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RESEARCH ARTICLE

Post-surgical resection prognostic value of combined OPN, MMP7, and PSG9 plasma biomarkers in hepatocellular carcinoma

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Abstract **Biomarkers for hepatocellular carcinoma (HCC) following curative resection are not currently sufficient for prognostic indication of overall survival (OS) and disease-free survival (DFS). The aim of this study was to investigate the prognostic performance of osteopontin (OPN), matrix metalloproteinase 7 (MMP7), and pregnancy specific glycoprotein 9 (PSG9) in patients with HCC. A total of 179 prospective patients with HCC provided plasma before hepatectomy. Plasma OPN, MMP7, and PSG9 levels were determined by enzyme-linked immunosorbent assay. Correlations between plasma levels, clinical parameters, and outcomes (OS and DFS) were overall analyzed. High OPN ($\geqslant$ 149.97 ng/mL), MMP7 ($\geqslant$ 2.28 ng/mL), and PSG9 ($\geqslant$ 45.59 ng/mL) were prognostic indicators of reduced OS ($P < 0.001$, $P < 0.001$, and $P = 0.007$, respectively). Plasma PSG9 protein level was an independent factor in predicting OS ($P = 0.008$) and DFS ($P = 0.038$). Plasma OPN + MMP7 + PSG9 elevation in combination was a prognostic factor for OS ($P < 0.001$). OPN was demonstrated to be a risk factor-associated OS in stage I patients with HCC and patients with low α-fetoprotein levels (< 20 ng/mL). These findings suggested that OPN, MMP7, PSG9 and their combined panels may be useful for aiding in tumor recurrence and mortality risk prediction of patients with HCC, particularly in the early stage of HCC carcinogenesis.**

Keywords biomarkers; OPN; MMP7; PSG9; HCC; prognosis

Introduction

Hepatocellular carcinoma (HCC) is the most common primary liver tumor, having an incidence virtually identical to mortality, resulting in up to 700 000 new diagnoses and 600 000 deaths per annum worldwide [1]. In many countries, HCC is the most common type of cancer, in part due to relatively high hepatitis B virus (HBV) infection rates and limited availability of recent antiviral agents. Although the mechanistic role of chronic HBV infection in HCC remains unclear, the relationship between HCC and chronic HBV infection is well documented [2]. Meanwhile, surgical resection can be an effective treatment for patients with HCC, and the efficacy of such treatment is limited in patients with late stage HCC diagnoses [3]. The reported 5-year disease-free survival (DFS) rate is only 25%–30%, and recurrence often leads to early mortality [4,5]. Current post-surgical prognostic techniques do not have sufficient ability to accurately predict patient outcomes. Thus, clinically useful biomarkers of survival and metastatic recurrence in HCC are urgently needed.

Recently, several studies have proposed α-fetoprotein (AFP) as a biomarker of post-surgical outcomes in HBV-associated HCC (HBV-HCC); the 5-year survival correlated with low (1.4–4.1 ng/mL) AFP levels, and thereafter, outcomes progressively declined with increasing AFP levels [6–8]. Clinical usefulness of plasma AFP detection is limited by the practical datable range (> 20 ng/mL), particularly in early-stage HCC, because even minute

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differences in AFP within the normal range can have an effect on prognosis and progression in HBV-HCC [8,9]. Numerous serum protein markers, such as lectin-bound AFP (AFP-L3), des-γ carboxyprothrombin (DCP), glypican-3, Dickkopf-1, and Golgi protein 73 (GP73), have been identified as alternative prognostic biomarkers for prediction of HCC diagnosis or prognosis [10–12]. Nonetheless, few prognostic markers have been widely adopted in regular clinical practice over the last decade although isolated examples exist on adoption of tumor serologic markers AFP-L3 and DCP used for clinical diagnostic purpose, particularly in Japan where such practice is endorsed by the Japan Society of Hepatology [13]. Meanwhile, AFP-L3% exceeding 5% can be used in the detection of small tumors and early stage HCC, and neither AFP-L3 nor DCP has been found to have greater prognostic value than AFP in early detection and recurrence prediction [10], necessitating development of several sensitive prognostic methods for clinical practice. HCC development has also been linked to OPN, a glycoprotein secreted into the extracellular matrix [14–17]. OPN has been shown to play an important role in tumor progression, especially in metastasis as an inducer of tumor-cell proliferation, migration, and extracellular matrix invasion [18]. Furthermore, downstream signaling pathways and effectors, including PI 3′-kinase/Akt, nuclear factor-κB, matrix metalloproteinases (MMPs), and urokinase-type plasminogen activator (uPA), are activated by OPN, which has been linked to the promotion of tumor metastasis in other cancer types [18]. Thus, OPN may be a promising candidate as a diagnostic and prognostic biomarker and may be an interesting therapeutic target for limiting metastasis.

Previously, the authors reported a novel approach for the detection of human blood proteins based on the secretome of cancerous tissues [19], a finding that may enable sensitive tumor-biomarker detection. Using existent data in an HCC secretome database of 1365 proteins, osteopontin (OPN), matrix metalloproteinase 7 (MMP7), and pregnancy-specific β-1-glycoprotein 9 (PSG9) were found to be the key participants in the transforming growth factor β pathway, a pivotal pathway that acts as a tumor suppressor in normal or dysplastic cells and a tumor promoter in advanced cancers [20]. Furthermore, plasma levels of OPN, MMP7, and PSG9 are readily assessed in patients with primary HCC, potentially providing a clinically applicable biomarker set.

Materials and methods

Patients and specimens

Patients with HCC who underwent hepatectomy were enrolled in this study. All subjects had definite post-operative pathological diagnosis and confirmed as HCC. The patients whose Child score was A underwent hepatectomy in this study. Plasma was collected prospectively from 179 patients with HCC (40 females, mean age 54.71 ± 11.24 years) prior to hepatectomy at the Cancer Hospital of Peking Union Medical College and Chinese Academy of Medical Sciences from March 2009 to June 2011. Peripheral blood samples were collected by venipuncture and preserved in EDTA-coated tubes. Samples were centrifuged at 4 °C for 10 min at 1000 *g* to separate plasma from blood cells. Supernatants were collected, divided into aliquots, and stored at − 80 °C until use [21]. All procedures related to human samples were approved by the Review Board of Chinese Academy of Medical Sciences Cancer Institute. All sample subjects provided informed consent, and the study was conducted under the approval of the Institutional Ethics Committee.

HCC diagnosis

HCC was diagnosed based on abdominal ultrasound, computed tomography (CT), magnetic resonance imaging (MRI), and/or biochemical profiles (AFP serology and liver function enzymes). Diagnoses were post-surgically confirmed by histopathology according to the criteria proposed by the American Association for the Study of Liver Disease guidelines [22]. HCC stage was defined according to the American Joint Committee on Cancer/Cancer Staging Manual (7th edition) by using tumor node metastasis (TNM) staging criteria, where stage I (single tumor, no vascular invasion) HCC was classified as early stage [23]. Diagnosis of chronic hepatitis B (CHB) and liver cirrhosis were conducted as previously described by Shen *et al*. [24].

Follow-up

Patients were followed until September 2015. Overall survival (OS) was defined as the interval between surgery and death. Only the patients died of tumor progression were enrolled. For patients suspected of recurrent cancers, recurrence or metastasis was verified by CT and/or MRI examinations. Recurrence or metastasis diagnoses were based on routine imaging and elevated AFP levels. Cause of death (recurrence, metastasis, or complicated liver cirrhosis) was recorded. DFS was defined as the interval between surgery and recurrence. If recurrence was not diagnosed, the date of death or last follow-up was recorded.

Plasma OPN, MMP7, and PSG9 assessment

OPN, MMP7, and PSG9 plasma protein concentrations were assessed using enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions.

ELISA kits for OPN and MMP7 were purchased from R&D Systems Inc. (Minneapolis, MN, USA). ELISA kits for PSG9 were purchased from BlueGene (Shanghai, China). In brief, 100 μL of diluted plasma for OPN (1:20) or MMP7 (1:2) was added to each well in the antibody precoated microtiter plate. For PSG9, 50 μL of diluted plasma (1:2) was added to each well. Then, conjugated antibodies were added to each well and mixed. The results were washed to remove excess plasma, and color development was achieved. Absorbance was measured immediately using a microplate reader (Bio-Rad Laboratory, Hercules, CA, USA). AFP levels were tested using a commercial electrochemiluminescent immunoassay kit (Roche Diagnostics, Mannheim, Germany) at the Clinical Diagnostic Laboratories at the Cancer Hospital of the Chinese Academy of Medical Sciences.

Statistical analysis

All statistical analyses were performed using SPSS software, version 17.0 (SPSS Inc., IL, USA) except for the time-dependent receiver operating characteristic (ROC) analysis, which was performed with R3.1.1. Continuous data for OPN, MMP7, and PSG9 plasma levels were presented as mean ± standard deviations, and relationships between plasma levels of the three proteins and clinical parameters were analyzed by Mann–Whitney test. DFS and OS rates by plasma levels of the selected proteins were assessed by log-rank test and the Kaplan–Meier curves. Variables determined by univariate analysis were entered in a multivariate analysis performed using a Cox multivariate analysis hazards model with forward stepwise selection. Optimal cutoff for OPN, MMP7, and PSG9 was determined with time-dependent ROC curve analysis, as previously described by Lambert *et al*. [25]. *P*-values less than 0.05 were considered statistically significant ($P < 0.05$).

Results

Plasma OPN, MMP7, and PSG9 levels and clinical parameters

Plasma OPN, MMP7, and PSG9 levels in patients with HCC were found to be 170.37 ± 145.44, 3.29 ± 10.40, and 30.69 ± 93.99 ng/mL, respectively. Plasma OPN and MMP7 levels were high in elderly patients, particularly in patients aged > 55 years ($P = 0.007$ and $P = 0.046$, respectively). Plasma OPN levels were significantly elevated in the patients with high TNM stages of II–IV ($P = 0.006$). OPN plasma levels were significantly low in patients with tumor size < 3 cm ($P < 0.001$), possibly indicative of a role in tumor growth. Furthermore, MMP7 plasma levels in patients with multiple tumors were significantly high ($P = 0.002$). The ELISA results and the patient characteristics are summarized in Table 1.

Prognostic value of plasma OPN, MMP7, and PSG9 levels

At the end of the follow-up period (5–76 months, mean 30 months), tumor recurrence was identified in 77 (43.02%) patients, and 29 (16.20%) patients died following tumor progression. Prognostic significance of plasma OPN, MMP7, PSG9, and AFP levels was determined by time-dependent ROC curve, where area under the ROC curve (AUC) corresponded to OS time (Fig. 1A). OPN AUC was highest, whereas PSG9 exhibited the lowest AUC. These findings indicated that AFP is a suboptimal prognostic biomarker for HCC. Cutoff values were calculated from AUC based on time-dependent ROC curves, with OPN, MMP7, PSG9 and AFP intervals at the 30th month (Fig. 1A). Based on the time-dependent ROC curve, cutoff values were determined for OPN (149.97 ng/mL), MMP7 (2.28 ng/mL), PSG9 (45.59 ng/mL), and AFP (95.97 ng/mL) (Fig. 1B–1E). AUC values for OPN, MM7, PSG9, and AFP were 0.704, 0.574, 0.542, and 0.571, respectively.

Comparison of Kaplan–Meier curves indicated low OS rates in patients with high OPN (⩾ 149.97 ng/mL), MMP7 (⩾ 2.28 ng/mL), and PSG9 (⩾ 45.59 ng/mL) (Fig. 2A–2C).

Two patient subgroups were readily identified in results, including (1) low, all three protein plasma levels were below cutoff ($n = 86$, 48.04%; OPN < 149.97 ng/mL, MMP7 < 2.28 ng/mL, PSG9 < 45.59 ng/mL) and (2) high, at least one of the three protein plasma level was above cutoff ($n = 93$, 51.96%). Low group ($n = 86$): median OS time was 30 months, and median DFS time was 26 months. High group ($n = 93$): median OS time was 21 months, and median DFS time was 17 months. Kaplan–Meier curves indicated low OS rates in the high group (Fig. 2D).

OPN ($P < 0.001$), MMP7 ($P = 0.009$), PSG9 ($P = 0.011$), and their combinations ($P < 0.001$) were potential predictive biomarkers of OS according to Cox univariate analyses. OPN ($P < 0.001$) and MMP7 ($P = 0.013$), but not PSG9 ($P = 0.059$), were potential biomarkers for DFS. Multivariate analyses of clinical parameters demonstrated that PSG9 was an independent prognostic factor for both OS ($P = 0.008$) and DFS ($P = 0.038$), and differentiation was an independent prognostic factor for DFS, $P = 0.019$ (Table 2).

OPN, MMP7, and PSG9 for prognostic prediction in early-stage HCC

An additional analysis was conducted on a subgroup of 86 early-stage (stage I) patients with HCC. Patients with high plasma OPN levels had significantly shorter OS compared

Table 1 Association of clinical parameters and plasma levels of OPN, MMP7, and PSG9 in HCC[a]

Clinical parameters		Plasma levels (mean±SD)		
		OPN	MMP7	PSG9
Age (year)	⩽55 (n = 91)	138.85±69.86	2.32±4.13	33.96±122.48
	>55 (n = 88)	202.95±190.05	4.28±14.20	27.30±50.33
	P value	0.007	0.046	0.969
Sex	Female (n = 39)	207.08±246.41	1.95±1.98	47.10±182.58
	Male (n = 140)	160.14±100.03	3.66±11.70	26.11±45.87
	P value	0.620	0.576	0.600
AFP (ng/mL)	<20 (n = 116)	167.55±127.00	2.38±3.51	27.47±50.96
	⩾20 (n = 63)	175.55±175.41	4.96±16.84	36.61±143.14
	P value	0.428	0.447	0.894
Hepatitis history	Yes (n = 165)	166.78±144.46	3.17±10.62	32.09±97.75
	No (n = 14)	212.60±155.86	4.62±7.55	14.11±8.93
	P value	0.302	0.597	0.480
Liver cirrhosis	Yes (n = 131)	162.48±114.50	3.84±12.05	26.54±46.97
	No (n = 48)	191.87±207.86	1.77±2.31	42.01±164.85
	P value	0.584	0.073	0.936
Tumor differentiation	Well/moderate (n = 140)	161.14±135.00	3.47±11.62	34.03±105.46
	Poor (n = 39)	203.48±176.01	2.64±3.48	18.67±22.63
	P value	0.086	0.146	0.962
Tumor size (cm)	⩽3 (n = 123)	120.18±57.59	2.69±4.05	24.82±52.583
	>3 (n = 56)	193.21±166.39	4.59±17.65	3.36±107.80
	P value	<0.001	0.241	0.251
TNM stage	I (n = 86)	137.15±69.55	2.10±3.27	34.38±127.76
	II + III + IV (n = 93)	201.08±185.67	4.38±14.03	27.27±44.57
	P value	0.006	0.285	0.382
Tumor number	Single (n = 156)	162.07±108.21	2.84±9.92	30.51±99.09
	Multiple (n = 23)	226.66±291.30	6.32±13.07	31.89±47.88
	P value	0.889	0.002	0.315
Vascular invasion	Yes (n = 66)	180.51±133.81	3.62±14.70	27.40±46.45
	No (n = 113)	164.43±152.09	3.09±6.82	32.60±113.04
	P value	0.094	0.095	0.747
Capsule invasion	Yes (n = 61)	150.55±100.41	2.06±2.52	29.48±59.54
	No (n = 118)	180.61±163.40	3.92±12.66	31.31±107.80
	P value	0.148	0.314	0.364
OS-Event (death)	Yes (n = 29)	200.80±107.73	2.41±2.70	63.23±209.37
	No (n = 150)	164.48±151.24	3.45±11.30	24.39±45.50
	P value	0.003	0.302	0.420
DFS-Event (recurrence)	Yes (n = 77)	190.23±144.22	2.70±3.99	38.02±133.17
	No (n = 102)	155.37±145.27	3.73±13.35	25.15±46.39
	P value	0.006	0.335	0.447

[a]Mann–Whitney tests were used to compare difference of subgroups in view of clinical parameters.

with patients with low OPN levels (Fig. 3A). Patients of the group "high" had a lower OS compared with patients of group "low." Notably, OPN was demonstrated to be a risk factor associated with OS in patients with AFP < 20 ng/mL (Fig. 3B). Furthermore, although plasma protein levels above the cutoff exhibited short OS time in patients with AFP < 20 ng/mL, the combination does not show better performance than OPN.

Discussion

The current study examined plasma levels of OPN, MMP7, and PSG9, revealing that some or all these indicators may be elevated in patients with poor OS. Furthermore, the combination of these three biomarkers could be used to predict early recurrence and survival, demonstrating to be effective in patients with early-stage (stage I) HCC. These

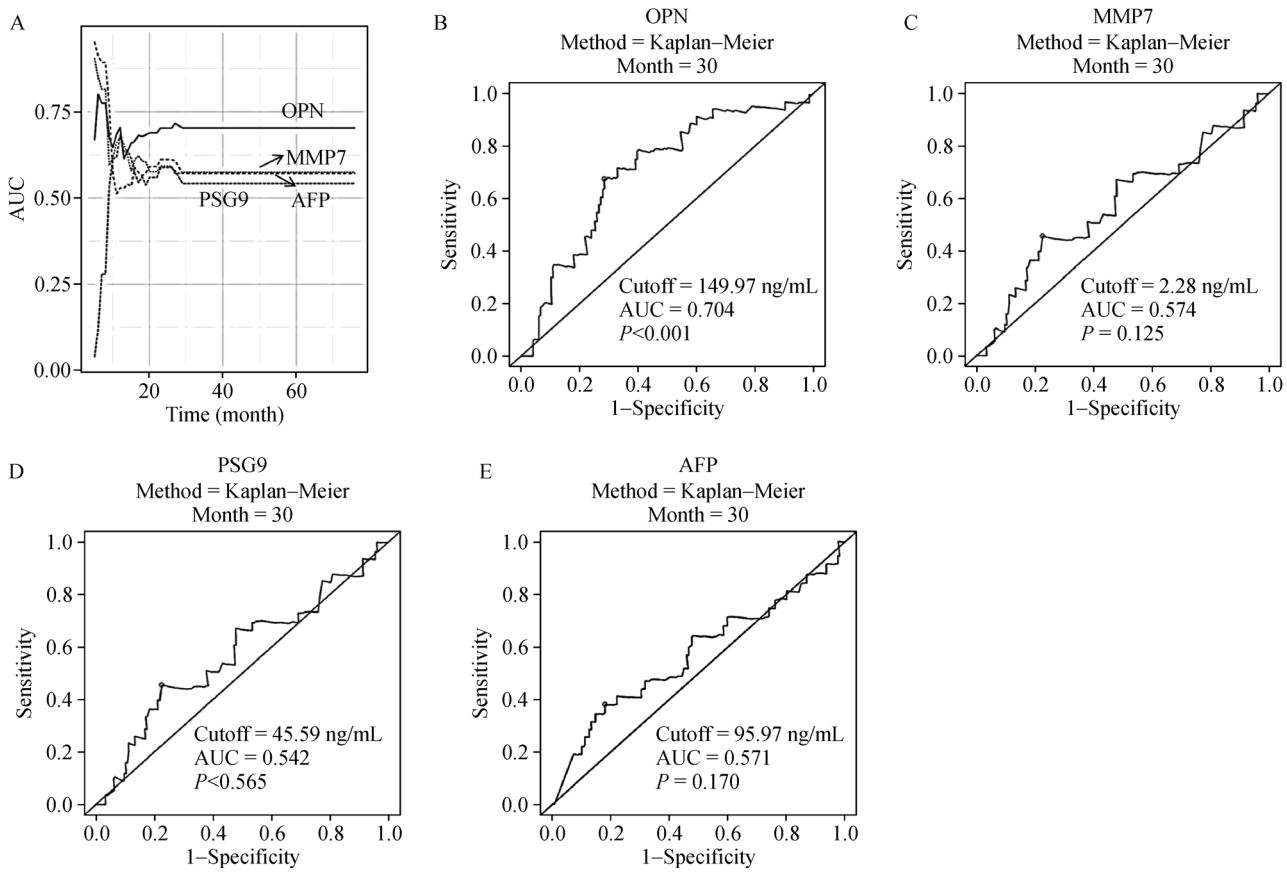

Fig. 1 Estimation of discriminatory performance of OPN, MMP7, PSG9, and AFP optimal cutoff values indicated by time-dependent receiver operating curve (ROC) corresponding to overall survival time. (A) Dynamic AUC plots for OPN, MMP7, PSG9, and AFP. Time-dependent ROC curves for determining optimal cutoff values of OPN (B), MMP7 (C), PSG9 (D), and AFP (E). AUC, area under the ROC curve.

findings suggested that combined plasma OPN, MMP7, and PSG9 levels may be useful targets in the development of prognostic panels that can aid in tumor recurrence and mortality-risk-prediction patients with HCC, particularly in the early stage of HCC carcinogenesis.

In clinical treatment of HCC, relatively high rates of metastasis and recurrence contribute to overall poor survival in these patients, and few treatments are currently available to limit metastasis-related recurrence [26]. One of the key limitations to improving clinical care is the inability to distinguish between patients that will exhibit recurrence after surgery, particularly in patients with early-stage disease without significant vascular invasion and regional or distant recurrence. As Cheng *et al*. [5] suggested, identifying risk factors for morality is important for effective surveillance and selection of adjunctive therapies in the postoperative period in patients with liver cancer. In the current study, 16.20% of patients died because of tumor progression during the follow-up period. In studies that have examined HCC over long time periods of up to 5 years, the OS rate progressively declines with time as tumor recurrence increases [27]. Meanwhile, similar initial OS rates were observed, and long follow-up would be necessary to demonstrate the sustained prognostic ability of these serum markers throughout the months and years of clinical surveillance following resection. Notably, the current study identified PSG9 as independent prognostic biomarkers for OS and DFS in HCC, a combination not previously described. Furthermore, these findings indicated that combined detection of these three proteins can improve prognostic detection of DFS and OS, and that detection of high OPN, MMP7, and/or PSG9 levels could be useful in detecting poor OS and recurrence.

Within the limited follow-up window of the present study, 43.02% of patients with HCC exhibited recurrence after curative resection surgery, with a large portion exhibiting elevated OPN levels. Previously, Sieghart *et al*. [27] reported that OPN expression could serve as a predictor of HCC recurrence following liver transplantation in patients with HCC. The results of this study confirmed that elevated plasma levels of OPN are associated with great recurrence and poor OS, particularly in patients with early stage cancer and AFP levels below 20

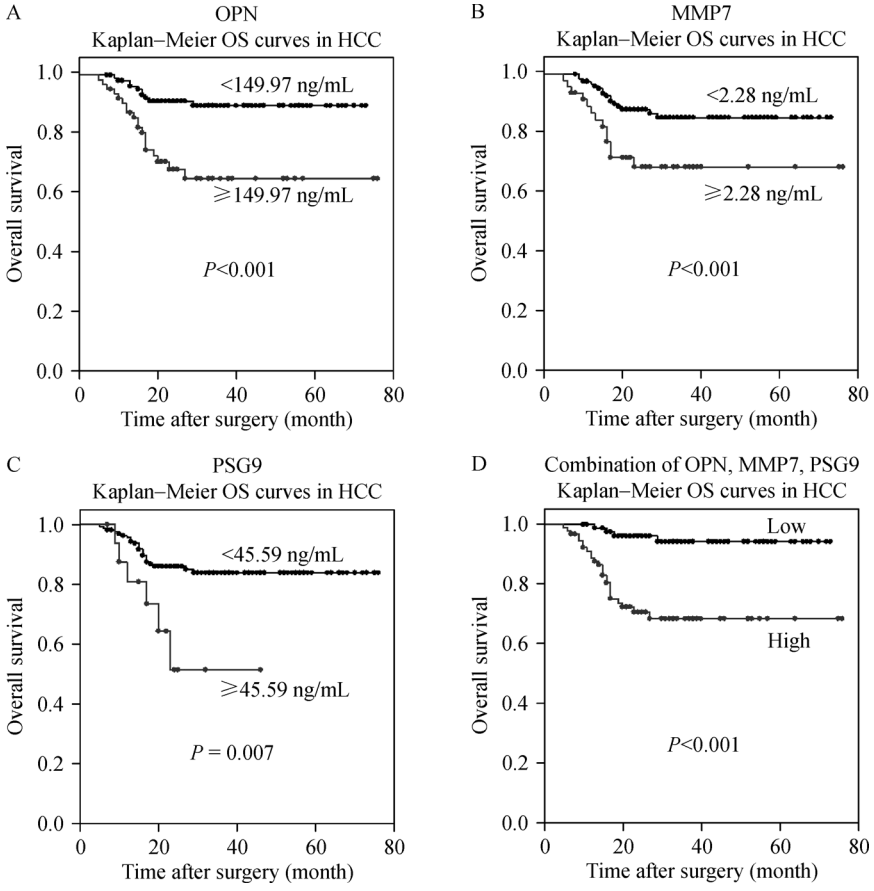

Fig. 2 Kaplan–Meier analysis indicated low overall survival (OS) rates with high plasma OPN, MMP7, and PSG9 levels in patients with hepatocellular carcinoma (HCC). Cumulative OS curves for OPN (A), MMP7 (B), and PSG9 (C). (D) Kaplan–Meier analysis of OS for combined plasma levels of OPN, MMP7, or PSG9. Low, all three protein plasma levels were below cutoff (OPN < 149.97 ng/mL, MMP7 < 2.28 ng/mL, PSG9 < 45.59 ng/mL, $n = 86$). High, at least one of the three protein plasma levels was above cutoff ($n = 93$).

ng/mL. In addition, these findings were well aligned with the results in other tumor types that indicate OPN may be linked recurrence and reduced OS in gastric, breast, lung, colon, and ovarian cancers [28]. The current study results provided novel evidence that plasma OPN levels may be indicators for tumor size, proliferation, and progression (Table 1), which are well aligned with prior mechanistic descriptions on the role of OPN in metastatic processes of tumor cell proliferation and migration [27–29]. Despite its potential as a prognostic marker, genetic heterogeneity provides reasonability to expect that abnormal OPN levels in the plasma will not always be apparent in early stage HCC for all patients.

Researchers have begun to propose combination methods for determining prognostic in patients with HCC that may be able to increase efficacy of these methods because no single serum biomarker elevation is detectable in all HCC cases [30]. In this study, serum OPN, MMP7, and PSG9 combinations were selected based on information from a previous study, providing evidence that these markers are routinely elevated in patients with HCC [20]. As demonstrated in the present study, previous studies of liver disease linked the MMP7 to capsular infiltration, portal vein invasion, and disease progression and recurrence although MMP7 levels may not always immediately be detectable in the peripheral blood supply at early disease stages [31,32]. MMP7 has also been identified as a regulator of liver matric turnover [33] and of metastasis-related genes [34], but it has rarely been reported in the context of HCC progression. According to the authors' previous work, MMP7 may also be a potential diagnostic marker for hepatic cirrhosis patients with HBV, consistent with the current study results [20].

Similarly, the carcinoembryonic antigen, PSG9, is a highly glycosylated protein known to be deregulated during colorectal carcinogenesis and active in regulating

Table 2 Univariate and multivariate analyses of factors associated with survival and recurrence

Variables	OS			DFS		
	Univariate	Multivariate		Univariate	Multivariate	
	P	HR (95% CI)	*P*	*P*	HR (95% CI)	*P*
Age, year (⩽55/>55)	0.330		NA	0.228		NA
Sex (female/male)	0.499		NA	0.323		NA
HBeAg (negative/positive)	0.628		NA	0.800		NA
Liver cirrhosis (yes/no)	0.655		NA	0.081		NA
Differentiation (well-moderate/ poor)	0.035	1.176 (0.787 – 3.739)	0.174	0.007	1.808 (1.103 – 2.964)	0.019
Tumor size (⩽3/>3, cm)	0.025	0.519 (0.169 – 1.597)	0.253	0.012	0.683 (0.390 – 1.199)	0.184
TNM stage (I/II + III + IV)	0.130		NA	0.295		NA
Tumor number (single/multiple)	0.117		NA	0.064		NA
Vascular invasion (yes/no)	0.759		NA	0.971		NA
Capsule invasion (yes/no)	0.112		NA	0.077		NA
OPN, ng/mL (low/high)	<0.001	2.331 (0.991 – 5.486)	0.053	<0.001	1.658 (0.995 – 2.763)	0.052
MMP7, ng/mL (low/high)	0.009	1.975 (0.893 – 4.370)	0.093	0.013	1.469 (0.884 – 2.441)	0.138
PSG9, ng/mL (low/high)	0.011	3.563 (1.384 – 9.172)	0.008	0.059	2.140 (1.042 – 4.398)	0.038
Combined[a]	<0.001		NA	<0.001		NA

HR, hazard ratio; NA, not adopted; CI, confidence interval; OS, overall survival; DFS, disease-free survival; HBeAg, hepatitis B e antigen.
[a]Group 1: all of three protein plasma levels were lower than cutoff values (OPN, 149.97 ng/mL, MMP7, 2.28 ng/mL, and PSG9, 45.59 ng/mL). Group 2: at least one of three protein plasma levels was higher than cutoff values.

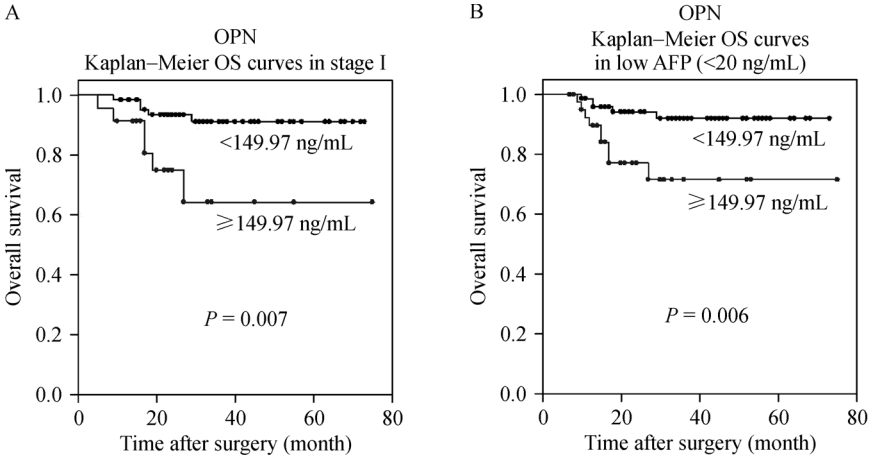

Fig. 3 Prognostic value of OPN in early-stage patients with HCC (stage I) and patients with low AFP levels (< 20 ng/mL). (A) Stage I HCC with high plasma OPN levels had significantly shorter OS compared with patients with low OPN levels. (B) OPN was demonstrated to be a risk factor associated with OS in patients with AFP < 20 ng/mL.

immunoreaction and angiogenesis, consistent with the current study findings [35,36]. PSG9 protein level was found to be an independent prognostic factor for OS and DFS in patients with HCC, a result not previously described and possibly mechanistically related to tumor cell invasion and migration. Notably, serum PSG9 elevation ($\geqslant$ 45.59 ng/mL) was observed in only 17 patients with HCC, and further research in large HCC patient cohorts would be necessary to verify trends in PSG9 and elucidate its full mechanism of action.

Our result showed that combined panels may be useful for aiding in tumor recurrence and mortality risk prediction of patients with HCC, particularly in the early stage of HCC carcinogenesis, because 60%–70% of patients with HCC still displayed low AFP levels during early stage disease, which can confound prognosis [37,38]. Additionally, some studies suggested that two biologically distinct recurrence mechanisms exist in HCC, responsible for early and late recurrence [39,40]. Discrimination between such recurrence mechanisms may not be apparent because this study only examined a relatively short follow-up period. Thus, further analysis is needed to validate these biomarkers in large-scale cohorts inclusive of robust follow-up periods. Moreover, the data of this study remain preliminary and will need to be re-evaluated by further studies.

In conclusion, high plasma levels of OPN, MMP7, and PSG9 in the peripheral blood were associated with high incidence of early recurrence and poor OS after curative resection for HCC. The combination of these three plasma protein biomarkers has superior prognostic predictive ability. Notably, this study is a valuable preliminary step toward achieving a conclusive evidence for combination serum biomarker prognostic use in HCC. Results may lead to the development of clinically viable techniques for detecting serum OPN, MMP7, and PSG9 levels as prognostic biomarkers of HCC.

Acknowledgements

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Compliance with ethics guidelines

Weiqi Rong, Yang Zhang, Lei Yang, Lin Feng, Baojun Wei, Fan Wu, Liming Wang, Yanning Gao, Shujun Cheng, Jianxiong Wu, and Ting Xiao declare no conflicts of interests. All procedures related to human samples were approved by the Review Board of Chinese Academy of Medical Sciences Cancer Institute. All sample donors provided informed consent, and the study was conducted under the approval of the Institutional Ethics Committee.

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