Pleomorphic adenoma gene 1 expression is associated with the diagnosis of hepatocellular carcinoma

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Abstract

Background: The pleomorphic adenoma gene 1 (*PLAG1*)) has been reported to be overexpressed in pleomorphic adenoma (PA). However, its expression and clinical significance in hepatocellular carcinoma (HCC) has not been investigated.

Methods: *PLAG1* protein levels in HCC serum and benign liver diseases (BLD) controls were measured by Western Blot, and α-fetoprotein (AFP) concentration was analyzed by enzyme-linked immunosorbent assay (ELISA). The relevance of *PLAG1*expression with the clinicopathological factors was assessed by Chi-square test. Furthermore, the receiver operating characteristic (ROC) curve was performed to investigate the values of the markers in diagnosis of HCC.

Results: Serum *PLAG1* protein level was significantly elevated in HCC group compared to that in controls (P<0.001). Furthermore, a significant association was found between *PLAG1* expression and clinical factors, such as tumor size (P=0.000), differentiation (P=0.014) and metastasis (P=0.001). ROC analysis showed that *PLAG1* could distinguish HCC patients from BLD controls with the area under the ROC curve (AUC) of 0.852 (95 % CI: 0.782-0.922; 78.8% sensitivity, 83.3% specificity; P<0.001), which had significantly superior discriminative ability than AFP (AUC=0.694, 67.3% sensitivity and 62.1 % specificity) or the combination of *PLAG1* and AFP (AUC=0.706, 69.2% sensitivity and 63.6 % specificity).

Conclusions: This study suggested that serum *PLAG1* might be a potential noninvasive tumor biomarker in the diagnosis of HCC.

Background

Hepatocellular carcinoma (HCC) is one of the most common and aggressive tumors, and the third leading cause of cancer-related death worldwide [1]. Cirrhosis, caused by alcohol abuse, nonalcoholic fatty liver disease and chronic infection with HBV or HCV, are regarded as the major etiological factors for HCC [2]. Despite the efforts in treatment of HCC including surgical resection, chemotherapy and liver transplantation have improved the condition of patients, the mortality still remain dismal because of relapse or intra-hepatic metastases after surgery [3–5]. Currently, the diagnosis of HCC mainly depends on imaging techniques, which are unsatisfactory, particularly for detection of HCC at early stages [6–8]. To date, plasma tumor marker detection has been the ideal method for screening patients at an early stage, and as we know, α-fetoprotein (AFP) level has been widely conducted for detection and monitoring of HCC. However, AFP is limited by the low sensitivity and specificity that nearly 40% of patients with HCC remain normal AFP levels [9, 10]. Therefore, there is still an urgent need to identify accurate diagnostic marker for HCC.

Pleomorphic adenoma gene 1 (*PLAG1*), located at 8q12, is a proto-oncogene and encodes a genuine transcription. Frequently, *PLAG1* is activated in pleomorphic adenoma (PA) of the salivary glands and rearranged by chromosomal aberrations which leading to gene fusion with partners of LLIR, CHCHD7, LIFR and CTNNB1 [11–15]. Furthermore, several previous studies have shown that tumor-specific genetic

alterations could be specifically identified through the proteins translated from the fusion gene transcripts [16–19]. Actually, *PLAG1* protein serves as an oncoprotein, which has been reported to function as a DNA-binding transcription factor. *PLAG1* is involved in multiple biological effects in regulating cell proliferation, induction of cell apoptosis and cell cycle arrest. However, the function of *PLAG1* in HCC is still unclear.

In the present study, we aim to determine the serum level of *PLAG1* in HCC and benign liver diseases (BLD) controls and to evaluate the relationship between its expression and the clinicopathologic factors of HCC. Finally, the diagnostic value of serum *PLAG1* in HCC was investigated.

Methods

Patients and serum samples

A total of 118 HCC patients who were pathologically confirmed after undergoing radical hepatectomy were enrolled in this study between The Third Hospital of Hebei Medical University. 56 patients with benign liver diseases (BLD) are defined as controls in this study. Blood samples were collected from all the subjects, and serum was separated from blood samples centrifuged at 3000 g for 15 min at 4℃, and then stored at -80℃ for further analysis. Demographic information and clinical features of all patients were summarized in Table 1. All procedures in this study were approved by the Ethical Committee of clinical research at The Third Hospital of Hebei Medical University, and written informed consent was obtained from each participant.

Table 1
The relationship between *PLAG1* level and the clinicopathological characteristics of HCC patients

Characteristic	NO.	*PLAG1* expression		*P*
	n = 118	Low	High	
Age				0.453
≥ 56	82	44	38	
< 56	36	22	14	
Gender				0.782
Male	72	41	31	
Female	46	25	21	
Tumor size (cm)				0.000
≤ 5	49	41	8	
> 5	69	25	44	
Liver cirrhosis				0.488
Presence	80	43	37	
Absence	38	23	15	
Differentiation				0.014
Well	44	31	13	
Moderate/Poor	74	35	39	
Hepatitis B/C				0.640
Yes	79	43	36	
No	39	23	16	
Metastasis				0.001
Yes	64	27	37	
No	54	39	15	
Drinking history				0.247
Yes	61	31	30	
No	57	35	22	

Western Blot analysis

Total proteins were extracted from serum samples by RIPA Lysis Buffer according to the manufacturer's instructions. Next, SDS-PAGE was performed to determine the level of *PLAG1* protein in serum. Rabbit anti-human monoclonal antibodies against *PLAG1*/ZAC (diluted 1:1,000) and specific HPR-conjugated goat anti-rabbit IgG secondary antibodies (1:40,000) were used. The relative protein expression was normalized to β-actin (ACTB).

Determination of AFP concentration

Serum AFP expression was measured by enzyme-linked immunosorbent assay (ELISA). According to the manufacturer's instructions, the normal range of AFP recommended is 0 ~ 2.85 ng/ml.

Statistical analyses

Statistical analyses were performed using SPSS Statistics version 18.0 (IBM, USA) and GraphPad Prism 5 (GraphPad software, USA). The correlation between two quantitative variables was analyzed using t-test, and the differences between *PLAG1* expression and clinicopathological factors of HCC patients were assessed by Chi-square test. Receiver operating characteristic (ROC) curves was used to estimate the diagnostic value of the markers. All experiments were in triplicate, and a $P < 0.05$ was considered to be statistical significant.

Results

Up-regulated expression of *PLAG1* in HCC serum

Western Blot assay was employed to measure serum *PLAG1* protein expression in HCC patients and controls. As shown in Fig. 1, we found that *PLAG1* levels was significantly elevated in patients with HCC compared to the BLD controls ($P < 0.001$). The result implicated that *PLAG1* might be an oncogene in the pathogenesis of HCC.

Relationship between *PLAG1* expression level and clinical characteristics of HCC patients

In order to examine whether *PLAG1* expression was associated with the clinical parameters, the patient were divided in to two groups (high and low) according to the mean value of *PLAG1* expression (Table 1). The outcome revealed that high *PLAG1* level was more frequently found in patients with larger tumor size ($P = 0.000$), moderate or poor differentiation ($P = 0.014$) and metastasis ($P = 0.001$). However, there was no significant correlation between *PLAG1* expression and other parameters, such as age, gender, liver cirrhosis, Hepatitis B/C infection and drinking history (all, $P > 0.05$). This suggests that serum *PLAG1* might play an important role in the development of HCC.

The value of serum *PLAG1* in HCC diagnosis

Next, we performed ROC analysis to determine the diagnostic significance of *PLAG1* for HCC. As shown in Fig. 2 and Table 2, the area under the curve (AUC) for *PLAG1* was 0.852 (95% CI: 0.782–0.922; $P <$

0.001) with 78.8% diagnostic sensitivity and 83.3% specificity, which was much higher than that for AFP 0.694 (95% CI: 0.600-0.789; *P*< 0.001) with 67.3% sensitivity and 62.1% specificity. However, the AUC of the combination of serum *PLAG1* and AFP for predicting HCC patients was 0.706 (95% CI: 0.613–0.799; *P*< 0.001) with 69.2% sensitivity and 63.6% specificity, which revealed that the combination of two factors do not improve the diagnostic accuracy for HCC.

Table 2
The diagnostic accuracy of *PLAG1* and AFP as biomarkers for detection in HCC patients

Variables	AUC	*P*	95% CI		Sensitivity	Specificity
			Lower	Upper		
PLAG1	0.852	< 0.001	0.782	0.922	0.788	0.833
AFP	0.694	< 0.001	0.600	0.789	0.673	0.621
Combined *PLAG1* and AFP	0.706	< 0.001	0.613	0.799	0.692	0.636

Discussion

With an extremely low 5 year survival rate, HCC has been regarded as one of the most severe type of cancers. Moreover, due to the difficulty in early discovery, diagnosis and treatment, HCC patients have suffered decreasing life expectancy [20, 21]. Therefore, finding non-invasive biomarkers is of great importance to improve the diagnostic accuracy in early detection of HCC. AFP is the most widely used tumor marker for HCC. However, the recent studies reported that AFP lacks adequate sensitivity and specificity for effective surveillance. In the study of Jong et al., they found that the combination of AFP-L3 and PIVKA-Ⅱ improved the diagnostic value for HCC detection in patients with or without AFP levels [22]. Moreover, Wang et al. have reported that serum GP73 is a potential tumor marker for HCC diagnosis and the combination of GP73 and AFP is more sensitive than AFP alone [23]. Therefore, in the present study, we explored the diagnostic role of *PLAG1* as well as the combination of *PLAG1* and AFP in patients with HCC.

As a transcription factor, *PLAG1* is composed of two putative nuclear localization signals, seven canonical C2H2 zinc finger domains and a serine-rich C terminus [24]. It has been reported that *PLAG1* activation was caused by cryptic rearrangements, and ectopic *PLAG1* overexpression had lead to particular tumors in human. It is well established that overexpression of *PLAG1* was identified in about 70% of all PA [15, 25]. In addition to PA, the overexpression of *PLAG1* is also found in other human solid tumors, such as lipoblastomas [26], hepatoblastomas [27], leiomyosarcomas [15], chronic lymphocytic leukemia [28], and pediatric gastrointestinal stromal tumors [29]. To our knowledge, *PLAG1* plays an important role in tumorigenesis. *PLAG1* not only upregulates expression of genes that contribute significantly to tumorigenesis, but also could downregulate genes to inhibit the proliferation of tumor cells. Taken together, these findings have determined that the *PLAG1* might serve as a proto-oncogene. Recent report indicates that *PLAG1* might be involved in the hepatoblastoma, malignant liver tumor

commonly occurred in childhood, suggesting a potential role of *PLAG1* in malignant liver diseases [30]. In the study of Hu et al., they found that *PLAG1* was associated with KPNA2 and the co-enrichment of KPNA2 and *PLAG1* in nucleus could distinguish HCC patients with the worst prognosis [31]. In accordance with previous studies, we found that *PLAG1* protein level was significantly elevated in HCC serum compared with controls.

In our study, we found that patients with HCC had significantly higher serum *PLAG1* expression in comparison to the controls. The data revealed that the expression of *PLAG1* was significantly correlated with tumor size, differentiation and metastasis. Furthermore, *PLAG1* has been reported to be useful for predicting mixed tumors exhibiting monotonous or unusual morphology. In view of these findings, we suggested that *PLAG1* expression might have diagnostic value for HCC. Therefore, our findings suggested that serum *PLAG1* could be a novel diagnostic biomarker for patients with HCC. Currently, as a conventional serum marker, AFP has been widely used in the diagnosis of HCC, however, it exhibited the dismal accuracy with the low sensitivity and specificity. In this study, our data suggested that *PLAG1* might serve as a non-invasive biomarker in the diagnosis of HCC, which was more accurate in detecting HCC than AFP.

Conclusions

In conclusion, our findings clearly demonstrated that *PLAG1* expression was up-regulated in serum of HCC. Moreover, serum *PLAG1* might potentially function as a novel and noninvasive biomarker for the diagnosis of HCC. However, to further confirm the diagnostic value of *PLAG1* in HCC, more deeply and larger-scale studies should be conducted in the future research.

Abbreviations

pleomorphic adenoma gene 1 (PLAG1))

pleomorphic adenoma (PA)

hepatocellular carcinoma (HCC)

benign liver diseases (BLD)

α-fetoprotein (AFP)

enzyme-linked immunosorbent assay (ELISA)

receiver operating characteristic (ROC)

Declarations

Ethics approval and consent to participate

This study was supported by the Ethics Committee of The Third Hospital of Hebei Medical University and also has been carried out in accordance with the World Medical Association Declaration of Helsinki.

The subjects had been informed the objective. Certainly, written consents were signed by every subject in this study.

Consent for publication

We obtaining permission from participants to publish their data.

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

X.Z. design of the work; X.Z. the acquisition, analysis, X.L., H.B. interpretation of data; G.L., N.L. the creation of new software used in the work; H.L., J.D. have drafted the work or substantively revised it. All authors read and approved the final manuscript.

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Figures

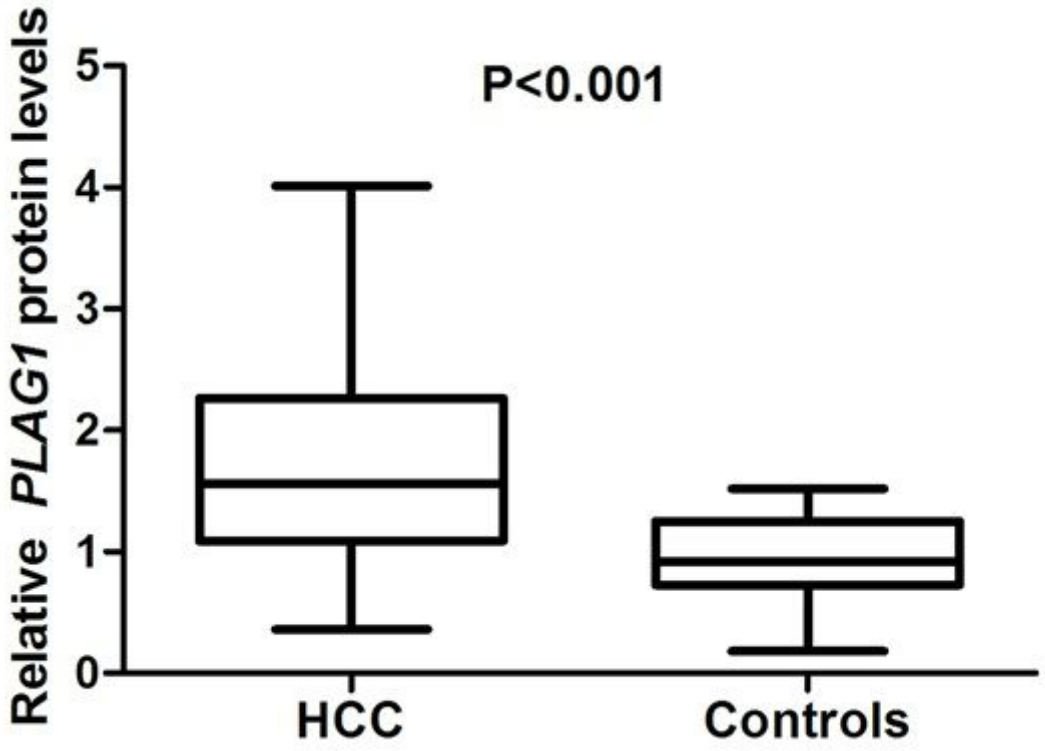

Figure 1

PLAG1 protein levels measured by Western Blot. Serum PLAG1 expression in HCC were significantly higher than that in controls (P<0.001).

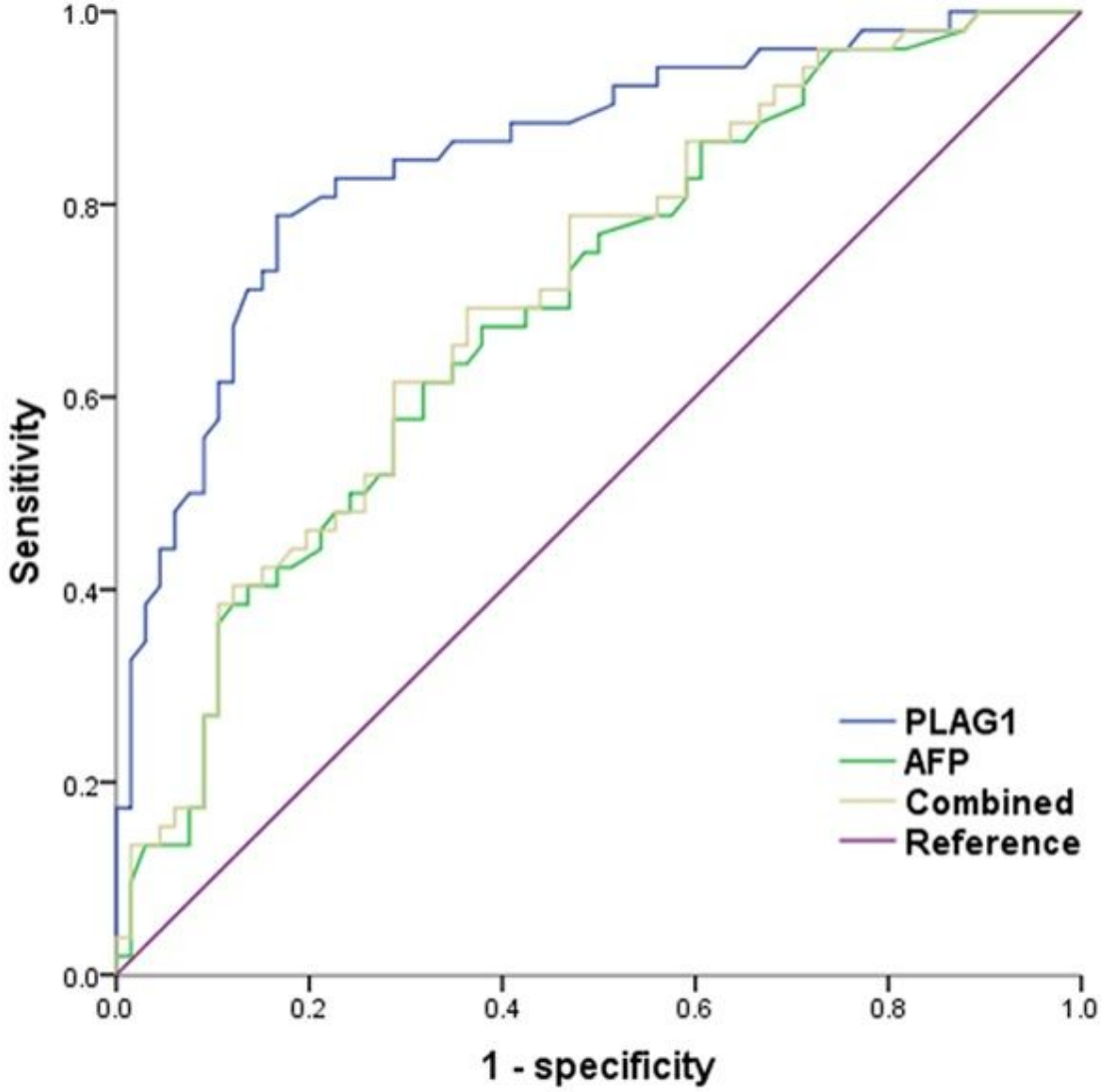

Figure 2

Receiver operating characteristic (ROC) curve analyses. ROC analysis for the diagnosis of HCC using PLAG1 (AUC: 0.852; 95% CI: 0.782-0.922; P<0.001), AFP (AUC: 0.694; 95% CI: 0.600-0.789; P<0.001) alone or the combination of them (AUC: 0.706; 95% CI: 0.613-0.799; P<0.001).