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Original Article

Correlations and prognostic values of contactin-1 and epithelial-mesenchymal transition marks in hepatocellular carcinoma

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Abstract: Contactin-1 (CNTN-1) is known to implicate the invasion and metastasis of many tumos, including Hepatocellular carcinoma (HCC). The epithelial-mesenchymal transition (EMT) was reported to be a critical step in HCC progression. However, the relationship of CNTN-1 and EMT in HCC remains unknown. This study aimed to investigate the expression and correlation of CNTN-1 and EMT, and to determine prognostic values of CNTN-1 and EMT marks in HCC. The expression of CNTN-1 and three EMT marks (E-cadherin, vimentin and Snail) was assessed in HCC specimens by immunohistochemistry. The correlations of CNTN-1 and three EMT marks were analyzed. The relationships of CNTN-1 and EMT marks with clinicopathological parameters and prognosis were also analyzed. Expression of CNTN-1, vimentin (VIM), and Snail was significantly higher in HCC tissues than in adjacent normal tissues, while E-cadherin (E-cad) expression was significantly lower in HCC tissues than in adjacent normal tissues. CNTN-1 expression was positively correlated with expression of VIM and Snail and was negatively correlated with E-cad expression. Kaplan-Meier analysis showed that high expression of CNTN-1, VIM, and Snail was significantly associated with reduced overall survival (OS) and disease-free survival (DFS), whereas opposite result was found for high E-cad expression. Moreover, multivariate analysis showed that high CNTN-1 expression, high Snail expression, and low E-cad expression were independent prognostic factors for poor prognosis in HCC patients. Overexpression of CNTN-1 correlates with abnormal expression of EMT marks. Abnormal expression of CNTN-1 and EMT marks is correlated with a poor prognosis in HCC. CNTN-1 may play a role in epithelial-mesenchymal transition in HCC.

Keywords: Contactin-1, epithelial-mesenchymal transition, prognosis, HCC

Introduction

Hepatocellular carcinoma (HCC) is the sixth common malignancy and the third most common cause of cancer death worldwide, with approximately 745,500 deaths in 2012 [1]. The high frequency of metastasis and recurrence and the difficulty of early diagnosis cause low survival rate in HCC patients. Nevertheless, the mechanism of the development, invasion and metastasis of HCC is still unclear.

Contactin-1 (CNTN-1), consisted of N-CAM, L1, and Nr-CAM, is a member of the immunoglobulin superfamily. As a neural cell adhesion molecule, CNTN-1 is implicated in neural cell migration and establishment of axon connection in the nervous system [2]. Currently, many studies revealed that CNTN-1 is involved in tumor invasion and metastasis in multiple cancers, such as gastric cancer [3], stomach cancer [4] and lung cancer [5], and correlates with poor prognosis of these tumors. Previously, we have found that CNTN-1 was overexpressed in HCC tissues and contributed to tumor metastasis and invasion in HCC [6]. Although the role of CNTN-1 is confirmed in the development of HCC, the relationship between CNTN-1 expression and EMT marks in HCC has not been reported.

Epithelial-mesenchymal transition (EMT), characterized by the loss or downregulation of epithelial differentiation marker E-cadherin (E-cad) and upregulation of mesenchymal markers Snail and vimentin (VIM) [7], is an evolutionari-

Table 1. Expression of CNTN-1, VIM, Snail and E-cad in hepatocellular cancer tissues and adjacent normal tissues

	n	Cancer tissues	Normal tissues	X^2	*p*
CNTN-1	81			7.14	0.008
Positive		50 (61.7%)	33 (40.7%)		
Negative		31 (38.3%)	48 (59.3%)		
Snail	81			8.10	0.004
Positive		45 (55.6%)	27 (33.3%)		
Negative		36 (44.4%)	54 (66.7%)		
E-cad	81			6.19	0.013
Positive		46 (56.8%)	61 (75.3%)		
Negative		35 (43.2%)	20 (24.7%)		
VIM	81			16.90	< 0.001
Positive		49 (60.5%)	23 (28.4%)		
Negative		32 (39.5%)	58 (71.6%)		

Abbreviations: CNTN-1, contactin-1; VIM, vimentin; E-cad, E-cadherin.

ly conserved developmental procedure which contributes to the constitution of the body plan, histogenesis, organogenesis and tumor invasion [8]. The importance of EMT in HCC development was reported in many current researches, suggesting that EMT is involved in HCC progression and metastasis [9-11].

In this study, we assessed the protein expression levels of CNTN-1, E-cad, Snail and VIM in 81 samples of HCC, and investigated values of their clinical pathology and prognosis.

Materials and methods

Patients and tissue samples

Tumor samples and matched adjacent normal tissue samples were acquired from 81 patients who were definitely diagnosed as HCC by postoperative pathological examination and underwent surgical resection at the affiliated provincial Hospital of Anhui Medical University (Hefei, China) from 2006 to 2010. These patients included 63 men and 18 women, between 19 and 74 years of age (mean age of 49 ± 16 years), none of them had accepted radiotherapy or chemotherapy before surgery. Clinical data and Detailed pathological were collected from every patient's medical records, such as age, gender, tumor size, tumor nodules, capsula, microvascular invasion, Edmondson grade, HbeAg, cirrhosis, Child-Pugh grade, a-fetoprotein (AFP) and TNM stage. Tumor differentiation was defined by the Edmondson grading system [12], and tumor stage was performed by the sixth edition of the tumor-node-metastasis (TNM) classification of the International Union against Cancer. The specimens fixed in formalin were embedded in confirmation of the diagnosis and paraffin for pathological analysis. Integrated clinical follow-up data was acquired from the HCC database of Anhui Provincial Hospital. This study obtained informed consent from each patient and was performed with approval of the Human Research Ethics Committee of Anhui Medical University.

Immunostaining and evaluation

Immunohistochemistry of each specimen for CNTN-1, E-cad, VIM and Snail was performed. The samples (4-μm thick) were sectioned onto silanized glass slides consecutively. Two-step immunohistochemical was used to exam these proteins expression. Briefly, Deparaffinized and hydrated sections were handled with 0.3% hydrogen peroxide for 15 min at room temperature to block endogenous peroxidase activity. Afterward, slides were washed in PBS (3 × 3 min) and placed in citrate buffer in citrate buffer (pH 6.0) for antigen retrieval. After washing with PBS (3 × 3 min), the slides were separately stained at 37°C for 2 h with the following antibodies: CNTN-1 monoclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA), Ecad monoclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA), Snail monoclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and VIM monoclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and then washed with PBS again (3 × 3 min). Slides were incubated with Universal IgG antibody-HRP polymer (Zhongshan Jinqiao Co, Beijing, China) at 37°C for 15 min, which were followed by 3 washes (PBS; 3 × 3 min). Finally, each slide was handled with 50 μl diaminobenzadine (DAB) which work solution for 3-10 min at room temperature, and then washed in PBS. All slides were counterstained with hematoxylin.

The fraction of stained tumor cells was categorized as follows: 0: none; 1: < 10%; 2: 10-50% and 3: > 50%. The staining intensity was scored

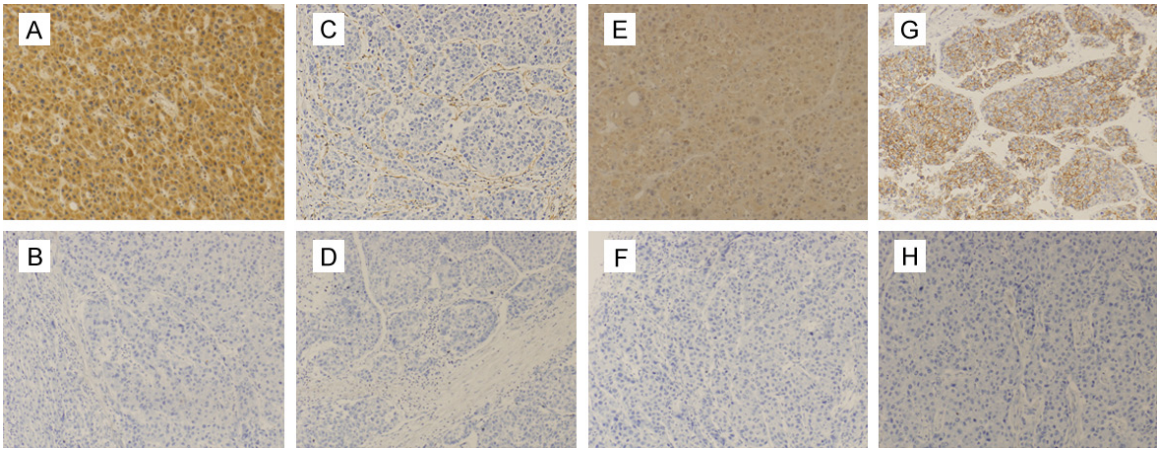

Figure 1. Immunostaining of CNTN-1, VIM, Snail and E-cad in HCC tissues. A. Positive immunostaining of CNTN-1 in HCC tissues. B. Negative immunostaining of CNTN-1 in HCC tissues. C. Positive immunostaining of VIM in HCC tissues. D. Negative immunostaining of VIM in HCC tissues. E. Positive immunostaining of Snail in HCC tissues. F. Negative immunostaining of Snail in HCC tissues. G. Positive immunostaining of E-cad in HCC tissues. H. Negative immunostaining of E-cad in HCC tissues. Abbreviations: CNTN-1, contactin-1; VIM, vimentin; E-cad, E-cadherin; HCC, hepatocellular carcinoma.

as follows: 0: negative; 1: weak staining; 2: moderate staining and 3: strong staining. The final immunostaining score was determined by their product. Accordingly, score < 3 was defined as low expression, and score ≥ 3 was high expression. The scoring of each sample was evaluated independently by two pathologists in Anhui Provincial Hospital independently without any knowledge of the clinicopathological findings.

Statistical methods

All analyses of statistics were performed by the statistical package SPSS 17.0 (SPSS, Inc, Chicago, IL). The chi-square test was used to analyze the difference of protein expression between the cancer group and normal group and the correlation between each protein expression and clinicopathological parameters. The Spearman rank correlation coefficient was applied to the analysis of the correlation between CNTN-1 and EMT marks in HCC. The Kaplan-Meier method and the log-rank test were used to analyze survival. The Cox regression model was used to evaluate the independent prognostic value. Statistically significant were considered at $P < 0.05$.

Results

High expression of CNTN-1, Snail and VIM and low expression of E-cad in HCC tissues

Among all of the 81 samples, positive CNTN-1 expression was detected in 50 (61.7%) cancer tissues and 33 (40.7%) normal tissues. The rate of tumors with negative Snail expression was 44.4% (36/81) and the rate of that is 66.7% (54/81) in normal samples. Positive E-cad expression and positive VIM expression were detected in 56.8% (46/81) and 60.5% (49/81) of tumor samples respectively, compared to 75.3% (61/81) and 28.4% (23/81) of adjacent normal tissues. By way of the chi-square test, a significant difference in CNTN-1 and EMT marks expression between the cancer group and the matched adjacent normal group ($P < 0.05$) was showed **Table 1**. The representative immunohistochemistry results were shown in **Figure 1**.

Negative correlation between CNTN-1 and E-cad and positive correlation betwen CNTN-1 and other EMT marks (VIM and Snail) in HCC

The Spearman rank test showed that expression of CNTN-1 was negatively associated with E-cad ($r = -0.277$, $P = 0.013$), and was positively correlated with VIM expression ($r = 0.247$, $P = 0.026$) and Snail expression ($r = 0.420$, $P < 0.001$) (**Table 2**).

Correlation between expression of CNTN-1, EMT marks and clinicopathological parameters in HCC

The correlations between the expression of CNTN-1 and EMT marks and the clinicopathological parameters of HCC patients were showed in **Table 3**. The overexpression of CNTN-

Table 2. Correlations of CNTN-1 with EMT marks in HCC

Immunoreactivity	CNTN-1				
	Negative	Positive	*r*	X^2	*p*
VIM					
Negative	17 (21%)	15 (18.5%)	0.247	4.94	0.026
Positive	14 (17.3%)	35 (43.2%)			
Snail					
Negative	22 (27.2%)	14 (17.3%)	0.420	14.31	< 0.001
Positive	9 (11.1%)	36 (44.4%)			
E-cad					
Negative	8 (9.9%)	27 (33.3%)	-0.277	6.20	0.013
Positive	23 (28.4%)	23 (28.4%)			

Abbreviations: CNTN-1, contactin-1; VIM, vimentin; E-cad, E-cadherin.

1 was closely related to tumor differentiation (P = 0.001), tumor size (P = 0.042), tumor nodule number (P = 0.009), vascular invasion (P = 0.006), tumor capsula (P = 0.023) and TNM stage (P < 0.001), but no significant correlation was present with patients'age, gender, Child-Pugh grade, HbeAg status, cirrhosis and AFP (P > 0.05).

The positive expression of Snail correlated with tumor differentiation (P = 0.006), HbeAg status (P = 0.021), vascular invasion (P < 0.001), tumor capsula (P = 0.002) and TNM stage (P < 0.001), but no correlation with patients' age, gender, cirrhosis, Child-Pugh grade, AFP, tumor size and tumor nodule number (P > 0.05). The downregulated expression of E-cadherin was correlated with tumor differentiation (P = 0.006), Child-Pugh grade (P = 0.043), AFP (P = 0.002), tumor size (P = 0.016), vascular invasion (P < 0.001) and TNM stage (P < 0.001), but no association was present with patients' age, gender, HbeAg status, cirrhosis, tumor nodule number, tumor capsula (P > 0.05). A significant association between the positive expression of VIM and tumor differentiation (P = 0.008), vascular invasion (P = 0.016) and TNM stage (P = 0.005), but no association existed with patients' age, gender, HbeAg status, cirrhosis, Child-Pugh grade, AFP, tumor size, tumor nodule number and tumor capsula (P > 0.05).

Survival analysis

Overexpression of Snail, VIM and CNTN-1 and down-regulation of E-cad indicate worse prognosis

Survival curves were analyzed by the Kaplan-Meier method (**Figure 2**). The result of log-rank test indicated that the disease-free survival (DFS) rate and overall survival (OS) rate of patients with positive CNTN-1 tumors were significantly lower than those of negative CNTN-1 group (P < 0.001, **Figure 2A** and **2B**). And, compared with the group with VIM positive expression, the DFS rate and OS rate of patients with VIM negative expression were significantly higher (P = 0.006 and P = 0.007 respectively, **Figure 2C** and **2D**). The DFS rate and OS rate of patients with Snail positive tumors were significantly lower when compared with Snail positive group (P < 0.001, **Figure 2E** and **2F**), whereas, the E-cad expression has an opposite result (P < 0.001, **Figure 2G** and **2H**). Results showed that overexpression of Snail, VIM and CNTN-1 and down-regulation of E-cad indicate low survival in HCC.

Univariate analysis of factors on the prognosis showed that CNTN-1, Snail, E-cad, VIM, Tumor size, Vascular invasion, Edmondson grade, Tumor capsula, Child-Pugh grade, AFP and TNM stage had significantly prognostic influences on OS and DFS (P < 0.05, **Table 4**). Furthermore, by multivariate survival analysis, CNTN-1, Snail and E-cad were found to be independent prognostic factors for the OS, and the independent prognostic factors for DFS were CNTN-1, Snail, E-cad and vascular invasion (P < 0.05, **Table 5**).

Discussion

In this study, the expression of CNTN-1 was significantly higher in HCC than in adjacent normal tissues, which was consistent with our previous study [6]. Moreover, overexpression of CNTN-1 was closely related to tumor differentiation, tumor size, tumor nodule number, vascular invasion, tumor capsula and TNM stage. Both our previous study [6] and this research showed that overexpression of CNTN-1 was correlated with poor prognosis in HCC, and the similar result was found in human gastric cancer [3], stomach cancer [4] and lung cancer [5]. Although EMT markers were also found to play important roles in tumor progression and metastasis of HCC [9-11], there are few studies investigating the correlations between CNTN-1 and EMT markers.

Table 3. Associations of CNTN-1, VIM, snail and E-cad expression with clinicopathologic variables

Variables	NO.	CNTN-1			VIM			Snail			E-cad		
		N	P	*P* value	N	P	*P* value	N	P	*P* value	N	P	*P* value
Age				0.537			0.354			0.095			0.963
< 60 years	53	19 (23.5%)	34 (42%)		19 (23.5%)	34 (42%)		20 (24.7%)	33 (40.7%)		23 (28.4%)	30 (37%)	
≥ 60 years	28	12 (14.8%)	16 (19.8%)		13 (16%)	15 (18.5%)		16 (19.8%)	12 (14.8%)		12 (14.8%)	16 (19.8%)	
Sex				0.951			0.114			0.591			0.337
Male	63	24 (29.6%)	39 (48.1%)		22 (27.2%)	41 (50.6%)		29 (35.8%)	34 (42%)		29 (35.8%)	34 (42%)	
Female	18	7 (8.6%)	11 (13.6%)		10 (12.3%)	8 (9.9%)		7 (8.6%)	11 (13.6%)		6 (7.4%)	12 (14.8%)	
Tumor size				0.042			0.631			0.129			0.016
> 5 cm	33	17 (21%)	16 (19.8%)		12 (14.8%)	21 (25.9%)		18 (22.2%)	15 (18.5%)		9 (11.1%)	24 (29.6%)	
≤ 5 cm	48	14 (17.3%)	34 (42%)		20 (24.7%)	28 (34.6%)		18 (22.2%)	30 (37%)		26 (32.1%)	22 (27.2%)	
Tumor nodule				0.009			0.501			0.089			0.134
Single	60	28 (34.6%)	32 (39.5%)		25 (30.9%)	35 (43.2%)		30 (37%)	30 (37%)		23 (28.4%)	37 (45.7%)	
Multiple	21	3 (3.7%)	18 (22.2%)		7 (8.6%)	14 (17.3%)		6 (7.4%)	15 (18.5%)		12 (14.8%)	9 (11.1%)	
Vascular invasion				0.006			0.016			< 0.001			< 0.001
Absent	56	27 (33.3%)	29 (35.8%)		27 (33.3%)	29 (35.8%)		34 (42%)	22 (27.2%)		17 (21%)	39 (48.1%)	
Present	25	4 (4.9%)	21 (25.9%)		5 (6.2%)	20 (24.7%)		2 (2.5%)	23 (28.4%)		18 (22.2%)	7 (8.6%)	
Edmondson grade				0.001			0.008			0.006			0.006
I-II	16	12 (14.8%)	4 (4.9%)		11 (13.6%)	5 (6.2%)		12 (14.8%)	4 (4.9%)		2 (2.5%)	14 (17.3%)	
III-IV	65	19 (23.5%)	46 (56.8%)		21 (25.9%)	44 (54.3%)		24 (29.6%)	41 (50.6%)		33 (40.7%)	32 (39.5%)	
Tumor capsula				0.023			0.612			0.002			0.066
Complete	53	25 (30.9%)	28 (34.6%)		22 (27.2%)	31 (38.3%)		30 (37%)	23 (28.4%)		19 (23.5%)	34 (42%)	
None	28	6 (7.4%)	22 (27.2%)		10 (12.3%)	18 (22.2%)		6 (7.4%)	22 (27.2%)		16 (19.8%)	12 (14.8%)	
HbeAg				0.219			0.248			0.021			0.323
Positive	68	28 (34.6%)	40 (49.4%)		25 (30.9%)	43 (53.1%)		34 (42%)	34 (42%)		31 (38.3%)	37 (45.7%)	
Negative	13	3 (3.7%)	10 (12.3%)		7 (8.6%)	6 (7.4%)		2 (2.5%)	11 (13.6%)		4 (4.9%)	9 (11.1%)	
Cirrhosis				0.794			0.318			0.377			0.106
Present	74	28 (34.6%)	46 (56.8%)		28 (34.6%)	46 (56.8%)		34 (42%)	40 (49.4%)		34 (42%)	40 (49.4%)	
Absent	7	3 (3.7%)	4 (4.9%)		4 (4.9%)	3 (3.7%)		2 (2.5%)	5 (6.2%)		1 (1.2%)	6 (7.4%)	
Child-Pugh grade				0.165			0.154			0.114			0.043
A	78	31 (38.3%)	47 (58%)		32 (39.5%)	46 (56.8%)		36 (44.4%)	42 (51.9%)		32 (39.5%)	46 (56.8%)	
B	3	0 (0)	3 (3.7%)		0 (0)	3 (3.7%)		0 (0)	3 (3.7%)		3 (3.7%)	0 (0)	
AFP				0.063			0.228			0.147			0.002
> 20 ng/mL	52	16 (19.8%)	36 (44.4%)		18 (22.2%)	34 (42%)		20 (24.7%)	32 (39.5%)		29 (35.8%)	23 (28.4%)	
≤ 20 ng/mL	29	15 (18.5%)	14 (17.3%)		14 (17.3%)	15 (18.5%)		16 (19.8%)	13 (16%)		6 (7.4%)	23 (28.4%)	
TNM stage				< 0.001			0.005			< 0.001			< 0.001
I-II	48	27 (33.3%)	21 (25.9%)		25 (30.9%)	23 (28.4%)		33 (40.7%)	15 (18.5%)		10 (12.3%)	38 (46.9%)	
III-IV	33	4 (4.9%)	29 (35.8%)		7 (8.6%)	26 (32.1%)		3 (3.7%)	30 (37%)		25 (30.9%)	8 (9.9%)	

Abbreviations: CNTN-1, contactin-1; VIM, vimentin; E-cad, E-cadherin; N, Negative; P, Positive; HBsAg, hepatitis B s antigen; AFP, alpha-fetoprotein; TNM, tumor-node-metastasis.

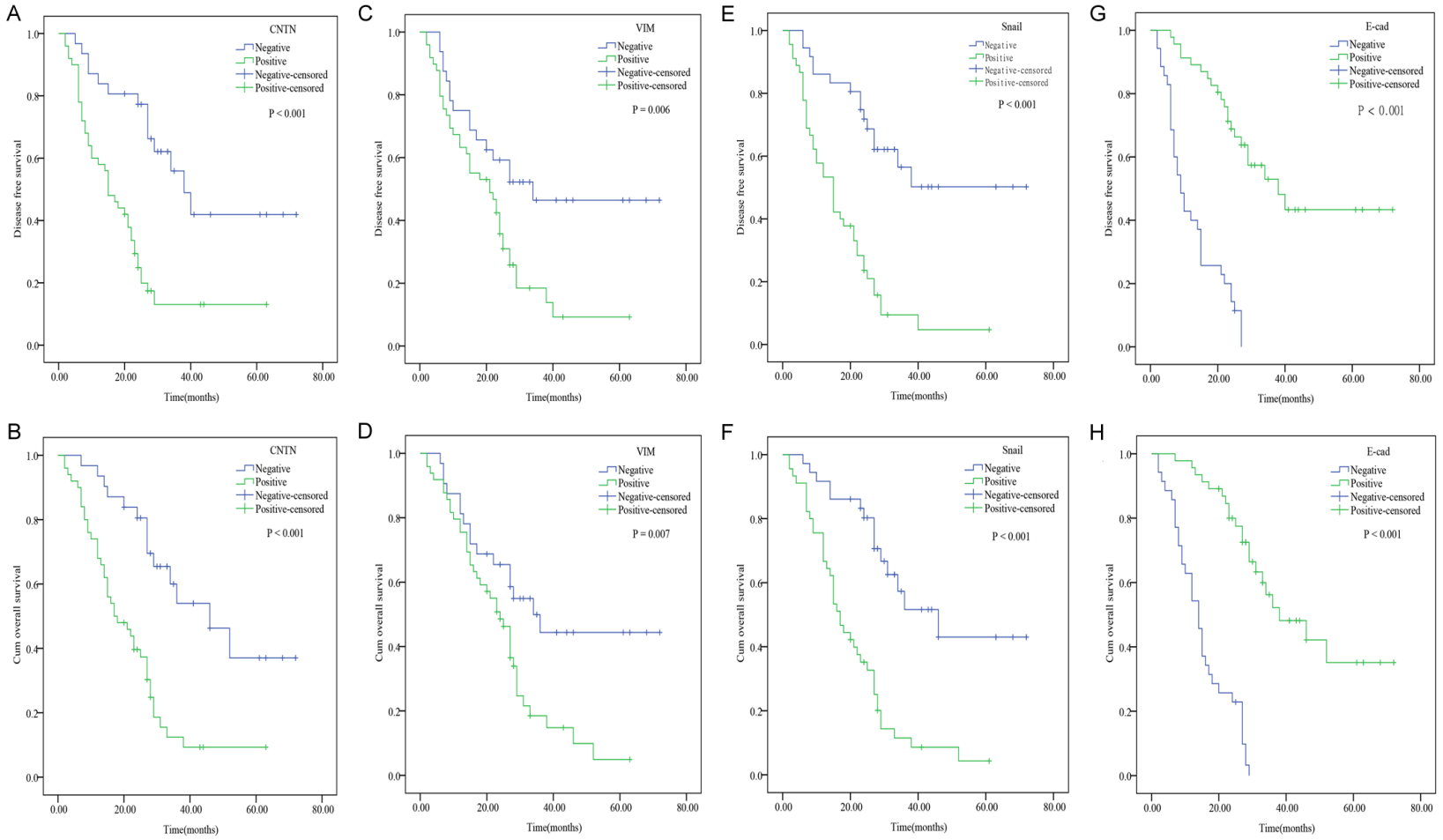

Figure 2. Kaplan-Meier analysis of DFS time and OS time of patients with HCC, based on immunostaining results of CNTN-1, VIM, Snail and E-cad. A, B. The DFS rate and OS rate of patients with positive CNTN-1 tumors were significantly lower than those of negative CNTN-1 group ($P < 0.001$). C, D. The DFS rate and OS rate of patients with VIM positive tumors were significantly lower than those of negative VIM group ($P = 0.006$ and $P = 0.007$ respectively). E, F. DFS rate and OS rate of patients with positive Snail tumors were significantly lower than those of negative Snail group ($P < 0.001$). G, H. The DFS rate and OS rate of patients with positive E-cad tumors were significantly higher than those of negative E-cad group ($P < 0.001$). Abbreviations: CNTN-1, contactin-1; VIM, vimentin; E-cad, E-cadherin; DFS, disease-free survival; OS, overall survival.

Table 4. Univariate analysis of factors associated with OS and DFS by Kaplan-Meier method and the log-rank test

Parameters	OS (month)			DFS (month)		
	Median	95% CI	*P* value	Median	95% CI	*P* value
CNTN-1						
Negative	46.182	36.896-55.468	< 0.001	44.571	34.428-54.713	< 0.001
Positive	22.136	17.355-26.918		20.584	15.111-26.057	
Snail						
Negative	46.599	37.298-55.901	< 0.001	47.052	37.506-56.598	< 0.001
Positive	20.593	16.283-24.903		17.410	13.149-21.670	
E-cad						
Negative	14.873	12.037-17.710	< 0.001	12.400	9.638-15.162	< 0.001
Positive	45.194	37.529-52.859		44.712	36.412-53.012	
VIM						
Negative	42.756	32.719-52.796	0.007	42.046	31.635-52.457	0.006
Positive	24.547	19.882-29.211		22.032	16.829-27.235	
Age						
≤ 60 years	31.310	24.550-38.069	0.725	29.914	22.719-37.113	0.755
> 60 years	30.853	22.953-38.753		29.332	20.501-38.163	
Gender						
Male	31.647	25.487-37.807	0.818	30.351	23.731-36.971	0.801
Female	30.663	20.223-41.104		29.421	17.954-40.887	
Tumor size						
≤ 5 cm	40.126	30.750-49.503	0.015	39.134	29.051-49.216	0.015
> 5 cm	25.774	19.868-31.680		23.756	17.320-30.192	
Tumor nodule						
Single	34.234	27.506-40.963	0.102	33.424	26.227-40.620	0.087
Multiple	24.558	16.763-32.353		22.397	13.582-31.212	
Vascular invasion						
Absent	40.236	32.906-47.566	< 0.001	39.893	32.180-47.605	< 0.001
Present	16.320	11.690-20.950		12.520	8.885-16.155	
Edmondson grade						
I-II	54.653	42.640-66.667	< 0.001	54.237	41.679-66.795	< 0.001
III-IV	24.922	20.405-29.440		23.236	18.129-28.343	
Tumor capsula						
Complete	38.057	30.590-45.524	0.001	37.721	29.765-45.677	0.001
None	20.879	14.853-26.904		18.049	11.970-24.128	
HbeAg						
Positive	32.248	26.158-38.338	0.906	30.879	24.334-37.424	0.75
Negative	25.068	17.101-33.035		22.769	14.794-30.745	
Cirrhosis						
Present	31.406	25.618-37.193	0.45	30.191	23.976-36.406	0.601
Absent	29.571	20.115-39.028		24.143	16.470-31.815	
Child-Pugh grade						
A	32.624	26.879-38.370	0.103	31.599	25.409-37.790	0.027
B	15.667	1.338-29.995		10.667	0.000-21.540	
AFP						
> 20 ng/mL	23.759	19.310-28.209	0.003	20.877	16.876-24.878	0.013
≤ 20 ng/mL	42.872	33.027-52.719		41.567	30.872-52.261	
TNM stage						
I-II	42.773	34.669-50.879	< 0.001	42.945	34.526-51.365	< 0.001
III-IV	18.061	13.491-22.630		14.394	10.147-18.641	

Abbreviations: CI, confidence interval; OS, overall survival; DFS, disease-free survival; CNTN-1, contactin-1; VIM, vimentin; E-cad, E-cadherin; N, Negative; P, Positive; HBsAg, hepatitis B s antigen; AFP, alpha-fetoprotein; TNM, tumor-node-metastasis.

Table 5. Multivariate Cox regression analysis of DFS and OS

Variables	OS (month)			DFS (month)		
	B	HR (95% CI)	*P*-value	B	HR (95% CI)	*P*-value
CNTN (Negative vs. Positive)	0.74	2.097 (1.063-4.137)	0.033	0.71	2.035 (1.027-4.031)	0.042
Snail (Negative vs. Positive)	0.777	2.174 (1.123-4.211)	0.021	0.729	2.073 (1.061-4.050)	0.033
E-cad (Negative vs. Positive)	-1.694	0.184 (0.093-0.364)	< 0.001	-1.42	0.242 (0.128-0.458)	< 0.001
VIM (Negative vs. Positive)	--	--	0.88	--	--	0.908
Tumor size (≤ 5 cm vs. > 5 cm)	--	--	0.825	--	--	0.627
Vascular invasion (Absent vs. Present)	--	--	0.162	0.636	1.890 (1.037-3.442)	0.038
Edmondson grade (I-II vs. III-IV)	--	--	0.193	--	--	0.26
Tumor capsula (Complete vs. None)	--	--	0.106	--	--	0.363
Child-Pugh grade (A vs. B)	--	--	--	--	--	0.888
AFP (> 20 ng/mL vs. ≤ 20 ng/mL)	--	--	0.646	--	--	0.872
TNM stage (I-II vs. III-IV)	--	--	0.843	--	--	0.328

Abbreviations: CNTN-1, contactin-1; VIM, vimentin; E-cad, E-cadherin; OS, overall survival; DFS, disease-free survival; HR, hazard ratio; CI, confidence interval.

E-cad, an important adhesion molecule in cell-cell adhesion, its loss is assured as a significant indication of EMT [13]. This research found that the down-regulated of E-cad expression in HCC was closely associated with tumor differentiation, Child-Pugh grade, AFP, tumor size, vascular invasion, TNM stage. Mima et al showed that decreased expression of E-cad is closely related to poor tumor differentiation and tumor invasion in HCC [14]. Furthermore, loss of E-cad, correlated with poor prognosis in HCC, is a prognostic factor for HCC patients [15]. Detecting missing E-cad may be helpful in predicting the ability of tumor metastasis in HCC.

As a zinc finger transcription factor, Snail was reported to play a critical role in the EMT progress [16]. Our immunohistochemistry results showed that Snail expression was significantly higher in HCC than in adjacent normal tissues and was closely related to tumor differentiation, HbeAg status, vascular invasion, tumor capsula and TNM stage. Previous reports [17, 18] suggested that the high expression of Snail correlates with poor prognosis in HCC and has a negative relationship with E-cad expression, and amassing evidence shows that Snail inhibit expression of E-cad to be involved in EMT in HCC [16, 18, 19].

VIM is a major intermediate filament protein of mesenchymal cells, which plays a vital role in tumor invasion and metastasis; and increased VIM expression contributes to tumor metastasis has also been reported in HCC [20]. VIM in fact functions as a positive regulator of EMT and upregulation of VIM appears to be a prerequisite for EMT induction [21]. Moreover, VIM identifying Axl as a key proximal component was considered as a molecular explanation for VIM-dependent cancer cell migration during EMT [22]. Our study demonstrated that VIM positive expression in HCC is associated with tumor differentiation, vascular invasion and TNM stage. These results suggest that VIM acts as a mesenchymal marker and may play an important role in tumor invasion and metastasis in HCC.

On the basis of the above results, we investigated the relationships between CNTN-1 and EMT marks (E-cad, VIM and Snail) and found that the expression of CNTN-1 was negatively associated with that of E-cad. A previous study suggested that CNTN-1 reduces E-cad expression via activating AKT by preventing PHLPP2-mediated AKT dephosphrorylation in lung cancer [23], whereas the mechanism of interaction between CNTN-1 and E-cad in HCC is yet unclear. Furthermore, CNTN-1 expression was positively correlated with the expression of Snail and VIM. To our knowledge, this is the first study that investigates the correlations between CNTN-1 and EMT marks in HCC.

The survival analysis demonstrated that the HCC patients with negative CNTN-1 expression had higher OS and DFS rates than those with positive CNTN-1 expression, and CNTN-1 expression served as an independent prognostic factor. Additionally, negative E-cad expres-

sion, positive Snail expression and positive VIM expression were associated with poor prognosis for HCC patients.

In conclusion, this study suggests that abnormal expression of CNTN-1, E-cad, VIM, and Snail predicts a poor prognosis of HCC. Furthermore, overexpression of CNTN-1 is closely associated with dysregulated expression of EMT marks in HCC, indicating CNTN-1 may play an important role in process of EMT event. Further studies are needed to support our findings.

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Disclosure of conflict of interest

None.

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