Original Paper

Prognostic and Diagnostic Significance of SDPR-Cavin-2 in Hepatocellular Carcinoma

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Key Words

Cavins • Cavin-2 • HCC • Prognosis • Diagnosis

Abstract

Background: Hepatocellular carcinoma (HCC) is a malignant tumor worldwide. Due to the lack of early prediction marker, numerous patients were diagnosed in their late stage. The family of cavins plays important roles in caveolae formation and cellular processes. Cavin-2, one of the members of cavins, has been reported as a suppresser in cancers. In this study, we have investigated its expression pattern and clinical significance in HCC. **Methods:** RT-qPCR was performed to detect the expression of cavin-2. **Results:** Cavin-2 was down-regulated in HCC and associated with tumor differentiation ($r=-0.275$, $P=0.013$) and tumor-node-metastasis (TNM) stage ($r=-0.216$, $P=0.035$). The Overall survival analysis showed that patients with lower cavin-2 expression had a relatively poor prognosis. Meanwhile, the multivariate analysis revealed that cavin-2 was an independent prognostic factor. The receiver operating characteristic curve analyses indicated that plasma cavin-2 presented a high accuracy (AUC=0.727, 0.865, 0.901) for diagnosing HCC cases from controls, hepatitis B and cirrhosis patients, respectively. Meanwhile, plasma cavin-2 showed a high sensitivity (88.4%, 89.9%) for detecting HCC with the serum α -fetoprotein (AFP) levels below 200 ng/ml from those hepatitis B and cirrhosis cases. **Conclusion:** Our data suggested that cavin-2 might be considered as a potential prognostic and diagnostic indicator in HCC.

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Introduction

Hepatocellular carcinoma (HCC) is one of the malignant tumors, which is also the second death-related diseases in humans worldwide [1]. HCC is the predominant form of liver cancer, accounting for 85% to 90% in all liver cancer cases [2]. 80% to 90% of the HCC

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patients have an established background of cirrhosis [3]. The major risk factor of cirrhosis is infected with hepatitis B virus (HBV), especially in Asia and Sub-Saharan Africa [4]. At present, HCC can be treated by kinds of treatments, including surgery, liver transplantation, chemotherapy and interventional therapy [5]. However, as most HCC is diagnosed in the advanced stage, the rate of 5-year survival is only 7% [6, 7]. Recurrence and metastasis are the main causes of low survival rate [8]. The serum α -fetoprotein (AFP) has long been used as a prognostic marker for HCC diagnosis. However, even in 15%-30% advanced patients, the AFP levels remain normal, leading to a high negative rate [9, 10]. Despite the specificity and sensitivity of the early diagnosis of HCC was improved quite bit by CT and MRI, the accuracy is still determined by the ability to discriminate between tumor and non-neoplastic lesions [11]. Up to now, we are short of good early diagnostic and predictive markers for HCC [12, 13]. Thus, it is necessary for us to seek a reliable marker.

Cavins are a new family of membrane proteins, which play an important role in caveolae formation [14, 15]. It has been reported that caveolae and its major components, known as caveolins, are involved in a variety of cellular processes including endocytosis, lipid homeostasis, signal transduction and tumorigenesis [16]. Recently, studies elaborated that cavins are able to stabilize the caveolae structure and regulate the availability of caveolins [17, 18]. So far, cavins have been identified with four different proteins, consisting of cavin-1 (polymerase transcript release factor, PTRF), cavin-2 (serum deprivation protein response, SDPR), cavin-3 (Sdr-related gene product that binds to c-kinase, SRBC), and cavin-4 (muscle-restricted coiled-coil protein, MURC) [19]. Comparing with cavin-1 and cavin-2, cavin-3's function was not well established and cavin-4 was related to caveolin-associated muscle disease [16, 20]. In 2015, Regazzetti et al. [21] found that cavin-1 and cavin-2 were necessary for the formation of caveolae. Therefore, researchers supposed that cavin-1 and cavin-2 may be related to tumorigenesis, one of the functions of caveolae. In 2012, Bai et al. [22] found that cavin-1 was inactivated in breast cancer, and the down-regulation of cavin-1 in breast cancer cells was associated with the promoter methylation. Meanwhile, Nassar et al. [23] demonstrated that the absence of cavin-1 in prostate cancer cells significantly led to tumor progression and metastasis in 2013. However, researches on cavin-2 have been limited to its role as a regulator of caveolae formation, while the potential value in tumors has not been deeply carried out [15].

Cavin-2 (serum deprivation protein response, SDPR) maps to chromosome 2q32-33, which shares more than 20% similarities with cavin-1, and is firstly shown *in vitro* to be a substrate for protein kinase C (PKC) isoforms by Burgener in 1990 [24]. The PKC was known to play an important role in progression of HCC [25] and Hansen et al. [15] demonstrated that cavin-2 was essential for the expression levels of cavin-1 protein which had a function in the development of tumors. Thus, we hypothesized that there was a correlation between cavin-2 and tumors. In 2013, Altintas et al. [26] found that cavin-2 could be considered as genes protective against prostate cancer and involved in the early stages of prostate carcinogenesis. Meanwhile, previous studies indicated that the levels of cavin-2 were not only down-regulated in prostate cancer but also in kidney and breast cancers [27, 28]. However, there has not been reported that the clinical and prognostic significance of cavin-2 expression in HCC.

In the present study, we investigated the levels of cavin-2 in HCC patients, then analyzed the relationship between cavin-2 expression and clinical characteristics, and evaluated the prognostic and diagnostic value of cavin-2. In conclusion, our aim was to provide an important basis for prognosis and diagnosis in HCC patients.

Materials and Methods

Specimens

91 patients (83 males and 8 females, mean age 55±10) with HCC and adjacent normal liver tissues were recruited in the Zhongnan Hospital of Wuhan University from 2011 to 2015, who underwent surgery

without preoperative chemotherapy or radiotherapy. All of the patients were selected based on pathology reports. Tumor staging (stage I, II, III and IV) was defined according to the seventh edition of the AJCC Cancer Staging Manual. Tumor specimens and corresponding adjacent non-tumor tissues were stored at -80 °C in RNAlater® RNA Stabilization Solution (Invitrogen, CA, USA). Follow-up data ranged from 2 to 48 months. Clinical and follow-up data were collected, and no information that could identify the patients was included.

Whole blood samples of 189 patients were obtained from Zhongnan Hospital of Wuhan University between 2014 and 2015, and divided into three groups: 71 patients with HCC (58 males and 13 females, mean age 57±13), 47 patients with hepatitis B (35 males and 12 females, mean age 52±14), and 71 the cirrhosis (52 males and 19 females, mean age 56±12), which were collected in EDTA tubes and centrifuged at 2,000 g for 5 min at 4 °C to spin down the blood cells. The supernatants were transferred to microcentrifuge tubes and centrifuged at 12,000 g for 5 min at 4 °C. The plasma was then stored at -80 °C until use. Patients who underwent preoperative chemotherapy or radiotherapy before were excluded. Meantime, 69 healthy blood samples (54 males and 15 females, mean age 54±11) were collected from the Physical Examination Center. All the controls were without hepatitis, hepatic diseases or abnormal liver biochemical outcome.

Ethical approval

Tissue and plasma specimens and clinical materials were collected after obtaining the informed consent of patients in accordance with institutional ethical guidelines, which was all approved by the Ethics Committee of Zhongnan Hospital of Wuhan University (Wuhan, China).

RNA extraction and reverse transcription

Trizol reagent (Invitrogen, CA, USA) was used to extract total RNA from tissues, and Total RNA Separate Extraction Kit (Biotek, Beijing, China) was used in plasma. The concentration and purity of RNA were quantified by NanoDrop ND2000 (Thermo, CA, USA). RNA was reverse transcribed to cDNA by using PrimeScript™ RT reagent Kit with gDNA Eraser (Takara, Japan), following: 42 °C for 2 min, and then 37 °C for 15 min, 85 °C for 5 sec.

Real-Time PCR Analysis

According to manufacturer's instructions, the levels of cavin-2 were performed on the Bio-Rad CFX96 (Bio-Rad Laboratories, Inc., Hercules, CA, USA) using SYBR-green I Premix EX Taq. The reactions started at 95 °C for 5 min, followed by 40 cycles of 95 °C for 30 s, 59 °C for 30 s and 72 °C for 30 s. In order to normalize the results for the qPCR, the GAPDH expression was used. The synthesized primers were as follows: cavin-2 (forward: 5'-AAGAGCGCATGGATAGGCAG-3' and reverse: 5'-TCATCGTGGGGCAAATCATCA-3'); GAPDH (forward: 5'-AGAAGGCTGGGGCTCATTG-3' and reverse: 5'-GCAGGAGGCATTGCTGATGAT-3'). All experiments were carried out in duplicate for each data point. Relative gene expression levels were calculated using the comparative Ct method formula $2^{-\Delta Ct}$.

Statistical Analysis

All statistical analyses were carried out using the SPSS version 17.0 (SPSS, Inc. Chicago, IL, USA) and GraphPad Prism 5.0 (GraphPad Software, La Jolla, CA, USA). All the data were presented in our study as mean ± standard deviation (M±SD) or median (QR). $P < 0.05$ was considered to be statistically significant. Statistical significance was assigned at $P < 0.05$ (*) or $P < 0.01$ (**). The Shapiro-Wilk test was carried out to check the normality of the distribution. The normally distributed numeric variables were evaluated by Student's *t*-test, while non-normally distributed variables were analyzed by Kruskal-Wallis variance analysis. Correlations were analyzed using the Spearman correlation. Survival curves were estimated by the Kaplan-Meier method and the log-rank test was used to estimate the statistical differences between survival curves. The multivariate analysis including the variables with a $P < 0.05$ in univariate analysis was used to assess the Cox proportional hazards model. Oneway ANOVA was used to validate the different cavin-2 expression among subgroups in plasma. The categorical variables were analyzed using Chi-square test. Finally, the receiver operating characteristic (ROC) curve analysis was performed to estimate the diagnostic values.

Results

Cavin-2 was significantly down-regulated in HCC tissue specimens

The expression levels of cavin-2 were measured by RT-qPCR in 91 paired clinical HCC tissues and adjacent normal liver tissues (Fig. 1). Expression of cavin-2 relative to GAPDH in tumor tissues was significantly down-regulated than in non-tumor tissues ($P < 0.01$; Fig. 1 A). The result indicated that cavin-2 may play a tumor suppressor's role in HCC.

Correlation between cavin-2 and clinic variables

Then studies were carried on assessing the correlation between cavin-2 and clinical characteristics. No statistically significant relevancies were found in gender, age, smoking, alcoholism, tumor size, cirrhosis, the serum α -fetoprotein (AFP), HBV-DNA and other biochemistry indexes. However, the levels of cavin-2 expression were correlated with differentiation ($r = -0.275$, $P = 0.013$) and tumor-node-metastasis (TNM) stage ($r = -0.216$, $P = 0.035$) (Table 1 and Table 2). Thus, down-regulation of cavin-2 might have an important role in HCC development and progression.

Association between cavin-2 levels and patients' survival

To answer whether the levels of cavin-2 expression correlated to the outcome of HCC patients, the 91 HCC patients were divided into two groups: relative high-cavin-2 group ($n = 46$, cavin-2 expression $\geq$ median) and relative low-cavin-2 group ($n = 45$, cavin-2 expression $<$ median) according to the median expression of cavin-2 (0.0092) (Fig. 2 A). Then survival signatures were analyzed in 67 patients, and 30 of them were in the high expression group, the others were in the low group. In patients' survival analyses, overall survival (OS) was calculated following the Kaplan-Meier method and log-rank test. Significantly, the low-cavin-2 group had a shorter survival time (median OS: 28 months), comparing with high-cavin-2 group (median OS: 40 months) (Fig. 2 B), suggesting the absence of cavin-2 expression could represent a novel indicator of poor prognosis in HCC.

In univariate analysis, differentiation, TNM stage and the levels of cavin-2 were significantly associated with overall survival of HCC patients ($P < 0.05$, Table 3). Then, we confirmed that differentiation, TNM stage and the levels of cavin-2 were independent prognostic predictors for overall survival of HCC patients by the multivariate analysis using Cox regression model ($P < 0.05$, Table 3).

Cavin-2 expression in plasma among subgroups

Table 4 summarized the main demographic and clinical characteristics of studied subjects. No difference was observed in important risk factors including gender, age,

Fig. 1. Cavin-2 expression in HCC tissues and adjacent normal liver tissues. A. Cavin-2 levels in tumor tissues were significantly lower than in nontumor tissues. B. The melt curve of cavin-2 and GAPDH in HCC tissues. C. The melt curve of cavin-2 and GAPDH in nontumor tissues. Results were expressed as mean $\pm$ SD. All data analyzed using Student's t test. ** $P < 0.01$.

Table 1. Association of cavin-2 expression with clinical parameters in HCC. Abbreviation: Data are mean $\pm$ SD. * $p < 0.05$, ** $p < 0.01$; HCC, hepatocellular carcinoma; TNM, tumor-node-metastasis; HBV, hepatitis B virus; AFP, α -fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ -glutamyl transferase; GLU, glucose

Characteristics	n	cavin-2 relative expression (-log) Mean $\pm$ SD	t	P
Tissue**			9.511	<0.001
HCC	91	2.04 $\pm$ 0.75		
Adjacent non-cancerous liver	91	1.30 $\pm$ 0.74		
Gender			-0.834	0.407
Male	83	2.02 $\pm$ 0.77		
Female	8	2.25 $\pm$ 0.57		
Age			-1.163	0.248
<55	41	1.95 $\pm$ 0.82		
≥ 55	50	2.14 $\pm$ 0.65		
Smoking			1.019	0.311
Negative	53	2.11 $\pm$ 0.77		
Positive	38	1.94 $\pm$ 0.73		
Alcoholism			1.402	0.164
Negative	68	2.10 $\pm$ 0.74		
Positive	23	1.85 $\pm$ 0.76		
Differentiation*			-2.541	0.013
High/ Moderate	68	2.02 $\pm$ 0.75		
Low	12	2.51 $\pm$ 0.66		
Tumor Size			-0.220	0.826
<10 cm	51	2.03 $\pm$ 0.77		
≥ 10 cm	40	2.07 $\pm$ 0.71		
Tumor nodes			0.707	0.482
Single	67	2.01 $\pm$ 0.78		
Multi	24	1.81 $\pm$ 0.63		
TNM stage*			-2.152	0.035
I ~ II	46	1.80 $\pm$ 0.64		
III ~ IV	45	2.16 $\pm$ 0.75		
HBV DNA (IU/ml)			-0.270	0.789
<500	12	2.09 $\pm$ 0.78		
≥ 500	26	2.17 $\pm$ 0.75		
Cirrhosis			1.785	0.078
Negative	47	2.17 $\pm$ 0.77		
Positive	44	1.89 $\pm$ 0.71		
AFP (ng/ml)			-0.610	0.543
<200	37	1.93 $\pm$ 0.79		
≥ 200	40	2.04 $\pm$ 0.72		
ALT (U/l)			-1.195	0.235
<46	49	1.95 $\pm$ 0.78		
≥ 46	42	2.14 $\pm$ 0.71		
AST (U/l)			-1.599	0.113
<46	44	1.91 $\pm$ 0.73		
≥ 46	47	2.16 $\pm$ 0.75		
GGT (U/l)			-0.079	0.937
<55	34	1.98 $\pm$ 0.69		
≥ 55	46	1.99 $\pm$ 0.79		
GLU (mmol/l)			-1.556	0.123
<6.2	67	1.96 $\pm$ 0.72		
≥ 6.2	24	2.24 $\pm$ 0.83		

Table 2. Correlation analysis of cavin-2 in relation to clinical parameters

Characteristics	Correlation Coefficient
Differentiation (High/ Moderate vs Low)	-0.275
TNM stage (I ~ II vs III ~ IV)	-0.216

smoking, alcoholism and glucose (GLU) in the four groups. There was a significant difference in alanine aminotransferase (ALT), aspartate aminotransferase (AST) among the groups.

To evaluate the value of cavin-2 as a biomarker, the levels of plasma target mRNA in 71 HCC patients, 47 hepatitis B, 71 cirrhosis patients, and 69 control cases were measured by RT-qPCR. The result indicated that the expression of cavin-2 in HCC was lower than that in hepatitis B, cirrhosis and control groups (HCC vs cirrhosis: $P < 0.001$; HCC vs hepatitis B: $P < 0.001$; HCC vs the controls: $P < 0.001$). However, comparing the levels of the controls,

Fig. 2. Overall survival according to cavin-2 expression levels. Patients with decreased cavin-2 expression (median OS: 28 months) showed reduced survival time compared with patients with high levels of cavin-2 expression (median OS: 40 months) ($P < 0.01$). Data analyzed using log-rank test.

Table 3. Prognostic factors in Cox proportional hazards model. Abbreviation: $P < 0.05$ was considered statistically significant; ALT, alanine aminotransferase; AST, aspartate aminotransferase; AFP, α -feto-protein; TNM, tumor-node-metastasis

Factors	Univariate analysis		Multivariate analysis	
	HR (95% CI)	P	HR (95% CI)	P
Gender	2.481 (0.511-4.291)	0.469		
Age	0.992 (0.955-1.031)	0.689		
Smoking	0.910 (0.454-1.827)	0.791		
Alcoholism	0.992 (0.458-2.148)	0.984		
Cirrhosis	1.049 (0.521-2.110)	0.894		
Tumor size	2.054 (0.943-4.471)	0.070		
Tumor number	1.471 (0.657-3.293)	0.348		
ALT	1.420 (0.697-2.892)	0.334		
AST	1.728 (0.859-3.474)	0.125		
AFP	1.546 (0.714-3.348)	0.269		
Differentiation (low vs high/ moderate)	3.141 (1.474-6.694)	0.003	2.504 (1.147-5.466)	0.021
TNM (III~IV vs I~II)	3.033 (1.360-6.767)	0.007	3.156 (1.389-7.170)	0.006
Cavin-2 expression (low vs high)	4.145 (1.940-8.853)	<0.001	4.225 (1.935-9.224)	<0.001

we found that the levels in hepatitis B and cirrhosis groups were higher than the controls (cirrhosis vs controls: $P < 0.001$; hepatitis B vs controls: $P < 0.05$). In addition, no remarkable difference was observed for cavin-2 between hepatitis B and cirrhosis (hepatitis B vs cirrhosis: $P = 0.16$) (Fig. 3).

Diagnostic value of cavin-2 in plasma

To assess whether plasma cavin-2 could be used as a potential diagnostic marker for HCC, ROC was constructed using 5 models: HCC vs the controls, HCC vs hepatitis B, HCC vs cirrhosis, hepatitis B vs controls and cirrhosis vs controls (Table 5). Result revealed that cavin-2 had a good sensitivity of 85.9% (AUC=0.727, 95%CI: 0.643-0.812) (Fig. 4A) in discriminating HCC patients from the controls. Meanwhile, the area under the ROC (AUC_{ROC}) showed that cavin-2 could be thought of as a better diagnostic value for differentiating HCC patients from hepatitis B (AUC=0.865, 95%CI: 0.796-0.934) (Fig. 4 B) and from cirrhosis (AUC=0.901, 95%CI: 0.849-0.952) (Fig. 4 D). Moreover, ROC result indicated that plasma

Table 4. Characteristics of the studied subjects. Abbreviation: # Median (25 Percentiles, 75 Percentiles); ^aChi-square test. ^bKruskal-Wallis; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GLU, glucose

Characteristics	HCC N=71	Hepatitis B N=47	Cirrhosis N=71	Control N=69	P
Gender					0.640 ^a
Male	58	35	52	54	
Female	13	12	19	15	
Age					0.710 ^a
<55	27	20	30	33	
≥55	44	27	41	36	
Smoking					0.748 ^a
Negative	44	30	39	40	
Positive	27	17	32	29	
Alcoholism					0.387 ^a
Negative	54	37	47	48	
Positive	17	10	24	21	
ALT (U/l)#	27(22,30)	136(79,260)	37(30,117)	28(20,29)	<0.001 ^b
AST (U/l)#	34(30,44)	57(44,74)	45(35,186)	23(21,26)	<0.001 ^b
GLU (mmol/l)#	4.69(4.61,4.81)	4.55(4.27,4.69)	5.73(4.63,6.83)	4.58(4.5,5.04)	0.083 ^b

Table 5. Comparisons of the AUC of the expression of cavin-2 for subgroups. Abbreviation: Se: Sensitivity; Sp: Specificity. #1 HCC patients with AFP levels below 200 ng/ml and Hepatitis B whose AFP levels also below 200 ng/ml. #2 HCC patients with AFP levels below 200 ng/ml and Cirrhosis whose AFP levels also below 200 ng/ml

Group	AUC	95%CI	P	Se(%)	Sp(%)
HCC vs Hepatitis B	0.865	0.796-0.934	<0.001	85.9	76.6
HCC vs Hepatitis B ^{#1}	0.870	0.799-0.938	<0.001	88.4	76.1
HCC vs Cirrhosis	0.901	0.849-0.952	<0.001	88.7	80.3
HCC vs Cirrhosis ^{#2}	0.899	0.847-0.952	<0.001	89.9	78.6
HCC vs Control	0.727	0.643-0.812	<0.001	85.9	55.1
Hepatitis B vs Control	0.628	0.526-0.729	0.0201	43.5	78.8
Cirrhosis vs Control	0.692	0.605-0.779	<0.001	63.8	69.0

Fig. 3. Cavin-2 levels in plasma among subgroups. Cavin-2 expression in HCC was lower than that in hepatitis B, cirrhosis and the controls. No differences were observed between hepatitis B and cirrhosis. The data analyzed using Oneway ANOVA. * $P < 0.05$, ** $P < 0.01$.

cavin-2 conferred a higher sensitivity for detecting HCC patients (n=69) with AFP levels below 200 ng/ml from those hepatitis B (n=46) (AUC=0.870, 95%CI: 0.799-0.938) (Fig. 4 C) and cirrhosis cases (n=70) (AUC=0.899, 95%CI: 0.847-0.952) (Fig. 4 E) whose AFP levels were also below 200 ng/ml. However, the diagnostic value of cavin-2 in another two groups was not obvious (Fig. 4 F-G).

Fig. 4. Receiver operating characteristic (ROC) curves. A. HCC vs Controls; B. HCC vs Hepatitis B; C. HCC (AFP levels below 200 ng/ml) vs Hepatitis B (AFP levels below 200 ng/ml); D. HCC vs Cirrhosis; E. HCC (AFP levels below 200 ng/ml) vs Cirrhosis (AFP levels below 200 ng/ml); F. Hepatitis B vs Controls; G. Cirrhosis vs Controls.

Discussion

HCC is the most common solid tumors in the global range, with highly aggressive malignancy and poor prognosis [29]. Because HCC could be diagnosed in the advanced stage after the related symptoms appear. Hence, a novel biomarker for diagnosing HCC at early stage is intensively desired in clinical. Over the past decade, numerous studies were undertaken to explore the biomarker for HCC [30]. However, the research progress remains quite slow. Caveolae are specialized plasma membrane subdomains implicated in cellular activities, such as migration, signaling, trafficking and tumorigenesis [23]. Cavins play important roles in caveolae biogenesis, regulating caveolar function and organization [31]. Evidence showed that cavin-2 (serum deprivation protein response, SDPR) was present in many cellular types, and down-regulated in several different cancers including breast, gastric, kidney, prostate and oral cancer [28, 32]. In 2016, Ozturk et al. [27] found that cavin-2 could be a potential prognostic biomarker and a therapeutic target for breast cancer. Therefore, it is particularly prospective to explore the application of cavin-2 in HCC.

In our present study, we investigated the prognostic, clinical and diagnostic value of cavin-2 in HCC patients for the first time. We found that cavin-2 was down-regulated in HCC tissues to a greater extent than in corresponding noncancerous tissues, and the results

indicated that the levels of cavin-2 were related to differentiation and TNM stage. In addition, comparing with the high cavin-2 group, the decreased levels of cavin-2 were associated with a poor prognosis and a shorter survival time. We also detected the expression of cavin-2 in plasma. Results showed that the levels of cavin-2 in HCC were lower than those in the controls, the hepatitis B and the cirrhosis. This result was consistent with the tissues. However, the levels of cavin-2 in the hepatitis B and the cirrhosis were higher than the controls, the reason for the results probably was that the cavin-2 was released during the destructions of normal tissues. However, the exact reasons needed further studies to explain. As we all know, HCC is a multi-factor, multi-step and complex process. Unfortunately, despite there was statistical significance in the two groups (HCC vs hepatitis B and HCC vs cirrhosis), no remarkable difference was found between hepatitis B and cirrhosis. This result indicated that cavin-2 could not be used as an indicator to monitor the process by which hepatitis B developed into cirrhosis. On account of these data, we found that cavin-2 could be used as a candidate prognostic and indicative biomarker for HCC.

Circulating RNA in plasma has been an emerging field for noninvasive diagnostic applications in HCC [33]. For the first time, we assessed the expression of cavin-2 in plasma to analysis the diagnostic value. The area under the ROC (AUC_{ROC}) elaborated that cavin-2 was helpful for differentiating HCC patients from the controls, with AUC of 0.727 and a good sensitivity of 85.9%. Infected with Hepatitis B virus is one of the most important risk factors for the occurrence of HCC. ROC curve analysis showed that circulating cavin-2 in plasma had a better diagnostic value to make a distinction between HCC and hepatitis B, with AUC of 0.865 (85.9% sensitivity, 76.6% specificity). Most importantly, cirrhosis acting as a premalignant disease of HCC, cavin-2 yielded an AUC of 0.901 (88.7% sensitivity, 80.3% specificity) for differentiating HCC from a total of 71 samples. These data showed that cavin-2 could be a good marker in HCC. When determining HCC cases with AFP<200 ng/ml from the cirrhosis whose AFP levels were also below 200 ng/ml, cavin-2 yielded the highest sensitivity (89.9%). This might be useful for determining HCC in early stages when the level of AFP is lower than the diagnostic standard. But the diagnostic value in other groups was not obvious. Our data demonstrated that cavin-2 was valuable to diagnosis in liver-related disease. However, the limitation of our research was that the sample size was relatively small; the present findings therefore should be validated in trials with more cases.

Ozturk et al. [27] suggested that the deletion of cavin-2 was likely to be mediated by promoter DNA hypermethylation during breast cancer progression. In 2016, Tian et al. [34] found that cavin-2 suppressed cell proliferation and invasion in breast cancer cells by regulating epithelial-mesenchymal transition (EMT), with up-regulating epithelial markers (E-cadherin and β -catenin) and down-regulating mesenchymal markers (Vimentin and N-cadherin). What is more, researchers suggested that transforming growth factor- β (TGF- β), which can induce EMT, was blocked by cavin-2 in breast cancer. In oral squamous cell carcinomas, Unozawa et al. [32] suggested that the ERK signaling pathway was attenuated frequently in the overexpression cavin-2 cells. In studied cancers, cavin-2 was a metastasis suppressor by inhibiting EMT, migration, and intravasation accompanied with promotion of apoptosis [27]. In HCC, numbers of studies reveal that it is believed that the EMT acts an important role, TGF- β signaling pathway and ERK signaling pathway have been considered as activators of cancer progression [35-38]. Therefore, we supposed that cavin-2 might be involved in HCC. These studies indicated that cavin-2 could suppress the development of HCC as well as other cancers. Unfortunately, the exact mechanism of cavin-2 is still unknown in HCC.

To sum up, our research provided insights into the expression levels of cavin-2 in HCC patients for the first time. We elaborated that the expression of cavin-2 was significantly lower in HCC than that in corresponding noncancerous tissues and relation to differentiation and TNM stage. Our study indicated that cavin-2 was an independent prognostic factor of HCC patients, and comparing with the low cavin-2 group, the high one had a good prognosis and a longer survival time. Then we detected in plasma, and found that cavin-2 expression was down-regulated in HCC group, and cavin-2 had a good diagnostic value to differentiating

HCC from cirrhosis, which was helpful to diagnose between premalignant lesions and HCC. These findings suggested for the first time that the expression of cavin-2 could be used as a novel prognostic and diagnostic marker for HCC. However, further analysis is still essential to test exact molecular mechanism of unregulated expression of cavin-2 in HCC.

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Disclosure Statement

The authors have declared that no conflict of interest.

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