·Clinical Research·

Expression and clinical significance of Ezrin and E-cadherin in esophageal squamous cell carcinoma

Jian-Wen Zhai, Xiao-Gang Yang, Fu-Shen Yang, Ji-Gang Hu, Wen-Xia Hua

Department of Cardiothoracic Surgery, the Affiliated Hospital of Hebei University of Engineering, Handan, Hebei 056002, P.R. China

[Abstract] Background and Objective: It has been proven that Ezrin protein may interact with E-cadherin protein and take part in metastasis of tumor cells. This study was to investigate the expressions of Ezrin and E-cadherin in esophageal squamous cell carcinoma (ESCC) and their relationship with the clinicopathologic factors, and analyze their diagnostic values for ESCC. **Methods:** The expression of Ezrin and E-cadherin in 72 specimen of ESCC and the paracancer normal squamous epithelium was detected using tissue array with SP immunohistochemistry. Their correlations to the clinicopathologic factors were analyzed statistically. **Results:** The positive rate of Ezrin was significantly higher in ESCC than in para-cancer normal squamous epithelium (90.7% vs. 46.0%, $P < 0.001$); the positive rate of E-cadherin was significantly lower in ESCC than in para-cancer normal squamous epithelium (27.6% vs. 97.4%, $P < 0.001$). Ezrin expression was related to the invasiveness and lymph node metastasis of ESCC ($P < 0.05$); E-cadherin expression was related to the differentiation and lymph node metastasis of ESCC ($P < 0.05$). The high expression of Ezrin was related to the low expression of E-cadherin ($P < 0.05$). **Conclusion:** The activation of Ezrin and the absence of E-cadherin contribute to the tumorigenesis and metastasis of ESCC.

Key words: Esophageal neoplasm, Ezrin, E-cadherin, tissue array, immunohistochemistry

Esophageal squamous cell carcinoma (ESCC) is a common gastrointestinal cancer with poor diagnosis due to metastasis. It has been found that Ezrin interacts with E-cadherin and plays a role in tumor cell metastasis. The present study detected the expressions of Ezrin and E-cadherin in ESCC using tissue array with immunohistochemistry, and analyzed their relations with malignancy degree and metastasis of ESCC, so as to provide new evidence for the diagnosis and prognosis prediction of ESCC.

Materials and Methods

Specimens

A tissue array consisting of 76 specimens of ESCC was prepared. The 76 patients, including 49 men and 27 women, underwent operation between July 2004 and July 2007 in Department of Thoracic Surgery of the Affiliated Hospital of Hebei University of Engineering. The median age was 62.5 years. None received radiotherapy or chemotherapy before operation. The diagnosis of ESCC was confirmed in all patients by postoperative pathologic examination. Of the 76 cases, 26 were well differentiated, 33 were moderately differentiated and 17 were poorly differentiated; 12 were at stage T1, 21 at stage T2, 29 at stage T3 and 14 at stage T4; 41 had lymphatic metastasis while 35 did not.

Methods

Preparation of tissue array Pathologic sections were prepared and HE staining was performed. One specimen of cancer tissue and one specimen of para-cancer normal tissue (3–5 cm from the cancer tissue) were obtained from each case to prepare 10 mm × 8 mm arrays with a thickness of 3–4 μm using an arrayer (Beecher, USA). The tissue array with HE staining was examined by pathologists.

SP immunohistochemistry Mouse anti-human Ezrin monoclonal antibody was produced by Neomarker Co., mouse anti-human E-cadherin monoclonal antibody by Santa Cruz Co., immunohistochemistry kit and diaminobenzidine (DAB) were purchased from Zymed Co. (USA). Immunohistochemical staining was performed according to the instructions of the kit. The sections were placed in an oven at 70℃ overnight, dewaxed and hydrated, then put into 3% H_2O_2 and incubated for 15 min to deactivate endogenous peroxidase, washed with PBS (pH 7.4) and put into citrate buffer (pH 6.0). Microwave or high pressure method was used to retrieve antigen for 15 min. The sections

Correspondence to: Jian-Wen Zhai; Tel: +86-310-3130889;
Email: zhaijw001@126.com

This paper was translated from Chinese into English by *CJC* Medical Translation and edited by Wei Liu on 2009-11-28.

The Chinese version of this paper is avaiable at http://www.cjcsysu.cn/cn/article.asp?id=16515.

Received: 2009-09-08; Accepted: 2009-11-16

Grant: Foundation of Hebei Province Science and Technology Office (No. 0720611027D)

were cooled at room temperature and incubated in closed normal goat serum for 15 min, then added with primary antibody and incubated at room temperature for 2 h or at 4℃ overnight, added with secondary antibody and incubated at room temperature for 15 min. After that, the sections were stained with DAB, washed under tap water and stained with hematoxylin. Positive and negative controls were set in the staining process; PBS was used instead of primary antibody in the positive control.

Evaluation criteria for immunohistochemical staining

The staining of Ezrin was positive when brown granules distributed diffusely in cytoplasm. The grading standards based on the staining intensity and number of positive cells as adopted by Mathew *et al*.[1] were as follows: (-), no expression; (+), less than 50% of the cells were positive or had weak staining; (++), no less than 50% of the cells were positive and had deep staining, which was also defined as overexpression.

The staining of E-cadherin was positive when yellow or brown granules appeared on cell membrane, negative when they appeared in cytoplasm but not on cell membrane. The grading standards based on the proportion of positive cells as adopted by Gonzalez *et al*.[2] were as follows: (-), no staining; (+), less than 75% of the cells were positive; (++), no less than 75% of the cells were positive. (++) was defined as normal expression; (-) and (+) were defined as weak or no expression, called abnormal expression.

Statistical analysis

SPSS13.0 statistical software was applied to analyze the relations of Ezrin and E-cadherin expression with clinicopathologic factors, such as patients' age, sex, tumor differentiation, invasiveness and lymph node metastasis, using Chi-square test and Wilcoxon test. $P < 0.05$ was considered significant.

Results

The expressions of Ezrin and E-cadherin in esophageal tissues

Ezrin was expressed in cancer cells, para-cancer normal squamous epithelial cells, macrophages and lymphocytes in interstitial tissues. In para-cancer normal squamous epithelial tissues, it was intensely expressed in prickle cells and corneocytes, weak or absent in basal cells (Figure 1A); in cancer

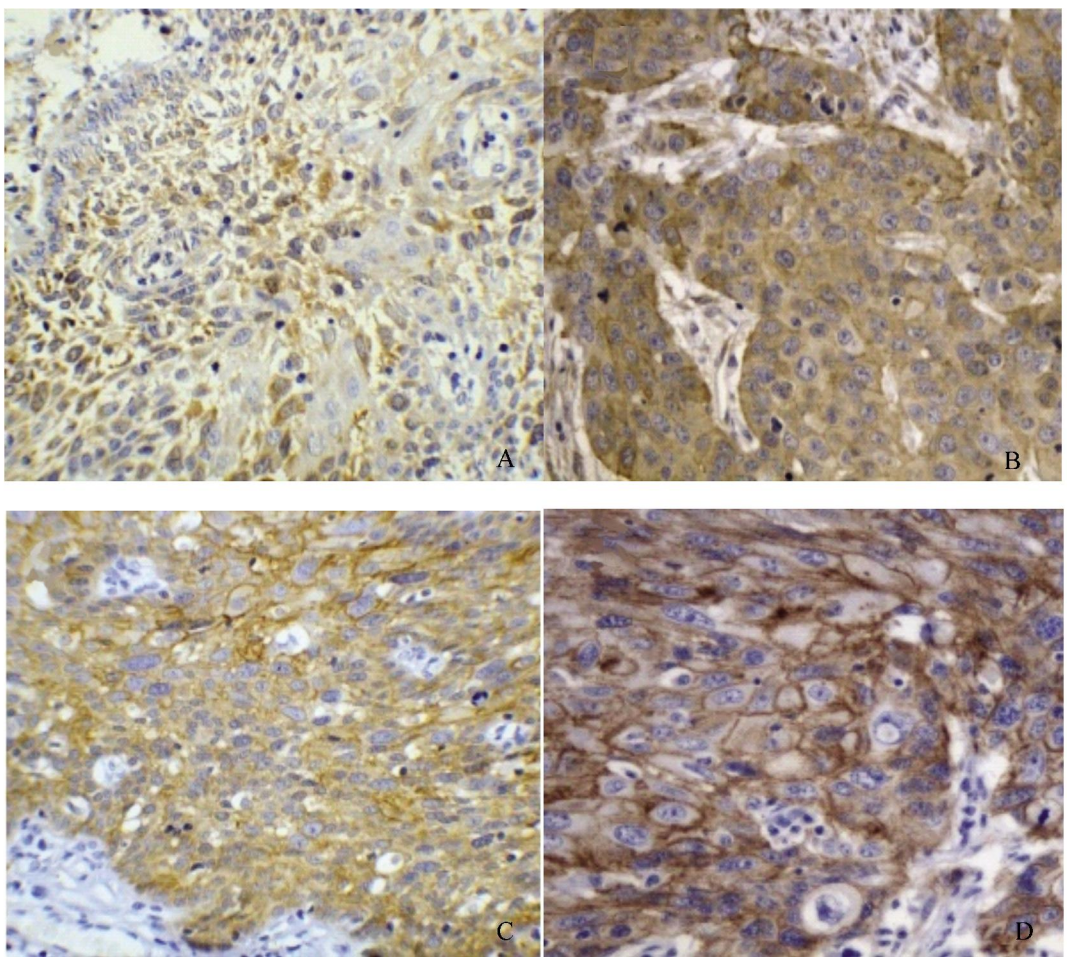

Figure 1 Expression of Ezrin and E-cadherin in esophageal squamous cell carcinoma and para-cancer normal tissues (SP ×400)

A, Ezrin is intensely expressed (in brown) in stratum spinosum and stratum corneum of para-cancer normal tissues; B, Ezrin is expressed (in brown) in cytoplasm of cancer cells; C, E-cadherin is intensely expressed (in brown) in stratum spinosum and horny layer of para-cancer normal tissues; D, E-cadherin is expressed (in brown) on membrane of cancer cells.

tissues, it was mainly expressed in the cytoplasm adjacent to cell membrane and rarely seen on cell membrane (Figure 1B).

E-cadherin was mainly expressed on cell membrane as brown granules. In para-cancer normal squamous epithelial tissues, it was intensely expressed in basal cells and prickle cells (Figure 1C); in cancer tissues, it was mainly expressed on cell membrane (Figure 1D). The positive rates of Ezrin and E-cadherin were significantly different between cancer tissues and para-cancer normal epithelial tissues (Table 1).

Table 1 Expression of Ezrin and E-cadherin in esophageal squamous cell carcinoma and para-cancer normal tissues

Group	Ezrin expression [cases (%)]		Statistic	E-cadherin expression [cases (%)]		Statistic
	Positive	Negative		Positive	Negative	
Para-cancer normal tissues	35(46.1)	41(53.9)	x^2 = 22.519	74(97.4)	2(2.6)	x^2 = 51.178
Cancer tissues	63(82.9)	13(17.1)	P < 0.001	34(44.7)	42(55.3)	P < 0.001

Relations between clinical parameters and the expressions of Ezrin and E-cadherin in ESCC

Ezrin expression was related with tumor invasiveness and lymph node metastasis, but had no relation to patients' age, sex, tumor size and differentiation; E-cadherin expression was related with tumor differentiation and lymph node metastasis, but had no relation with patients' age, sex, tumor size and invasiveness (Table 2).

Table 2 Relationships between expression of Ezrin, E-cadherin and clinical parameters of esophageal squamous cell carcinoma

Parameter	Case number	Ezrin expression (cases)				Statistic	E–cadherin expression (cases)				Statistic
					tive rate (%)		–	+	++	Positive rate (%)	
Sex	49	9	19	21							
Male	27	4	10	13	86.1	zc = –0.494	25	10	14	48.9	zc = –0.786
Female					85.1	P = 0.621	17	3	7	37.0	P = 0.432
Age (years)	44	6	18	20							
< 60	32	7	11	14	86.3	zc = –0.479	27	4	13	38.6	zc = –0.698
⩾ 60					78.1	P = 0.632	15	9	8	53.1	P = 0.485
Tumor size	33	6	14	13							
< 5 cm	43	7	15	21	81.8	zc = –0.716	16	8	9	51.5	zc = –0.695
⩾ 5 cm					83.7	P = 0.474	26	5	12	39.5	P = 0.487
Differentiation	26	4	12	10							
Well	33	7	11	15	88.4		8	6	12	69.2	
Moderate	17	2	6	9	78.7	χ^2 = 1.537	21	5	7	36.3	χ2 = 10.586
Poor					88.2	P = 0.464	13	2	2	23.5	P = 0.005
Infiltration degree	31	10	11	10							
Not invaded adventitia	45	3	18	24	67.7	zc = –2.528	16	5	10	48.3	zc = –0.654
Passed through adventitia					93.3	P = 0.011	26	8	11	42.2	P = 0.513
Lymph node metastasis	41	5	13	23							
Positive	35	8	16	11	87.8	zc = –2.136	28	7	6	31.7	zc = –2.758
Negative	–	+	++	Posi-	77.1	P = 0.033	14	6	15	60.0	P = 0.006

Relations between Ezrin expression and E-cadherin expression in ESCC

Among the 76 cases of ESCC, 21 were positive for E-cadherin, 16 of which were positive for Ezrin; 45 were negative for E-cadherin, 43 of which were positive for Ezrin. The positive rate of Ezrin was significantly lower in E-cadherin-positive cases than in E-cadherin-negative cases (χ^2 = 6.99, P < 0.05).

Discussion

The invasion and metastasis of malignant tumors are the direct causes of death for tumor patients. Recent studies have showed that Ezrin plays an important role in tumor metastasis[3]. Ezrin connects cell membrane and actin cytoskeleton in cytoplasm[4], and the actin-based cytoskeleton plays a pivotal role in cell movement and cellular morphogenesis. It has been proven that Ezrin is involved in the interaction between cells and the interaction between cells and stroma through regulating adhesion molecules and signal transduction, and plays a crucial role in tumor development, invasion and metastasis [5]. Studies showed that the high expression of Ezrin can induce tumor metastasis, therefore, is related with poor prognosis of tumor[6].

E-cadherin is a calcium-dependent glycoprotein which widely distributes in epithelial cells. E-cadherin mainly mediates adhesion between homogenous cells, functions as cytoskeleton to maintain structural integrity and epithelial polarity. The absence or decrease of cell adhesion mediated by E-cadherin is an important step in metastasis of most tumors. The decrease or absence of E-cadherin expression weakens cell adhesion, making

tumor cells easy to separate and grow invasively, leading to metastasis[7].

Pujuguet *et al.*[8] found that Ezrin regulates the transmission of E-cadherin to cell membrane. That mechanism may help to explain the roles of Ezrin and E-cadherin in tumor progression, that is, the abnormal expression of E-cadherin (decrease or absence of expression on cell membrane, or only expressed in cytoplasm) and the overexpression of Ezrin are the features of invasive tumors. The activation of Rac 1 pathway by Ezrin causes hepatocyte growth factor (HGF) to induce tyrosine phosphorylation of E-catenin and X-catenin, inhibits the function of E-cadherin and leads to redistribution of E-cadherin in cytoplasm and on cell membrane. In other words, HGF activates Met, regulates the phosphorylation of catenins and inhibits the adhesion of E-cadherin, leads to instable intercellular adhesion, induces the secretion of urokinase-type plasminogen active factor and hydrolysis of periplasmic protein. That may be one possible pathway leading to the structural and functional abnormality of E-cadherin. Therefore, it is proved that Ezrin and E-cadherin interact with each other and play important roles in tumor invasion and metastasis.

The present study showed that Ezrin expression was related with the invasiveness and lymph node metastasis of ESCC, but had no relation to patients' age, sex, tumor size and differentiation. Higher expression of Ezrin in cancer cells than in para-cancer normal epithelial tissues indicates that Ezrin might be crucial in tumorigenesis and progression of ESCC. Detecting Ezrin expression might have potential diagnostic and prognostic value for ESCC. We also found that E-cadherin expression was related with the differentiation and lymph node metastasis of ESCC, but had no relation to patients' age, sex, tumor size and invasiveness. The positive rate of E-cadherin was lower in cancer tissues of patients with lymph node metastasis than in those without ($P < 0.05$), and was lower in poorly differentiated cancer tissues than in well differentiated ones ($P < 0.05$); the difference between the cases with invasion and those without was not significant. This study showed that combined examination of Ezrin and E-cadherin may be helpful in evaluating the differentiation, invasiveness and metastasis of ESCC, thus providing a new index for tumor prognosis.

The invasion and metastasis of tumor cells is a multi-stage, multi-step and multi-factor process, which is related with the characteristics of tumor cells, the overall immune status of the host and the characteristics of the local tissue being metastasized. Ezrin and E-cadherin are both related with the genesis and metastasis of ESCC, and they may have negative cooperativity. However, it does not prove that Ezrin inhibits the expression of E-cadherin or suppresses the adhesion function of E-cadherin by promoting the expression of cytoskeleton protein. Therefore, the relations between Ezrin and E-cadherin need to be further explored.

References

[1] Mathew J, Hines JE, Obafunwa JO, et al. CD44 is expressed in hepatocellular carcinomas showing vascular invasion [J]. J Pathol, 1996,179(1):74-83.

[2] Gonzalez MA, Pinder SE, Wencyk PM, et al. An immunohistochemical examination of the expression of E-cadherin, alpha- and beta/gamma-catenins, and alpha2- and beta1-integrins in invasive breast cancer [J]. J Pathol, 1999,187(51): 523-529.

[3] McClatchey AI. Merlin and ERM proteins: unappreciated roles in cancer development? [J]. Nat Rev Cancer, 2003,3(11): 877-883.

[4] Yonemura S, Tsukita S, Tsukita S. Direct involvement of Ezrin/radixin/moesin (ERM)-binding membrane proteins in the organization of microvilli in collaboration with activated ERM proteins [J]. J Cell Biol, 1999,145(7): 1497-1509.

[5] Martin TA, Harrison G, Mansel RE, et al. The role of the CD44/ezrin complex in cancer metastasis [J]. Crit Rev Oncol Hematol, 2003,46(2): 165-186.

[6] M kitie T, Carpé O, Vaheri A, et al. Ezrin as a prognostic indicator and its relationship to tumor characteristics in uveal malignant melanoma [J]. Invest Ophthalmol Vis Sci, 2001,42(11):2442-2449.

[7] Santos-Garcí A, Abad-Hernáandez MM, Fonseca-Sánchez E, et al. E-cadherin, laminin and collagen IV expression in the evolution from dysplasia to oral squamous cell carcinoma [J]. Med Oral Patol Oral Cir Bucal, 2006,11(2):100-105.

[8] Pujuguet P, Del Maestro L, Gautreau A, et al. Ezrin regulates E-Cadherin-dependent adherens junction assembly through Racl activation [J]. Mol Biol Cell, 2003,14(5):2181-2191.