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Original Article

Correlation of CD133, E-cadherin, and β-catenin with metastasis and prognosis in esophageal squamous cell carcinoma

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Abstract: Background: CD133, a biomarker of cancer stem cells, is often detected in highly aggressive cancers, including esophageal squamous cell carcinoma (ESCC). E-cadherin, a suppressor of tumor metastasis, and β-catenin, a key factor in the Wnt signaling pathway, play important roles in ESCC. However, the evaluation of the correlation of them in the prediction of prognosis and metastasis in ESCC was unknown. In this study, we analyzed the associations among CD133, E-cadherin, and β-catenin in ESCC and their correlations with clinicopathological factors and survival in ESCC. Methods: Immunohistochemistry (IHC) was used to assess the expression of CD133, E-cadherin, and β-catenin in 110 specimens of ESCC and 50 normal esophageal tissues. Each patient's clinical data, such as gender and age, were also collected. Results: Levels of CD133 and β-catenin were significantly higher, and levels of E-cadherin were significantly lower, in ESCC tissues than in normal esophageal tissues. Moreover, the expression of CD133 was positively correlated with β-catenin but negatively correlated with E-cadherin. Univariate analysis indicated that the postoperative 5-year overall survival (OS) time of patients was related to the expression of CD133, E-cadherin and β-catenin, lymph node metastasis (LNM), depth of invasion, and tumor-node-metastasis (TNM) stage. Multivariate regression revealed that LNM was an independent prognostic factor for ESCC patients. Conclusions: Our study suggested that CD133 might regulate the expression of E-cadherin and β-catenin in ESCC. Combined detection of these factors may be of certain value for predicting metastasis and prognosis in ESCC patients.

Keywords: Esophageal squamous cell carcinoma, CD133, E-cadherin, β-catenin

Introduction

Esophageal cancer is a malignant tumor that occurs in the esophageal squamous epithelium. About 300,000 people die of this condition each year. In China, the incidence of esophageal cancer accounted for one-fourth of all malignant tumors [1]. ESCC is the most important histological type in esophageal cancer. The study of molecular biology of esophageal cancer has attracted increasing amounts of attention from researchers.

CD133, also called prominin-1, is one of the most reliable surface markers for cancer stem cells [2]. Tumor stem cell theory has become a hot topic in recent years in the study of the molecular biology surrounding the origins of tumors. Cancer stem cells (CSCs), also called tumor-initiating cells, have received a great deal of attention because they play a pivotal role in tumor invasion, metastasis, growth, and resistance to conventional therapy [3-5]. Currently known TSC markers include CD44, CD133, ABCG-2, Bim-1, Oct4, and similar substances. CD133, a pentaspan transmembrane protein, is considered to be a stem cell marker used to identify tumor-initiating cells [6]. CD133 has been found to be one of the most reliable surface markers for CSCs in several cancers, such as melanoma [7], lung [8], colon [9], breast [10], and ovarian cancer [11]. Studies of various malignancies have shown high levels of expression of CD133 in cancer tissues [12].

Table 1. Clinicopathological factors

Patient characteristics	Numbers (n)	Percentage (%)
Gender		
Male	76	69.1
Female	34	30.9
Age (years)		
<60	50	45.4
≥60	60	55.6
Position		
Upper	13	11.8
Middle	71	64.6
Lower	26	23.6
Gross		
Medullary	60	54.6
Ulceration	22	20.0
Narrow	6	5.4
Fungating	22	20.0
Diameter (cm)		
<3.8	54	49.1
≥3.8	56	50.9
LNM		
Negative	59	53.6
Positive	51	46.4
Depth of invasion		
Over serous membrane	65	59.1
Above serous membrane	45	40.9
Tumor grade		
Good	12	10.9
Moderate	55	50.0
Poor	43	39.1
TNM stage		
I and II	68	61.8
III and IV	42	38.2

Hang reported the presence of CD133-positive cancer cells in ESCC in 2012 [13].

E-cadherin has traditionally been considered a tumor suppressor, and defects in its structure and function and downregulation due to somatic or germline mutations have been linked to the ability of epithelial cancer cells to survive, migrate, invade, and undergo epithelial-mesenchymal transition (EMT) [14, 15]. EMT refers to the epithelial cells and extracellular factors in the process of interaction between the loss of polarity and intercellular connection, epithelial cell migration, and the development of processes characteristic of interstitial cells. E-cadherin deficiency is a marker of EMT. Abnormal activation of the Wnt signaling pathway can mediate the abnormal expression of the corresponding target genes and cause tumorigenesis, in which β-catenin is a key factor. β-catenin is bound to E-cadherin on the cell membrane under normal conditions. When β-catenin enters the nucleus and binds to LEF/TCF, this process can lead to carcinogenesis of the esophageal squamous epithelium.

At present, CD133, E-cadherin and β-catenin have not been reported in ESCC. We here investigated the roles of CD133, E-cadherin, and β-catenin, which may lead to the discovery of candidate genes for clinical molecular treatment of ESCC patients.

Materials and methods

Specimens

We collected 110 cases ESCC samples from January 2011 to December 2012 in the Department of Pathology of the First Affiliated Hospital, Bengbu Medical College. We randomly selected distal esophageal margin tissue samples of 50 cases (at least from the edge of cancer tissue >5 cm). None of these patients had undergone chemo- or radio-therapy before surgery. To discern complete clinic-pathological and follow-up data for these patients, the results of pathological diagnosis were determined by two high-grade clinical pathologists. This experiment was approved by the Bengbu Medical College human ethics committee. All patients were followed up until January 2017 (rang 2-110 months). Specific parameters are shown in **Table 1**.

Immunohistochemistry

Concentrated CD133 mouse anti-human monoclonal antibody was purchased from Abcam Corporation (dilution ratio 1:200), an anti-human E-cadherin, β-catenin polyclonal antibody, Elivision™ Plus Kit and DAB reagent were purchased from Fuzhou Maixin Biotechnology Development Ltd. in China.

All the experimental specimens were fixed with 4% neutral formalin. The paraffin-embedded tissue was obtained by continuous slicing of sections 4-μm thick, which were dried, dewaxed in xylene solution and gradient ethanol solu-

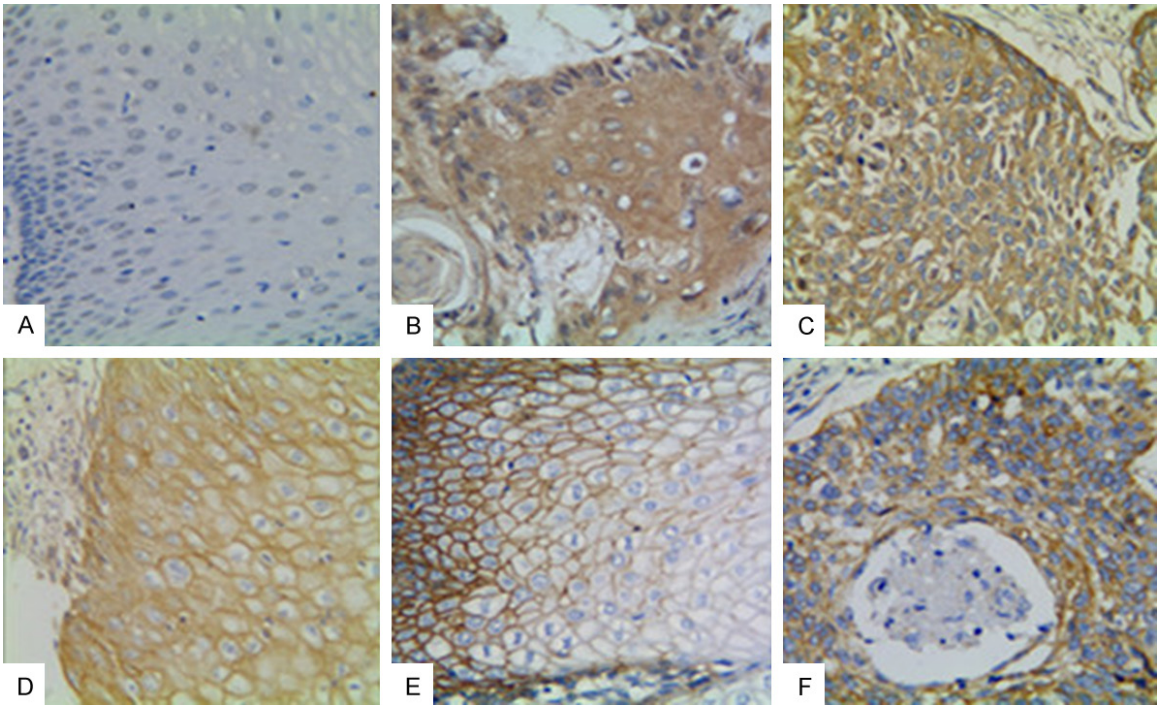

Figure 1. Expression of CD133, E-cadherin, and β-catenin proteins in ESCC or normal tissue (× 400 magnification). A: Negative staining of CD133 in the normal tissue. B: Positive staining of CD133 in ESCC. C: Negative staining of E-cadherin in ESCC. D: Positive staining of E-cadherin in the normal tissue. E: Negative staining of β-catenin in the normal tissue. F: Positive staining of β-catenin in ESCC.

tion, and then washed. Immunohistochemical analysis was then performed. These specific references to the immunohistochemical staining steps kit instructions.

Evaluation of staining

The results were expressed using the following method: color intensity and color ranges. Color intensity: colorless = 0; light yellow = 1; dark yellow = 2; brown = 3. Color range (percentage of positive cells under high-power fields, HPF): <10%, 1; 11%-50%, 2; 51%-75%, 3; >75%, 4. The color intensity and the range was multiplied as final scores: <2 was negative; >2 was positive. Each sample was randomized by 10 HPF (× 400 times), taking the median of the count.

Statistical analysis

Data analysis was performed using SPSS version 19.0 software for Windows (Chicago, IL, US). The relationship between CD133, E-cadherin, β-catenin, and clinicopathological parameters was analyzed using χ^2 and Fisher exact tests. Association between CD133, E-cadherin, and β-catenin was detected through Spearman correlation analysis. The Kaplan-Meier method with log-rank test was used for univariate analysis. Multivariate analysis, including age, gender, position, gross, tumor diameter, LNM, depth of invasion, tumor grade, TNM stage, CD133, E-cadherin and β-catenin, were assessed using by Cox's regression model. A value of $P<0.05$ was considered statistically significant.

Results

Association between CD133, E-cadherin and β-catenin expression in ESCC and clinicopathological factors

The expression of CD133 in ESCC cytoplasm was observed by IHC. The positive expression rates of CD133 were 53.6% (59/110) and 16% (8/50), respectively, in ESCC tissues and the distal esophageal margin of normal esophageal mucosa (**Figure 1A**, **1B**), with statistically significance ($P<0.05$). This study revealed that positive rate of CD133 was positively related to LNM, depth of invasion, tumor grade and TNM stage but not to age, gender, position, gross, or tumor diameter (**Table 2**).

Table 2. Associations of CD133, E-cadherin, and β-catenin expression with the clinicopathological characteristics of ESCC

Variable	CD133		χ^2	*P*	E-cadherin		χ^2	*P*	β-catenin		χ^2	*P*
	+ (%)	- (%)			+ (%)	- (%)			+ (%)	- (%)		
Gender			0.532	0.466			1.995	0.158			0.234	0.628
Male	39 (51.3)	37 (48.7)			19 (25.0)	57 (75.0)			44 (57.9)	32 (42.1)		
Female	20 (58.8)	14 (41.2)			13 (38.2)	21 (61.9)			18 (52.9)	16 (47.1)		
Age (years)			0.487	0.485			3.673	0.055			0.005	0.944
<60	25 (50.0)	25 (50.0)			10 (20.0)	40 (80.0)			28 (56.0)	22 (44.0)		
≥60	34 (56.7)	26 (43.3)			22 (36.7)	38 (70.9)			34 (56.7)	26 (43.3)		
Position			0.465	0.793			1.619	0.445				0.131
Upper	8 (61.5)	5 (38.5)			2 (15.4)	11 (84.6)			9 (69.2)	4 (30.8)	4.067	
Middle	38 (53.5)	33 (46.5)			23 (32.4)	48 (67.6)			35 (49.3)	36 (50.7)		
Lower	13 (50.0)	13 (50.0)			7 (26.9)	19 (73.1)			18 (69.2)	8 (30.8)		
Gross			2.151	0.542			4.591	0.204			5.741	0.125
Medullary	33 (55.0)	27 (45.0)			17 (28.3)	43 (71.7)			36 (60.0)	24 (40.0)		
Ulceration	9 (40.9)	13 (59.1)			5 (22.7)	17 (77.3)			8 (36.4)	14 (63.6)		
Narrow	4 (66.7)	2 (33.3)			4 (66.7)	2 (33.3)			5 (83.3)	1 (16.7)		
Fungating	13 (59.1)	9 (40.9)			6 (27.3)	16 (72.7)			13 (59.1)	9 (40.9)		
Diameter (cm)			1.285	0.257			0.089	0.766			0.305	0.581
<3.8	26 (48.1)	28 (51.9)			15 (27.8)	39 (72.2)			29 (53.7)	25 (46.3)		
≥3.8	33 (58.9)	23 (41.1)			17 (30.4)	39 (69.6)			33 (58.9)	23 (41.1)		
LNM*			39.320	0.000*			11.811	0.001*			17.162	0.000*
Negative	48 (81.4)	11 (18.6)			9 (15.3)	50 (84.7)			44 (74.6)	15 (25.4)		
Positive	11 (21.6)	40 (78.4)			23 (45.1)	28 (54.9)			18 (35.3)	33 (64.7)		
Depth of invasion			22.274	0.000*			40.524	0.000*			13.406	0.000*
Over Serous membrance	47 (72.3)	18 (27.7)			4 (6.2)	61 (93.8)			46 (70.8)	19 (29.2)		
Above serous membrance	12 (26.7)	33 (73.3)			28 (62.2)	17 (37.8)			16 (35.6)	29 (64.4)		
Tumor grades			7.710	0.021*			1.718	0.424			12.511	0.002*
Well	3 (25.0)	9 (75.0)			5 (41.7)	7 (58.3)			4 (33.3)	8 (66.7)		
Moderate	27 (49.1)	28 (50.9)			17 (30.9)	38 (69.1)			25 (45.5)	30 (54.5)		
Poor	29 (67.4)	14 (32.6)			10 (23.3)	33 (70.9)			33 (76.7)	10 (23.3)		
TNM stage			0.487	0.000*			0.114	0.735			2.932	0.087
I and II	22 (32.4)	46 (67.6)			19 (27.9)	49 (72.1)			34 (50.0)	34 (50.0)		
III and IV	37 (88.1)	5 (11.9)			13 (31.0)	29 (69.0)			28 (66.7)	14 (33.3)		

*P<0.05.

Table 3. Correlation between CD133, E-cadherin, and β-catenin expression in ESCC

Variable	CD133		*rs*	*P*	E-cadherin		*rs*	P
	+ (%)	- (%)			+ (%)	- (%)		
β-catenin								
+	49 (44.5)	13 (11.8)	0.597	0.000#	8 (7.3)	54 (49.1)	-0.433	0.000*
-	10 (9.1)	38 (34.6)			24 (21.8)	24 (21.8)		
E-cadherin								
+	12 (10.9)	20 (18.2)	-0.207	0.030*				
-	47 (42.7)	31 (28.2)						

#Positive association; *Negative association.

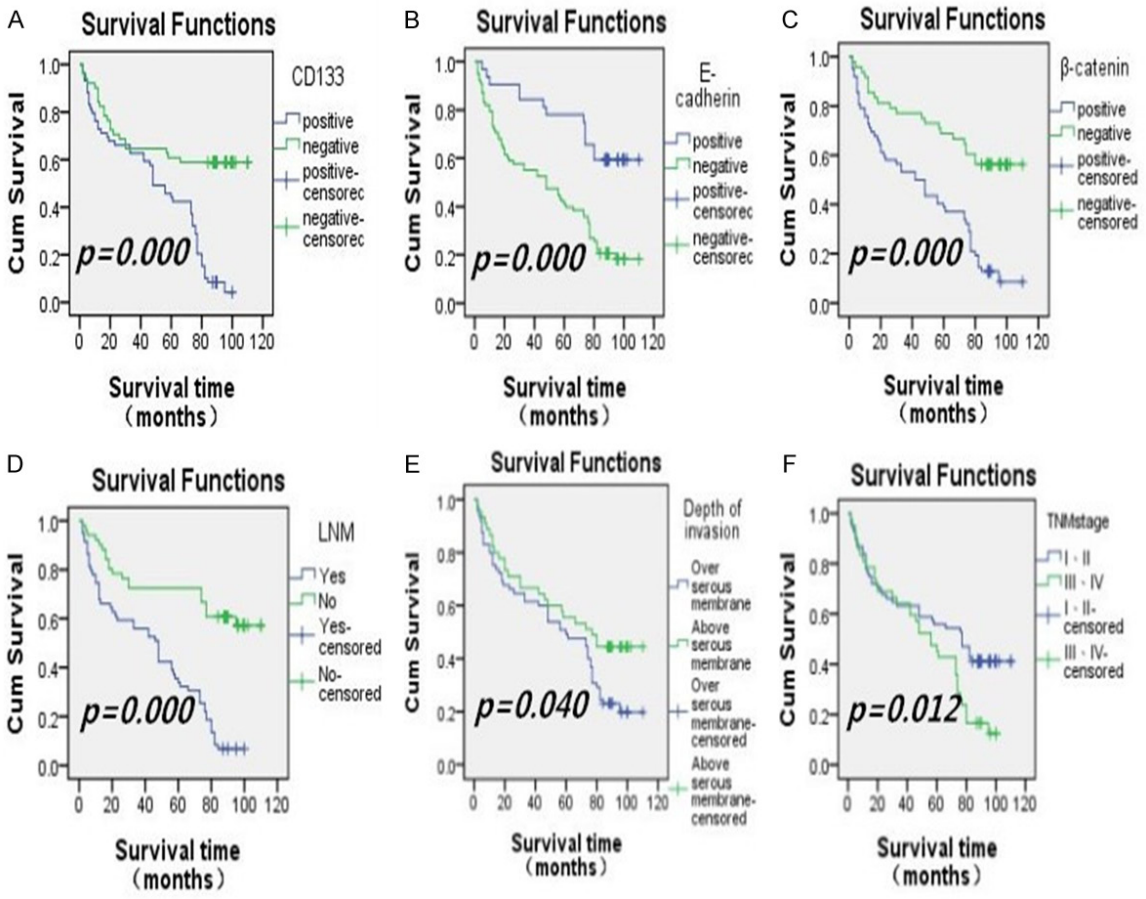

Figure 2. The survival of patients of ESCC patients by Kaplan-Meier analysis. x-axis, OS time (months); y-axis, the percentage of patients. (A) OS of the ESCC patients in relation to CD133 (log-rank = 22.449, *P* = 0.000); (B) OS of the ESCC patients in relation to E-cadherin (log-rank = 22.206, *P* = 0.000); (C) OS of the ESCC patients in relation to β-catenin (log-rank = 15.352, *P* = 0.000); (D) OS of the ESCC patients in relation to LNM (log-rank = 32.069, *P* = 0.000); (E) OS of the ESCC patients in relation to depth of invasion (log-rank = 4.199, *P* = 0.040); (F) OS of the ESCC patients in relation to TNM stage (log-rank = 6.343, *P* = 0.012); In (A-C) analyses, the green line means patients with negative CD133, or E-cadherin, or β-catenin and the blue line representing patients with positive CD133, or E-cadherin, or β-catenin group. In (D) analyses, the green line representing patients without LNM group and the blue line representing patients with LNM group. In (E) analyses, the green line representing patients above serous membrane group and the blue line representing patients over serous membrane group. In (F) analyses, the green line represents patients in the stages III and IV group, and the blue line represents patients in TNM stages I and II.

cadherin was found was absent on the cell membrane in ESCC (**Figure 1C**) but was positive in the normal esophageal mucosa epithelium (**Figure 1D**). The positive rates of E-cad-

Table 4. Results of univariate logistic regression analyses of overall survival (OS) time

Variable	n	Mean five-year OS time (months)	*Log-rank*	*P* value
Gender			0.007	0.935
Male	76	61.276±4.634		
Female	34	55.794±6.637		
Age (years)			0.351	0.554
<60	50	58.517±5.743		
≥60	60	62.267±5.339		
Position			0.269	0.874
Upper	13	55.308±9.478		
Middle	71	62.127±4.869		
Lower	26	57.962±8.369		
Gross			0.673	0.880
Medullary	60	54.532±5.139		
Ulceration	22	61.000±7.400		
Narrow	6	67.500±8.881		
Fungating	22	60.603±3.917		
Diameter (cm)			0.610	0.435
<3.8	54	61.519±4.922		
≥3.8	56	57.074±6.575		
LNM			32.069	0.000
Negative	59	43.559±4.180		
Positive	51	79.542±5.746		
Depth of invasion			4.199	0.040
Over serous membrane	65	55.782±4.850		
Above serous membrane	45	67.467±6.380		
Tumor grade			0.639	0.726
Well	12	66.583±12.103		
Moderate	55	62.018±5.508		
Poor	43	54.926±5.801		
TNM stage			6.343	0.012
I and II	68	65.221±5.303		
III and IV	42	51.911±5.115		
CD133			22.449	0.000
Negative	51	73.549±6.306		
Positive	59	48.822±4.226		
E-cadherin			15.352	0.000
Negative	78	50.581±4.430		
Positive	32	85.063±6.197		
β-catenin			22.206	0.000
Negative	48	78.667±5.777		
Positive	62	46.419±4.560		

herin protein in ESCC and normal mucosa of esophageal margin were 29.1% (32/110) and 80% (40/50), respectively, and the difference was statistically significant ($P<0.05$). The positive rate of E-cadherin over serous membrane was significantly lower than that above the serous membrane; meanwhile, it was significantly lower with LNM than without ($P<0.05$). However, the expression of E-cadherin in ESCC was not related to age, gender, tumor position, gross, tumor grade, tumor diameter, or TNM stage (**Table 2**).

β-catenin was positively localized in tumor cell nucleus and cytoplasm, but it was seen in the cell membrane of the normal tissue. The positive rate of β-catenin was 56.4% (62/110) and 30% (15/50) in ESCC and normal mucosal tissue, respectively (**Figure 1E** and **Figure 1F**). The difference between them was statistically significant ($P<0.05$). β-catenin protein showed no significant correlation with age, gender, tumor position, gross, tumor diameter, or TNM stages. The positive rate of β-catenin was inversely associated with LNM, depth of invasion or tumor grade (**Table 2**).

Correlation of CD133, E-cadherin, and β-catenin in ESCC

Spearman correlation analysis showed CD133 expression to be negatively correlated with E-cadherin expression (rs = -0.207, $P<0.05$); CD133 protein expression was positively correlated with β-catenin protein expression (rs = 0.597, $P<0.05$). The expression of E-cadherin was negatively correlated with β-catenin (rs = -0.433, $P<0.05$) (**Table 3**).

Univariate analysis for ESCC

Our experiment found that the 5-year OS time was related to CD133, E-cadherin, β-catenin, LNM, depth of invasion, and TNM stage of ESCC patients. The 5-year OS time of 59 patients (48.822±4.226 months) with CD133+ group was significantly lower than that of 51 patients (73.549±6.306 months) with CD133- group (log-rank = 22.449, $P = 0.000$; **Figure 2A**). Similarly, the 5-year OS time

Table 5. Results of multivariate logistic regression analyses of overall survival (OS) time

	B	SE	Wald	*P*	Exp (B)	95.0% CI for Exp (B)	
						Lower	Upper
Gender	0.215	0.267	0.648	0.421	1.240	0.735	2.093
Age	0.060	0.245	0.061	0.806	1.062	0.657	1.719
LNM	-0.794	0.379	4.389	0.036	0.452	0.215	0.950
Depth of invasion	0.384	0.407	0.888	0.346	1.467	0.661	3.258
Tumor grade	-0.191	0.217	0.776	0.378	0.826	0.539	1.264
Position	-0.115	0.215	0.284	0.594	0.892	0.585	1.359
Gross	-0.129	0.106	1.488	0.223	0.879	0.714	1.081
Diameter (cm)	-0.036	0.260	0.019	0.889	0.964	0.579	1.606
TNM stage	0.204	0.289	0.501	0.479	1.227	0.697	2.160
CD133	-0.292	0.507	0.330	0.566	0.747	0.276	2.020
E-cadherin	0.856	0.469	3.334	0.068	2.354	0.939	5.901
β-catenin	-0.645	0.404	2.551	0.110	0.525	0.238	1.158

of 62 patients (46.419±4.560 months) with β-catenin+ group was lower than that in the β-catenin- group (78.667±5.777 months) (log-rank = 22.206, *P* = 0.000; **Figure 2C**). In addition, the 5-year survival time with E-cadherin+ group (85.063±6.197 months) was significantly higher than in the of E-cadherin- group (50.581±4.430; log-rank = 15.352, *P* = 0.000; **Figure 2B**). The 5-year OS time in the group without LNM (79.542±5.746 months) was longer than in the LNM group (43.559±4.180 months) (**Figure 2D**). The serous membrane group showed a 5-year OS time of 67.467± 5.746 months, which was been higher than that under the serous membrane (55.782± 4.850 months) (**Figure 2E**). The 5-year OS time of the patients with low TNM stage group (65.221±5.303 months) was longer than that of the patients in the high TNM stage group (51.911±5.115 months) (**Figure 2F**). These data are shown in **Table 4**.

Multivariate analysis for ESCC

Age, gender, position, gross, tumor diameter, LNM, depth of invasion, tumor grade, TNM stage, CD133, E-cadherin, and β-catenin were introduced into the Cox's regression model for multivariate analysis. The results showed that LNM could be used as an independent prognostic factor for ESCC patients (*P*<0.05, **Table 5**).

Discussion

In this experiment, CD133 was expressed in the cytoplasm of cancer cells, and the positive expression rate of CD133 in ESCC tissues was 53.6%, which was significantly higher than in the normal tissues (16%). Although there have been few reports on the expression and mechanism of CD133 in esophageal cancer, the literature indicates that CD133 expression has been reported in many tumors. Kazama and colleagues analyzed CD133 expression in both primary tumors and lymph node metastases in stage III colorectal cancer by immunohistochemically examining tumor-initiating cells [16]. In a study of forty patients with colorectal cancer, Kashihara also concluded that CD133 expression is correlated with poor prognosis in colorectal cancer patients [17]. Huang et al. found that high levels of CD133 expression in NSCLC are correlated with certain clinical pathologic parameters, including tumor differentiation and the 5-year survival rate [5]. In this study, we demonstrated that the positive expression rate of CD133 in ESCC patients with LNM (81.3%, 48/59) was significantly higher than without LNM (21.6%, 11/51); the TNM stage of CD133 was higher, and the expression of CD133 protein was higher; the histological differentiation of cancer tissue was higher, and the expression of CD133 protein was higher; and when the invasiveness of tumor tissue was stronger, CD133 protein was more easily expressed. These findings suggest that the expression of CD133 is closely related to the development and progression of ESCC, which can lead to increases in the invasiveness and rapid metastasis of cancer cells. This is consistent with CD133 in other tumors [5, 16, 17]. This indicates that CD133 expression is closely related to the occurrence and development of ESCC, which led to the increased invasion of cancer cells. Then, after cancer cells became prone to distant metastasis, the disease worsened.

E-cadherin is a transmembrane glycoprotein that mediates calcium-dependent cell-to-cell adhesion, which is fundamentally important to the generation of a polarized epithelial phenotype [18]. This is consistent with the experimen-

tal results reported by Zha et al. [19]. E-cadherin is regarded as an invasion and metastasis suppressor [20]. Tamara M H Gall et al. reported that downregulation of E-cadherin is a hallmark of the epithelial-mesenchymal transition (EMT), an essential step in the metastatic progression of human cancer [21]. The E-cadherin/β-catenin complex plays an essential role in maintaining epithelial integrity. Aberrant expression and disruption of this complex affects the adhesive repertoire of a cell and are associated with a variety of malignancies resulting from EMT [18]. In our study, 110 patients with ESCC were analyzed by IHC: the protein of E-cadherin was not significantly correlated with age, sex, tumor location, gross, tumor grade, tumor diameter, and TNM stage; the positive rate over the serous membrane (6.2%, 4/65) was significantly lower than that above the serous membrane (62.2%, 28/45); the expression of lymph node metastasis in LNM was significantly lower than that in patients without LNM ($P<0.05$). We here hypothesized that E-cadherin plays an important role in the normal physiological function of cells in esophageal squamous epithelial cells, and the expression of E-cadherin on the cell membrane is affected by some other factors. This process induces EMT, leading to the carcinogenesis of epithelial cells, rendering the tumor cells prone to invasion and metastasis.

β-catenin serves as a transcriptional cofactor in Wnt signaling [22, 23]. Expression of β-catenin is correlated with increased invasion and metastasis in a number of human cancers [19]. β-catenin is involved in the process of cell adhesion, the maintenance of cell polarity, and the differentiation of normal epithelial tissue. β-catenin participates in the early process of malignant tumor formation by activating apoptosis-related proteins for activation of the Wnt pathway [24, 25]. β-catenin was found in 50 cases of normal esophageal squamous epithelium, mainly located in the cell membrane, but when the squamous cell carcinoma occurred in this experiment, it was concentrated largely in the cytoplasm and nucleus. Analysis of 110 patients with ESCC through SPSS 19.0 software found that the protein expression of β-catenin was closely related to the degree of differentiation and infiltration depth of ESCC ($P<0.05$). The expression of β-catenin protein (75.6%, 44/59) in patients with LNM was higher than that in patients without LNM (35.3%, 18/51). These results are consistent with those reported by Mahler-Araujo B et al. [20].

Spearman's analysis showed that CD133 protein expression and β-catenin were positively correlated, but E-cadherin, CD133, and β-catenin were negatively correlated with each other. This suggests that CD133, β-catenin, and E-cadherin may have a certain relationship in ESCC. Although the relationship between these three proteins in ESCC has not been reported in the literature, it has been discussed in other malignancies. E-cadherin is a key cell adhesion molecule that binds to its cytoplasmic adaptor β-catenin protein. This association between E-cadherin and β-catenin makes them partners in cell-cell adhesion as well as in signal transduction involving several functions, including polarity, growth and differentiation, [26]. E-cadherin or β-catenin can affect cell adhesion and contribute to tumorigenesis via EMT [27]. In early human hair follicle placodes, CD133 expression correlated with Wnt activation, as determined by translocation of β-catenin to nuclei and expression of the Wnt target LEF1 [28]. CD133 has been identified as a potential target gene of Wnt signaling [29]. Sun et al. found that the expression of CD133 was negatively correlated with WWOX and E-cadherin, and there was a positive correlation between WWOX and E-cadherin in colorectal samples, indicating a definite relationship between the three biomarkers in colorectal cancer, which will facilitate the prediction of the progression and prognosis of colorectal cancer by physicians [30]. We speculated that when the esophageal carcinoma epithelial cells carcinogenesis, the following events occur: Wnt signaling pathway activation, EMT, E-cadherin protein deletion, β-catenin translocates to the nucleus and accumulates in the cytoplasm, and overexpression of CD133, the Wnt signal pathway target gene. These events can predispose tumor cells to invasiveness, transfer and proliferation.

We found the 5-year OS rate of CD133-positive expression (6.8%, 4/59) to be significantly lower than that of CD133-negative patients (58.8%, 30/51), as indicated by Kaplan-Meier survival analysis. The results of this experiment are consistent with the experimental results of Huang M [12]. The Cox's regression model for multivariate analysis, LNM, can be used as an independent prognostic factor for ESCC. D

Hang did not find evidence to support CD133 as a predictor of ESCC patients' survival in relatively early or late stages after dividing ESCC patients into two groups according to TNM stage [13]. This is consistent with our experimental results. However, Huang M et al. proved that the life span of patients with negative CD133 expression was longer than that of patients with positive expression by Kaplan-Meier analysis [12]. CD133, β-catenin and E-cadherin can be used to assess the prognosis of patients to a certain extent by assessing their levels of expression in ESCC, although the three genes cannot be used as independent prognostic factors.

Conclusions

We have demonstrated that CD133 and β-catenin are associated with low levels of E-cadherin by measuring levels of these three proteins in ESCC patients. Although these factors cannot be used as independent prognostic factors for patients, the high expression of CD133 and β-catenin and the low expression of E-cadherin decreased the patient's 5-year OS. This indicates that the combined detection of these three factors has a certain value for guiding the clinical evaluation of patients with poor prognosis. Our experiment can provide some candidates for clinical molecular therapy, such as CD133, β-catenin, E-cadherin, or all three together. We will complete the follow-up experiments through protein and gene quantification in order to confirm the results of this experiment and make it more illustrative.

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Disclosure of conflict of interest

None.

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