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• ESOPHAGEAL CANCER •

Prognostic and clinicopathological features of E-cadherin, α-catenin, β-catenin, γ-catenin and cyclin D_1 expression in human esophageal squamous cell carcinoma

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Abstract

AIM: To investigate the expression of E-cadherin, α-catenin, β-catenin, γ-catenin and cyclin D_1 in patients with esophageal squamous cell carcinoma (ESCC), and analyze their interrelationship with clinicopathological variables and their effects on prognosis.

METHODS: Expression of E-cadherin, α-catenin, β-catenin, γ-catenin and cyclin D_1 was determined by EnVision or SABC immunohistochemical technique in patients with ESCC consecutively, their correlation with clinical characteristics was evaluated and analyzed by univariate analysis.

RESULTS: The reduced expression rate of E-cadherin, α-catenin, β-catenin and γ-catenin was 88.7%, 69.4%, 35.5% and 53.2%, respectively. Cyclin D1 positive expression rate was 56.5%. Expression of γ-catenin was inversely correlated with the degree of tumor differentiation and lymph node metastasis (χ^2 = 4.183 and χ^2 = 5.035, respectively, P<0.05), whereas the expression of E-cadherin was correlated only with the degree of differentiation (χ^2 = 5.769, P<0.05). Reduced expression of E-cadherin and γ-catenin was associated with poor differentiation of tumor, reduced expression of γ-catenin was also associated with lymph node metastasis. There obviously existed an inverse correlation between level of E-cadherin and γ-catenin protein and survival. The 3-year survival rates were 100% and 56% in E-cadherin preserved expression group and in reduced expression one and were 78% and 48% in γ-catenin preserved expression group and in reduced expression one, respectively. The differences were both statistically significant. Correlation analysis showed the expression level of α-catenin correlated with that of E-cadherin and β-catenin (P<0.05).

CONCLUSION: The reduced expression of E-cadherin and γ-catenin, but not α-catenin, β-catenin and cyclin D1, implies more aggressive malignant behaviors of esophageal carcinoma cells and predicts the poor prognosis of patients.

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INTRODUCTION

Esophageal squamous cell carcinoma (ESCC) is one of the most common malignant tumors in China[1]. In recent years, the postoperative survival of patients with esophageal carcinoma has been improved. However, the overall prognosis for esophageal cancer patients remains poor, the 5-year survival rate of post operative advanced esophageal carcinoma patients was 20-35%. Although surgical techniques and preoperative management have progressed, early diagnosis and treatment are still important[2-5]. The prognostic clinical characterization of esophageal carcinoma remains inadequate using conventional histological grading and staging systems. Recently, various attempts have been made to investigate the relationship between certain molecular markers and the clinical course of squamous cell carcinoma of esophagus. In fact, the biological factors that determine a different individual outcome (recurrent, survival) at an analogous stage of disease are obscure[3-6].

E-cadherin and catenin are important adhesion molecules in normal epithelial tissue. Catenins, including α-catenin, β-catenin, γ-catenin, play an important role in the E-cadherin mediated intercellular signal transduction and cell adhesion. Loss of normal cellular adhesion plays a critical role in many aspects of tumor biology. For instance, alterations in cell-cell adhesion in cancer cells are reflected at the microscopic level in degree of cohesiveness and pattern of tumor growth. Detachment of cancer cells is an initial step in invasion of surrounding tissues and in spread to distant organs, and altered tumor cell adhesion is important in these processes. Several studies examined the role of the E-cadherin/catenin complex in growth mediation and maintenance of cell-cell adhesion in various tumors[7-19]. The expression of adhesion molecules may reflect biological behaviors and characteristics of tumors and are conducive to predict and evaluate the risk of relapse and metastasis in patients with postoperative esophageal carcinoma, thus having practical significance in guiding individualized treatment[3,18,20-23].

Cyclin D1 encodes a cell-regulatory protein that is expressed at high level during the G1 phase of the cell cycle. Cyclin D1 binds to cyclin-dependent kinases and proliferating cell nuclear antigens. The formation of these complexes has been implicated in the control of cell proliferation[24]. Cyclin D1 is the target gene of beta-catenin, overexpression of the latter in the cytoplasm may promote malignant transformation by triggering cyclin D1 expression in a number of cancers. It was regarded by several reports that cyclin D1 could predict the prognosis in some cancers, including esophageal cancer[25-28].

In this study, the expression of E-cadherin, α-catenin, β-catenin, γ-catenin and cyclin D1 in 62 ESCC patients was

analyzed, concerning the histopathological and survival data, effects on progression of cancer and their prognostic value in ESCC. The results may provide some suggestions for clinical treatments.

MATERIALS AND METHODS

Materials

Specimens of cancer tissues were taken from 62 consecutive patients with squamous cell carcinoma of the thoracic esophagus who had undergone esophagectomy with regional lymph node dissected from January to December of 1996 at the Department of Thoracic Surgery, Cancer Hospital of Shantou University Medical College. None of them received irradiation or chemotherapy preoperatively. The patients included 49 men and 13 women with a mean age of 54 (range 35-79) years. Three tumors were located in the upper thorax, 36 in the middle thorax and 23 in the lower thorax (Table 1). The removed specimens were examined histological with hematoxylin and eosin staining, and then the clinicopathologic stage was determined according to TNM classification. Survival time was calculated from the date of operation to death or the date of last follow-up. Follow-up time ranged from 6 to 54 mo with an average of 36 mo.

Table 1 Background data of patients

Term	No. of cases (%)
Total	62
Age (yr)	
<50	22 (35.5)
⩾50	40 (64.5)
Sex	
Male	49 (79.1)
Female	13 (20.9)
Location	
Upper thoracic	3 (4.8)
Middle thoracic	36 (56.5)
Lower thoracic	23 (37.1)
Histological grade	
I	16 (25.8)
II	35 (56.5)
III	11 (17.7)
Depth of invasion	
T1	2 (3.2)
T2	10 (16.1)
T3	32 (51.6)
T4	18 (29)
Lymph node metastasis	
Positive	35 (56.5)
Negative	27 (43.5)

Immunohistochemical staining

Immunohistochemical analysis was done retrospectively. Resected esophageal specimens, including both tumor and normal mucosae, were fixed in a 40 g/L formaldehyde solution and embedded in paraffin. The following antibodies were used in this study: mouse monoclonal anti-human cyclin D1 antibody (M-0024C, Antibody Company USA, diluted 1:50 in PBS), rabbit polyclonal anti-human E-cadherin antibody (BA0475, Antibody Company USA, diluted 1:100 in PBS), rabbit polyclonal anti-human α-catenin antibody (C-2081, Sigma Bioscience Company, USA, diluted 1:1 000 in PBS), rabbit polyclonal anti-human β-catenin antibody (C-2206, Sigma Chemical Company, USA, diluted 1:2 000 in PBS), goat polyclonal anti-human γ-catenin antibody (C-20 Santa Cruz Biot Co, USA, diluted 1:200 in PBS). Four μm thick sections of formalin-fixed paraffin-embedded tissue blocks of esophageal tumors were cut. The sections were deparaffinized, dehydrated and blocked to remove endogenous peroxidase activated by 3 mL/L H_2O_2 in methanol for 30 min. The sections were treated with microwave in 0.l mol/L citrate buffer pH 6.0 at 750 W for 12 min. After incubation with 100 mL/L normal goat serum to block non-specific binding, they were then incubated with the primary antibodies overnight at 4 ℃. After antibody was washed with PBS, the sections were incubated with the secondary antibody and immunostained by SABC method (γ-catenin, Boster Company, China) and EnVision method (E-cadherin, α-catenin, β-catenin and cyclin D1; EnVision, Cat. No. D-3001, 3002, Antibody Diagnostic Inc) according to the manufacturer's instructions, and finally DAB was visualized. Tissues were counterstained with hematoxylin. Negative control was designed by using PBS instead of primary antibody. Adjacent normal squamous epithelium served as an internal positive control of E-cadherin and catenin protein expression. Known immunostained-positive sections were used as positive control of cyclinD1 protein expression.

Positive criterion of immunohistochemical staining

Tumor sections were scored by light microscopy by 2 independent observers without knowledge of the stage and patient profiles. The percentage of positively stained cells was calculated after 100 cells were counted at more than 5 high-power (40×) fields. The following definitions were made: Cyclin D1: more than 10% positive staining in nuclei was defined as positive staining; E-cadherin and catenin: more than 10% positive staining in cell membrane was defined as positive staining; less than 50% positive staining in cell membrane was defined as reduced expression, more than 50% positive staining in cell membrane was defined as preserved expression.

Statistical analysis

χ^2 test or Fisher's exact probability test and Spearman rank correlation coefficient analysis were used to assess the association between immunohistochemical features and clinicopathological characteristics. The cumulative survival rate was calculated by the Kaplan-Meier method, and statistical significance was analyzed by the log-rank test. A P value less than 0.05 was considered statistically significant. All the statistical analyses were performed using the SPSS 10.0 V for Windows.

RESULTS

Expression of E-cadherin, α-catenin, β-catenin, γ-catenin and cyclin D1 in esophageal squamous cell carcinoma

The positive expression rate of E-cadherin, α-catenin, β-catenin, γ-catenin and cyclin D1 in 62 esophageal cancer patients was 62.9% (39/62), 79% (49/62), 95.2% (59/62), 75.8% (47/62) and 56.5% (35/62), respectively. The reduced expression rate of E-cadherin, α-catenin, β-catenin and γ-catenin was 88.7%, 69.4%, 35.5%, 53.2%, respectively. Cyclin D1 positive expression showed brown stained signals in the nuclei (Figure 1), only a small number of expressions in cytoplasm or membrane of cells. E-cadherin, α-catenin, β-catenin and γ-catenin positive expression showed brown stained signals in membrane of cells and the intercellular junctions (Figure 2A-C).

Relationship between expressions of E-cadherin, α-catenin, β-catenin , γ-catenin and cyclin D1 in esophageal squamous cell carcinoma

Significant positive correlation was found between the intensity of α-catenin and β-catenin (r = 0.274, P<0.05), E-cadherin and α-catenin (r = 0.279, P<0.05). No significant differences were seen in other protein expressions.

Table 2 The relationship between clinicopathology and the expression of cyclin D_1 E-cad and catenins

Type	Cases	CyclinD1		P	E-cad		P	α-cat		P	β-cat		P	γ-cat		P
		Positive	Negative		Preserved	Reduced		Preserved	Reduced		Preserved	Reduced		Preserved	Reduced	
Histological grade																
I	16	6	10		5	11		7	9		10	6		8	8	
II	35	15	20	>0.05	5	30	<0.05	11	24	>0.05	24	11	>0.05	19	16	<0.05
III	11	6	5		0	11		1	10		6	5		3	8	
Depth of invasion																
T_3	12	6	6	>0.05	4	8	>0.05	4	8	>0.05	6	6	>0.05	5	7	>0.05
T_4	50	31	19		6	44		15	35		34	16		25	25	
Lymph node metastases																
Positive	35	14	21	>0.05	6	29	>0.05	9	26	>0.05	23	12	>0.05	12	23	<0.05
Negative	27	13	14		4	23		10	17		17	10		18	9	

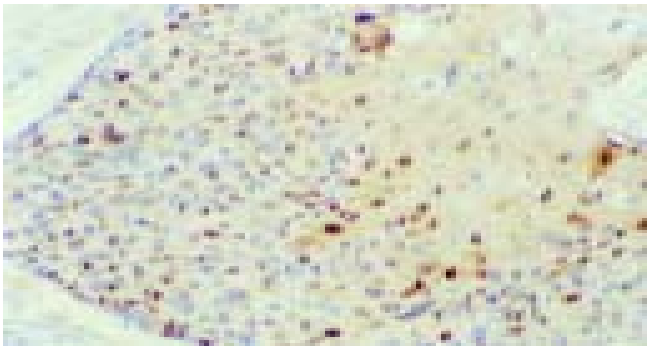

Figure 1 Positive expression of cyclinD1 protein in nuclei of esophageal squamous cell carcinoma. IHC×200.

Relationship between E-cadherin, α-catenin, β-catenin, γ-catenin and cyclin D1 expression and clinicopathologic variables in esophageal squamous cell carcinoma

Expression of E-cadherin correlated significantly only with histological grade. Poor differentiation was associated with reduced or loss of E-cadherin expression (P<0.05). Significant inverse correlation existed between the intensity of γ-catenin expression and histological grade, and lymph node metastasis (P<0.05). No significant correlation was found between abnormal expression of other proteins and histological grade, lymph node metastasis and depth of invasion (Table 2).

Relationship between E-cadherin, α-catenin, β-catenin, γ-catenin, and cyclin D1 expression and survival

Analysis of the 3-year survival after operation showed that the overall survival rate was 62% in 62 cases of esophageal cancer. Univariate analysis showed that the survival time was associated with the histological grade, depth of invasion, lymph node metastasis, expression of E-adherine and γ-catenin. Reduced E-cadherin or γ-catenin expression was correlated with poor prognosis. The mean survival time of grades I, II, III was 41, 45 and 12 mo (P<0.05), respectively. The 3-year survival rate was 67.1% and 49.4% in T3 and T4 patients (P<0.05), respectively, and was 47.8% and 80.3% in patients with positive and negative lymph node metastases (P<0.05), respectively. The median survival time was 54 mo and 37 mo in patients with preserved and reduced or loss of E-cadherin expression, the 3-year survival rate was 100% and 56% (P<0.05), respectively. The median survival time was 42 and 33 mo in patients with preserved and reduced or lost expression of γ-catenin, the 3-year survival rate was 78% and 48% (P<0.05), respectively. No difference in survival curves was seen between reduced expression of α- and β-catenins compared with preserved expression. Similar results were found in the positive and negative expressions of cyclin D1. The median survival time was 39 and 38 mo in the patients with preserved and reduced or lost expression of α-catenin, the 3-year survival rate was 65%. The median survival time was 36 and 39 mo in the patients with preserved and reduced or loss expression of β-catenin, the 3-year survival rate was 65%. The median survival time was 40 and 34 mo in the patients with positive and negative expressions of cyclin D1, the 3-year survival rate was 68% and 58% (P>0.05), respectively.

DISCUSSION

The main causes of treatment failure are recurrence and metastasis in resectable esophageal cancer. Modern molecular biology studies have demonstrated that invasion and metastasis of tumors as a continuous process, include three steps: a reduced cell-cell adhesion, alterations in the interaction of tumor cells with extracellular matrix, and invasion into surrounding

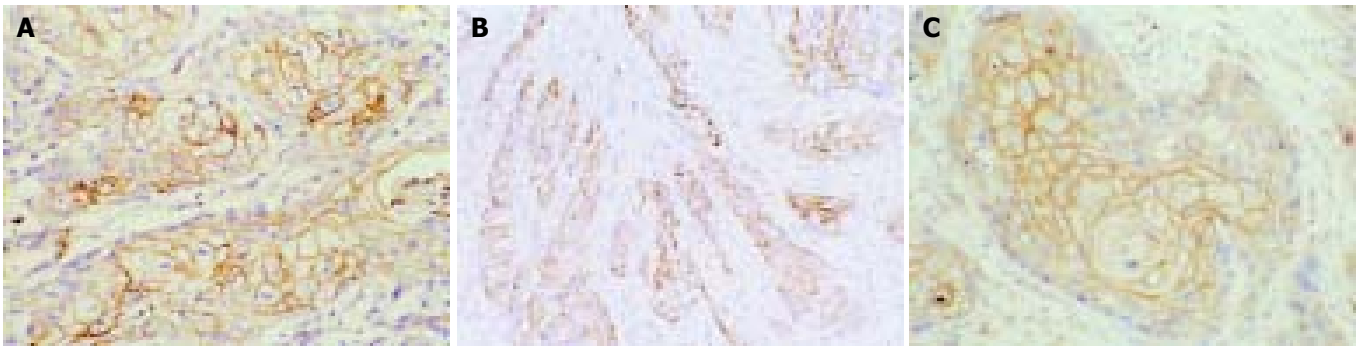

Figure 2 Positive expression of E-cadherin and γ-catenin proteins in membrane of esophageal squamous cell carcinoma. A: Positive expression of E-cadherin protein in membrane of esophageal well differentiated squamous cell carcinoma. IHC ×200; B: Positive expression of γ-catenin protein in membrane of esophageal squamous cell carcinoma. IHC×200; C: Positive expression of γ-catenin protein in membrane of esophageal squamous cell carcinoma. IHC×400.

tissues including blood vessels and lymph duct. Thus the first and critical step is that the tumor cells could detach from primary foci and re-adhere to metastatic position[12,29,30]. E-cadherin is a calcium-dependent cell-cell adhesion transmembrane glycoprotein, maintaining normal epithelial polarity, and intercellular adhesion, which are present in almost all normal epithelial cell surfaces. It is anchored to the cytoskeleton via cytoplasm proteins, including alpha and beta catenin[13,14,31]. E-cadherin, therefore, is one of the most important adhesion molecules expressed by epithelial cells and is regarded as an invasion suppressor molecule[13,14]. In this study, overall survival was inversely corrected with E-cadherin expression. Patients with preserved E-cadherin expressing tumor had a better prognosis than those with reduced expression of E-cadherin. This was in agreement with previous studies on a variety of cancers, such as cancer of head and neck[15,16], breast[17,18], stomach[19,32,33], bladder[27]. In all these studies, reduction or loss of E-cadherin expression was significantly associated with dedifferentiation, increased invasiveness, and high incidence of lymph node metastasis, hematogenous recurrence and poor prognosis in a number of human carcinomas, including esophageal cancer. But some other studies did not acquire the same results[3]. There were different results of reduced E-cadherin protein expression in specimens from patients with ESCC in various researches[21,23,32-35]. While in our investigation 88.7% of ESCC showed reduced expression of E-cadherin. It was postulated that selection of the patients entering into the study, immunohistochemical method, antibody origination, tumor heterogeneity and differences in staining evaluation might individually or in combination hold responsibility. As a marker associated with squamous cell differentiation[36], the level of E-cadherin expression had an inverse correlation with histological grade. Reduced or loss of E-cadherin expression was correlated with poor differentiation, but not with lymphatic metastases and depth of tumor invasion, suggesting that the reduction of E-cadherin expression is associated with loss of the ability of adhesion and facilitate to blood vessel metastases, as previously reported[21].

Catenins are a family of proteins including α-(102 ku), β-(88 ku), γ-(82 ku) catenins. The cytoplasmic domain of E-cadherin could bind directly to either β-catenin or γ-catenin, whereas α-catenin could link E-cadherin- (β, γ)-catenin complex to acting cytoskeleton. The integrity of the adhesion function of E-cadherin also depended on an intact catenin system[15]. β-catenin could also play a role in intracellular signaling and function as an oncogene when it bound to T-cell factor 4 (Tcf4)-binding site in the promotor region of cyclin D1 and transactivated genes after translocation to the nuclei[37-39]. Catenins had different clinicopathological roles in various cancers. In many epithelial carcinomas including carcinoma of the esophagus[3,6,20,22,23,33], head and neck[16], breast[17-19,25], stomach[19], colon[24] and bladder[27], catenins had a prognostic significance in survival. Some investigators reported that abnormal expression of α-catenin was associated with the prognosis of esophageal cancer. It also had predicative values for lymph node metastasis in esophageal carcinoma. Several reports suggested that abnormal expression of β-catenin could indicate poor prognosis in a number of tumors, including esophageal cancer[17,20,25,33]. γ-catenin was found to be more important in nodal metastasis in tongue cancer. It was also predictive of the presence of subclinical nodal metastasis in clinically node-negative neck[16]. The expression of α-catenin but not β- or γ-catenin was found to be correlated with the expression of E-cadherin in this study. The reduction or loss of γ-catenin expression was associated with more lymph node metastases than the preserved expression (P<0.05). There was a correlation between poor differentiation of tumor and reduction or loss of γ-catenin expression. The reduction or loss of γ-catenin expression was in association with shorter median survival time and lower 3-year survival rate. All these suggested that γ-catenin might be one of the prognostic factors in esophageal cancer. Howeres, the expression of α-catenin and β-catenin was not related to the histological grade, depth of invasion, lymph node metastases and survival time.

The clinical significance of cyclin D1 expression was different in various tumors. It has shown that cyclin D1 gene amplification or enhanced expression was correlated with higher histological grade of tumor, lymphatic or hematogenous metastasis and poor prognosis[40-43]. A controversial report, however, existed[6]. Some investigators thought cyclin D1 was the target gene of β-catenin. Although a positive correlation between β-catenin activation and cyclin D1 expression was reported, our study did not show such a result. Furthermore, cyclin D1 expression was not associated with the extent of tumor infiltration, grade of differentiation, lymphatic metastases and survival time. These inconsistencies with other authors may be associated with location of tumor, pathologic classification, biologic behaviors, examination methods and evaluating criteria.

Our study showed that the main prognostic factors of postoperative survival time were histological grade, depth of tumor invasion and lymph node metastasis. The reduced expression of E-cadherin or γ-catenin was associated with poor differentiation of tumor cells. Reduced or loss of γ-catenin expression also had predictive values for nodal metastasis. The reduction or loss of E-cadherin and γ-catenin expression could predict the shorter survival time. Therefore we suggest that adjuvant radiation or chemotherapy should be considered in esophageal carcinoma patients with reduced expression of E-cadherin and γ-catenin in T4 stage, poor-differentiation in histopathology, and lymph node metastases in order to improve the survival rate.

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