification of novel potential serum biomarkers for detecting early-stage HCC remains a challenge. Research has revealed that lncRNAs are stably present in serum or plasma, and can be detected in patients with different types of cancer (15). Notably, several HCC-related lncRNAs have been detected in the serum of patients with HCC, including lncRNA HULC, HEIH, SCARNA10 and lnc34a (34-37). Taken together, these findings suggested that serum lncRNAs may be potential noninvasive biomarkers for HCC diagnosis and prognosis.

Our previous study indicated that the interaction between lncRNA PTTG3P and mRNA PTTG1 served a vital role in the occurrence and development of HCC (24). A schematic

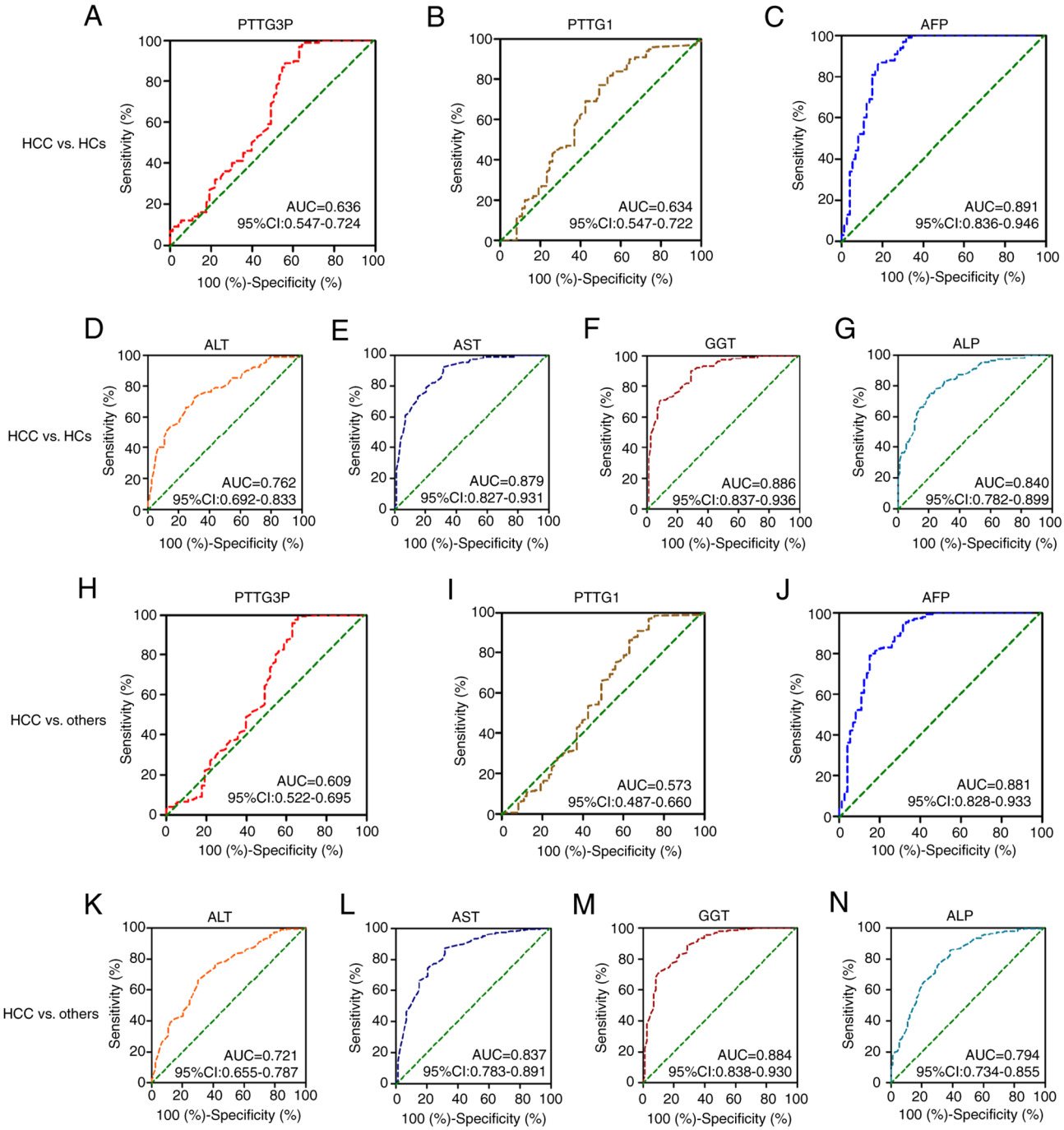

Figure 3. Diagnostic value of serum lncRNA PTTG3P and mRNA PTTG1 in patients with HCC. The diagnostic value of (A) PTTG3P, (B) PTTG1, (C) AFP, (D) ALT, (E) AST, (F) GGT and (G) ALP was estimated by receiver operating characteristic curve analysis when comparing patients with HCC with HCs. Diagnostic efficacy of (H) PTTG3P, (I) PTTG1, (J) AFP, (K) ALT, (L) AST, (M) GGT and (N) ALP to discriminate patients with HCC from those with liver cirrhosis and chronic hepatitis B, and HCs. lncRNA, long noncoding RNA; HCC, hepatocellular carcinoma; HC, healthy control; AFP, α-fetoprotein; GGT, γ-glutamyl transpeptidase; AST, aspartate transaminase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; AUC, area under the curve.

model of the functions of lncRNA PTTG3P during HCC tumor growth and metastasis is shown in Fig. S2. PTTG3P may enhance cellular proliferation and metastatic capabilities by increasing the expression of PTTG1 and stimulating the PI3K/AKT signaling pathway. Subsequently, this modulation could affect its downstream signaling cascades, encompassing various cell-cycle regulators and epithelial-mesenchymal transition-associated factors in HCC. In the present study, the diagnostic efficacy of serum lncRNA PTTG3P, mRNA PTTG1 and their combinations for the diagnosis of HCC was evaluated. The results indicated that the expression levels of lncRNA PTTG3P and mRNA PTTG1 were markedly elevated in patients with HCC and CHB compared with those in HCs. In addition, the preoperative and postoperative expression levels of lncRNA PTTG3P and mRNA PTTG1 were compared in 36 patients with HCC. The data showed that the postoperative expression levels of lncRNA PTTG3P and mRNA PTTG1 were significantly lower than the preoperative levels, indicating that they may be applied as promising prognostic markers for HCC. lncRNA PTTG3P is a processed

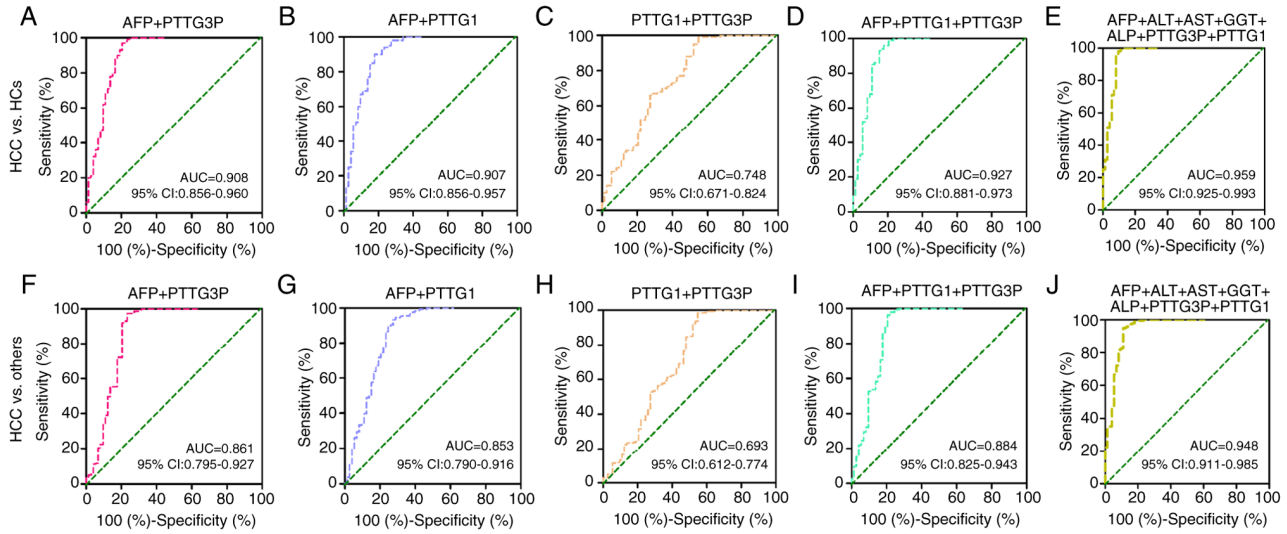

Figure 4. Diagnostic efficacy of the combinations of lncRNA PTTG3P and mRNA PTTG1 with other biomarkers in patients with HCC. The diagnostic value of the combinations of (A) PTTG3P and AFP, (B) PTTG1 and AFP, (C) PTTG1 and PTTG3P, (D) PTTG3P, PTTG1 and AFP, and (E) PTTG3P, PTTG1, AFP, ALT, AST, GGT and ALP were estimated by receiver operating characteristic curve analysis when comparing patients with HCC and HCs. Diagnostic efficacy of the combinations of (F) PTTG3P and AFP, (G) PTTG1 and AFP, (H) PTTG1 and PTTG3P, (I) PTTG3P, PTTG1 and AFP, and (J) PTTG3P, PTTG1, AFP, ALT, AST, GGT and ALP to discriminate patients with HCC from patients with liver cirrhosis and chronic hepatitis B, and HCs. lncRNA, long noncoding RNA; HCC, hepatocellular carcinoma; HC, healthy control; AFP, α-fetoprotein; GGT, γ-glutamyl transpeptidase; AST, aspartate transaminase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; AUC, area under the curve.

pseudogene, which has been confirmed to serve a key role in the development and progression of cancer. Liu *et al* (38) reported that the lncRNA PTTG3P levels were markedly elevated in colorectal cancer (CRC), and were closely related to the incidence of lymph node metastasis and distant metastasis in patients with CRC. Furthermore, lncRNA PTTG3P could downregulate the expression of microRNA-155-5P to promote the invasion and migration of CRC. Huang *et al* (23) demonstrated that PTTG3P was associated with non-small cell lung cancer (NSCLC) cell proliferation; these previous results suggested that PTTG3P could serve as a new therapeutic and prognostic target for NSCLC. In addition, previous studies have regarded mRNA PTTG1 as an oncogenic factor in various types of cancer, such as pancreatic adenocarcinoma and lung adenocarcinoma (39,40). A long follow-up of patients could provide valuable information on disease progression and recurrence; however, the present study recruited patients between July 2022 and March 2023. Furthermore, at the time of writing, 90% of patients recruited were alive, which is not suitable for survival analysis. We aim to follow up the patients recruited between July 2022 and March 2023 to provide valuable information on disease progression and recurrence in a further study.

To investigate the prognostic impact of PTTG3P and PTTG1 on HCC, the present study used TCGA database. Data from TCGA program showed that low expression of PTTG1 indicated improved prognosis compared with high expression of PTTG1; thus, high expression of PTTG1 appears to be a risk factor for HCC prognosis. The interaction between lncRNA PTTG3P and mRNA PTTG1 crucially participates in the development of cancer. The specific mechanisms underlying the interaction between lncRNA PTTG3P and mRNA PTTG1 in cancer have been reported in previous studies. Huang *et al* (41) suggested that PTTG3P can promote the resistance of prostate cancer cells to androgen-deprivation therapy via upregulating PTTG1. Guo *et al* (42) indicated that PTTG3P may promote the growth and metastasis of cervical cancer through PTTG1. Zhang and Shi (43) showed that PTTG3P is distinctly upregulated and may serve an oncogenic role in a PTTG1 and PTTG2-mediated manner in esophageal squamous cell carcinoma. Moreover, Huang *et al* (25) reported that the levels of serum PTTG3P were significantly higher in patients with HCC than in patients with benign liver diseases and HCs, which is in accordance with the present data. Overall, the aforementioned studies further confirm the present results that serum lncRNA PTTG3P and mRNA PTTG1 may have potential as novel biomarkers for HCC.

Subsequently, the associations between the serum levels of lncRNA PTTG3P and clinical parameters of patients with HCC were investigated in the present study. The results showed that serum lncRNA PTTG3P expression was slightly associated with AST. AST is an enzyme mainly used to evaluate hepatic outcomes. Previous research has revealed that high levels of AST may be indicative of liver damage (44); therefore, monitoring the levels of AST may be an appropriate way to evaluate liver function (45). Previous studies have reported that patients with HCC exhibiting high AST levels show poorer survival (46,47). Additionally, the current data indicated that patients with HCC aged >55 years exhibited elevated expression levels of lncRNA PTTG3P, whereas younger patients tended to have lower expression levels of lncRNA PTTG3P, thus suggesting that there may be a certain association between lncRNA PTTG3P levels and the age of onset of HCC. However, there were no associations detected between serum lncRNA PTTG3P expression and sex, HBsAg, AFP level, cirrhosis, tumor size, tumor number, Edmondson grade, lymph node metastasis, portal vein invasion, ALT, GGT, ALB, ALP, TP, TBIL or DBIL, which was consistent

with the results of Huang *et al* (25). The aforementioned negative results may be due to the limited sample size of the present study. Furthermore, the clinical features of 36 patients with HCC before and after surgery were explored in the present study, and a significantly decreased AFP level was observed in patients post-operation. AFP is the most frequently used marker for the screening and diagnosis of HCC in clinical practice (48,49). Tian *et al* (50) reported that the concentration of AFP in the HCC group was significantly higher than that in the other groups, while the levels of AFP after liver cancer surgery were significantly lower than those before surgery, which is in agreement with the present data. Accordingly, it may be concluded that increased serum expression levels of lncRNA PTTG3P could serve as an unfavorable prognostic factor for HCC.

To explore the diagnostic efficacy of lncRNA PTTG3P and mRNA PTTG1, ROC curve analysis was applied. The present data indicated that both serum lncRNA PTTG3P and mRNA PTTG1 exhibited good diagnostic accuracy in distinguishing patients with HCC from HCs. The diagnostic value of PTTG3P exhibited 37.0% sensitivity and 97.0% specificity, whereas that of mRNA PTTG1 showed 50.7% sensitivity and 97.0% specificity. Moreover, ROC curve analysis showed that AFP, ALT, AST, GGT, ALP and PTTG3P had predictive value for discriminating patients with HCC from others (CHB, LC, and HCs). However, mRNA PTTG1 showed no diagnostic efficacy for distinguishing patients with HCC from others (CHB, LC and HCs).

In recent decades, the early diagnosis of HCC has relied on surveillance with serological assessments of AFP; however, the specificity and sensitivity of AFP is not sufficiently satisfactory to detect early-onset HCC (51). Thus, the combinations of lncRNA PTTG3P and mRNA PTTG1 with AFP for HCC diagnosis were estimated in the present study. The data indicated that the AUC values of the combinations were greater than that of AFP alone for discriminating HCC from HC. Moreover, ALT, AST, GGT and ALP have also been recognized as prognostic biomarkers for HCC (52-54). In the present study, the diagnostic efficacy of the combination of the aforementioned biomarkers with lncRNA PTTG3P and mRNA PTTG1 was investigated. The combination of lncRNA PTTG3P and mRNA PTTG1 with AFP, ALT, AST, GGT and ALP exhibited the best diagnostic efficacy, yielding an AUC of 0.959 with 90.4% sensitivity and 98.0% specificity. The present results are consistent with the literature (25). Thus, the current study demonstrated that serum lncRNA PTTG3P and mRNA PTTG1 could be potential biomarkers for the prognosis and diagnosis of HCC.

Notably, there are certain limitations in the present study. Firstly, the present study was conducted with a limited sample size, since it is a single-center study. Secondly, no validation cohort was included in the study. Finally, the present study lacks follow-up data. Therefore, large-scale multicenter studies and further research are recommended to evaluate the potential of serum lncRNA PTTG3P and mRNA PTTG1 as novel biomarkers for the diagnosis and prognosis of HCC.

In conclusion, the current study demonstrated that the combinations of serum lncRNA PTTG3P, mRNA PTTG1, AFP, ALT, AST, GGT and ALP exhibited a good performance for the diagnosis of HCC. Moreover, the postoperative expression levels of lncRNA PTTG3P and mRNA PTTG1 were significantly lower than the preoperative levels in 36 paired patients with HCC. Thus, it was concluded that serum lncRNA PTTG3P and mRNA PTTG1 may be novel and noninvasive biomarkers for the diagnosis and prognosis of HCC.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

SWC conceived and proposed the idea. SWC and PFK designed the work. FZ, XYC and SKK contributed to acquisition, analysis and interpretation of data. XRZ, TTL, YHS and CMK contributed to collection of clinical samples and interpretation of data. SQ, HMW, YW and SZL contributed to the interpretation of data, and drafted and revised the manuscript. SWC and FZ confirm the authenticity of all the raw data. All authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

The present study was performed according to the principles of The Declaration of Helsinki. Each subject provided written informed consent and the research protocol was approved by the Ethics Committee of the Second Affiliated Hospital of Guangzhou University of Chinese Medicine (Guangzhou, China; approval no. BE2020-211-01).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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