Expression of E-Cadherin in Oral Squamous Cell Carcinoma is Associated with Clinical Prognosis

Jianming Zhang[1]
Wei Zhang[2]
Ping Gao[1]
Yanqiu Li[1]
Changyi Li[1]
Sakan Maeda[3]

[1] Department of Emergency, Stomatology Hospital of Tianjin Medical University, Tianjin 300070, China.
[2] Tianjin University of Chinese Traditional Medicine, Tianjin 300070, China.
[3] Division of Molecular Pathology, Kobe University Graduate School of Medicine, Kobe, Japan.

Correspondence: Jianming Zhang
Tel:86-22-23332011
E-mail: zjming17@hotmail.com

Received May 9, 2006; accepted June 7, 2006.

OBJECTIVE To investigate the correlation of E-cadherin expression with clinicopathological parameters and prognosis of patients with oral squamous cell carcinoma.

METHODS We examined the expression of the protein E-cadherin in 43 oral squamous cell carcinoma (SCC) surgical speciments by SABC immunohistochemistry.

RESULTS There was a significant correlation between the level of E-cadherin expression and tumor stage (P=0.024), invasive pattern (P=0.009) and lymph node metastasis (P=0.023). No relation was found with age (P=0.084), sex (P=1.356) and differentiation (P=0.877). Using the Kaplan-Meier method we demonstrated that those cases which showed E-cadherin expression (-) or (+) had a significantly poorer prognosis compared those cases which showed expression (++) or (+++) (P=0.0146).

CONCLUSION E-cadherin, is an important indicator of clinical diagnoses and prognositic marker for oral SCC patients.

KEYWORDS: E-cadherin, metastasis, prognosis, squamous cell carcinoma.

E-cadherin is a member of the cadherin superfamily, which are calcium-dependent intercellular adhesion molecules believed to play a role in cell recognition and segregation, morphogenetic regulation, and tumor suppression,[1] Reduced expression of E-cadherin is associated with invasion and metastasis in different human cancers, such as primary ovarian carcinomas,[2] esophageal carcinoma,[3] cervical carcinoma,[4] human laryngeal cancer,[5] and rectal cancer [6] etc.

Squamous cell carcinoma (SCC), the most frequent malignancy of the oral cavity, is associated with a poor clinical outcome. Investigation of the expression of proteins associated with clinical characteristics and pathology in SCC would be useful in assessment of tumor behavior, accurately predicting prognosis, and improving the ability to determine the most appropriate therapies for each patient.[7]

In this study, we examined the expression of E-cadherin in surgical resected specimens of oral SCC, and investigated the correlation of E-cadherin expression and clinicopathological parameters and prognosis of patients with oral SCC.

MATERIALS AND METHODS

Patients

We collected 43 surgically treated cases of oral SCC from September 1991 to July 1997 in the Kobe University Hospital. The cases consisted of 32 males and 11 females, ranging in age from 41 to 81 years (av-

erage age of 59.6 years). Tumors were classified as well differentiated (n=22), moderately differentiated (n=18), or poorly differentiated (n=3), according to the International Histological Classification of Tumors.[8] Based on the TNM tumor stage classification,[9] our cases were classified as Stage I (5 cases), Stage II (14 cases), Stage III (9 cases), and Stage IV (15 cases).

All patients were treated initially with surgery, after which 17 received postoperative radiotherapy or chemotherapy. Resected specimens were examined in our study.

Carcinoma invasion was classified according to the mode of invasion by Yamamoto et al.[10]: mode 1, well defined margin; mode 2, cords, less obvious margin; mode 3, groups of cells, no distinct margin; mode 4c, diffuse invasion of cord-like type; mode 4d, diffuse invasion of diffuse type. The 43 cases in this study were divided into mode 1 (n=0), mode 2 (n=6), mode 3 (n=18), mode 4c (n=15), and mode 4d (n=4).

Immunohistochemical analysis

Immunohistochemical studies were performed using an indirect streptavidin-biotin immunoperoxidase technique. Sections were dewaxed and rehydrated in concentrated sodium citrate buffer (20×), then heated for 10 min at 750 W output in a microwave oven. Sections were treated with methanol for 20 min to inhibit endogenous peroxide activity, then blocked with serum blocking solution to reduce nonspecific labeling. Sections were incubated with 1:400 diluted HECD-1 (MAb to human E-cadherin, Takara Shuzo, Japan) for 1 h at room temperature. The sections were also treated with irrelevant primary antibody as a negative control. The sections were treated with diluted biotinylated anti-mouse and anti-rabbit Ig antibody for 15 min, then reacted with horseradish peroxidase-conjugated streptavidin (Dako Japan, Kyoto, Japan) for 15 min. After each step, the sections were rinsed in phosphate-buffered saline (PBS) for 15 min. To visualize immunoreactivity, the sections were treated with DAB. Following hematoxylin counterstaining, the slides were permanently mounted. Immunostaining scores were determined by a researcher with no knowledge of the patient clinical status.

The degree of staining for E-cadherin was scored as described by Hiraki et al.[11] (+++): extensive staining comparable to control epithelium at the invasion front of SCC. (++): staining reduced from control levels, but greater than 50 % of the level of positive staining. (+): positive staining, but reduced to less than 50 % of control levels. (-): very little or no staining. Normal epithelium proximal to the tumor within the specimen was used as a control.

Statistical analysis

The χ^2-test was used to assess the statistical significance of E-cadherin expression in relation to clinicopathological parameters. Survival curves were obtained using the Kaplan-Meier method. P values < 0.05 were considered significant.

RESULTS

Under light microscopy, E-cadherin immunostaining in tumor cells was observed not only in the membrane but also in the cytoplasm (Fig.1).

E-cadherin expression (+++) was observed in 10 cases (23.3%), expression (++) in 11 (25.6%), expression (+) in 13 (30.2%), and no expression in 9 cases (20.9%). E-cadherin expression (+) and (-) was significantly associated with more advanced stage tumors (P=0.024), demonstrating a diffusely invasive pattern (P=0.009) and lymph node metastasis (P=0.023). No relationship was observed between E-cadherin expression and age (P=0.084), sex (P=1.356) or tumor differentiation (P=0.877, Table 1). The survival curve demonstrated a significant difference between the cases with expression (-) or (+) of E-cadherin from the expression (++) or (+++) population (P=0.0146, Fig. 2).

DISCUSSION

E-cadherin is an important suppressor molecule of tumor development. E-cadherin functions by enhancing adhesion between the cells and thereby inhibits cellular proliferation and growth of tumors. The human E-cadherin gene is located at chromosome16q22.1. The expression of E-cadherin in human tumors has been reported by multiple researchers. Though the data from these reports are not very consistent, E-cadherin has been shown to be related to tumor biological behavior. The relationship is that down-regulation of E-cadherin expression weakens adhesion between cells, followed by the infiltration, dissemination and metastasis. This event contributes to the poorer prognosis of the patients.[2-7]

In our study, the down-regulation of E-cadherin expression in oral SCC correlated with increased lymph node metastasis and poorer prognosis, as seen in previous reports. Furthermore, reduced expression of E-cadherin correlated with tumor stage and invasive

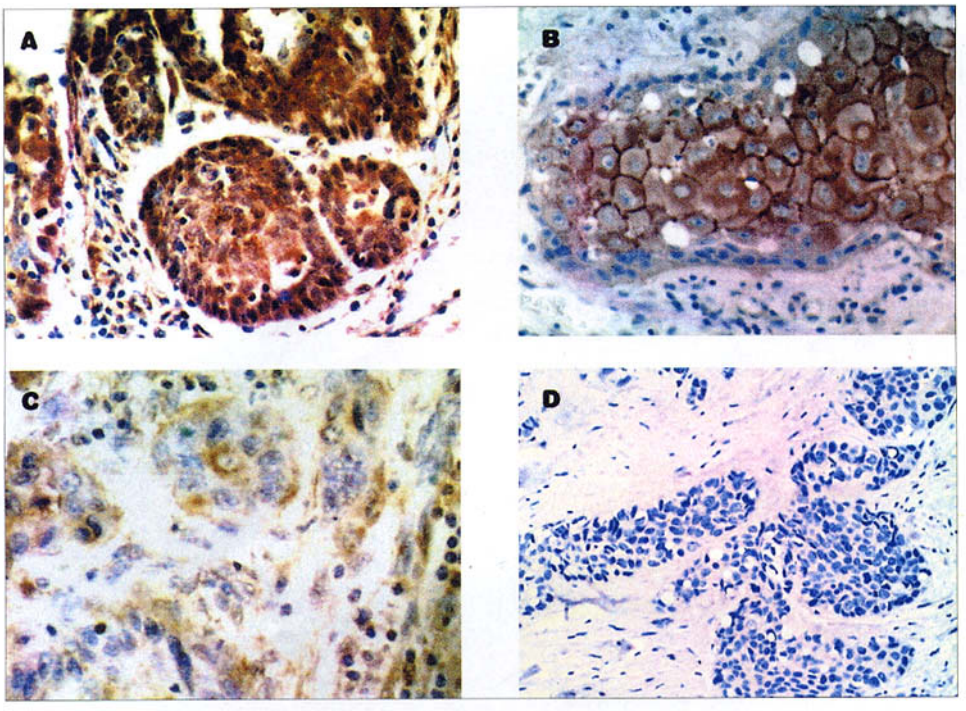

Fig.1. E-cadherin immunohistochemical staining of oral SCC. A: E-cadherin (+++) expression (original magnification, × 200); B: E-cadherin (++) expression (original magnification, × 200); C: E-cadherin (+) expression (original magnification, × 200); D: E-cadherin (-) expression (original magnification, × 200).

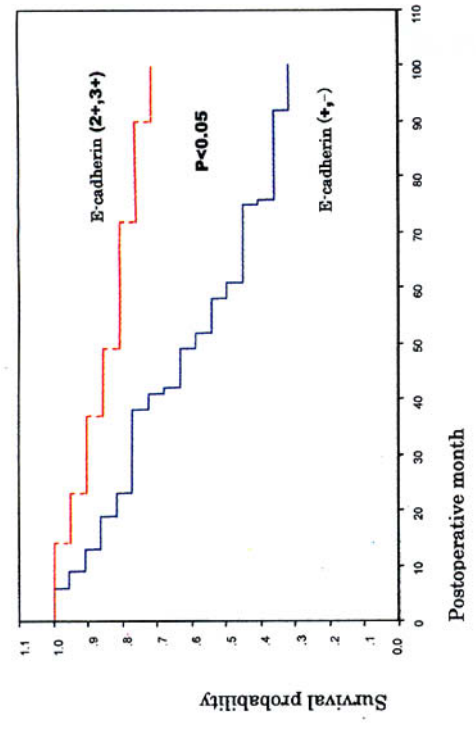

Fig.2. Kaplan-Meier survival curves according to the tissue status of E-cadherin in oral SCC. The cases with E-cadherin expression (-,+) were significantly poorer in prognosis compared to those with E-cadherin expression (++, +++)(P=0.0146).

Table. 1. Relationship between the expression status of E-cadherin and clinicopathological parameters of oral SCC

Factor	E-cadherin (++, +++) N(%)	E-cadherin (-,+) N(%)	*P*
Age			
≥ 60	15 (62.5%)	9 (37.5%)	
<60	6 (31.58%)	13 (68.42%)	0.084
Sex			
Male	15 (46.88%)	17 (53.12%)	
Female	6 (54.54%)	5 (45.46%)	1.356
Differentiation			
W	11 (50%)	11 (50%)	
M	9 (50%)	9 (50%)	
P	1 (33.33%)	2 (66.67%)	0.877
Tumor stage			
1~2	9 (42.86%)	7 (35%)	
3~4	8 (34.78%)	15 (65.22%)	0.024
Invasive pattern			
1~3	16 (66.67%)	8 (33.33%)	
4c, 4d	5 (26.32%)	14 (73.68%)	0.009
Lymph node metastasis			
Negative	13 (68.42%)	6 (31.58%)	
Positive	8 (33.33%)	16 (66.67%)	0.023

W: well differentiated M: moderately differentiated P: pooly differentiated

pattern, but not with age, sex and differentiation, results similar to those of Shinohara et al.(12) who reported no significant relationship between down-regulation of E-cadherin and differentiation in oral SCC .

Recently, Bosch et al.[13] showed that E-cadherin, is an important indicator of clinical diagnoses and prognositic marker of head and neck SCC patients. The prognostic strength of E-cadherin was independent of, and stronger than histological grading, N stage, tumor site, and even stronger than the TNM stage. Based on these results, evaluation of E-cadherin in SCC by immunostaining is recommended as a significant prognostic marker. In our studies of 43 cases of OSCC after surgery over a period of 8 years, we evaluated E-cadherin expression using an immunohistochemical staining technique. Our findings support the belief that assessment of E-cadherin expression, only by immunostaining, has prognostic importance in patients with oral SCC.

Studies relating to how E-cadherin may influence tumor behavior, have shown that E-cadherin gene mutations were not identified in human oral SCC cells,[14] and that the interaction between α-, β-and γ-catenin,[15] S100A4,[9] dysadherin[16] and E-cadherin may affect the metastatic potential of tumor cells and, consequently, the prognosis of the cancer patients. Thus, to elucidate the mechanism underying the effect of E-cardhrin on tumor prognosis will be the next focus of our research.

In conclusion, we believe E-cadherin is an independent important indicator for clinical diagnoses, a prognositic marker and a useful factor for the prediction of metastatic potential of oral SCC patients. E-cadherin status will be of great value to clinical oncologists in determining the best therapeutic methods for patients to obtain improved outcomes.

REFERENCES

1 Hazan RB, Qiao R, Keren R, et al. Cadherin switch in tumor progression. Ann N Y Acad Sci 2004;1014:155–163.

2 Faleiro–Rodrigues C, Macedo–Pinto I, Pereira D, et al. Prognostic value of E–cadherin immunoexpression in patients with primary ovarian carcinomas. Ann Oncol 2004; 15:1535–1542.

3 Feith M, Stein HJ, Mueller J, et al. Malignant degeneration of Barrett's esophagus: the role of the Ki–67 proliferation fraction, expression of E–cadherin and p53. Dis Esophagus 2004;17:322–327.

4 Carico E, Atlante M, Bucci B, et al. E–cadherin and α–catenin expression during tumor progression of cervical carcinoma. Gynecologic Oncol 2001;80:156–161.

5 Sun WH, Liu HM, Li YJ, et al. A study of the relationship between the expression of connexin43, E–cadherin and biological behaviors of human laryngeal cancer. Chinese Journal of Otorhinolaryngology 2004;39:293–297.

6 Fernebro E, Bendahl PO, Dictor M, et al. Immunohistochemical patterns in rectal cancer: application of tissue microarray with prognostic correlations. Int J Cancer 2004;111:921–928.

7 Zhang J, Nakamura T, Ito R, et al. The evaluation of both metastasis and prognosis of oral squamous cell carcinoma by S100A4 and E–cadherin immunostaining. Oral Med & Patho 2004;9:67–74.

8 Pindborg JJ, Reichart PA, Smith CJ, et al. Histological Typing of Cancer and Precancer of the Oral Mucosa. WHO, Springer–Verlag, Berlin 1997;35–38.

9 International Union Against Cancer, TNM classification of malignant tumours. Springer–Verlag, Berlin. 1987.

10 Yamamoto E, Kohama G, Sunakawa H, et al. Mode of invasion, bleomycin sensitivity, and clinical course in squamous cell carcinoma of the oral cavity. Cancer 1983;51: 2175–2180.

11 Hiraki A, Shinohara M, Ikebe T, et al. Immunohistochemical staining of desmosomal components in oral squamous cell carcinoma and its association with tumour behaviour. Br J Cancer 1996;73:1491–1497.

12 Shinohara M, Hiraki A, Ikebe T, et al. Immunohistochemical study of desmosomes in oral squamous cell carcinoma: correlation with cytokeratin and E–cadherin staining, and with tumour behaviour. J Pathol 1998; 184: 369–381.

13 Bosch FX, Andl C, Abel U, et al. E–cadherin is a selective and strongly dominant prognostic factor in squamous cell carcinoma: a comparison of E–cadherin with desmosomal components. Int J Cancer 2005;114: 779–790.

14 Saito Y, Takazawa H, Uzawa K, et al. Reduced expression of E–cadherin in oral squamous cell carcinoma: relationship with DNA methylation of 5′ CpG island. Int J Oncol 1998; 12:293–298.

15 Lin YC, Wu MY, Li DR, et al. Prognostic and clinicopathological features of E–cadherin, alpha–catenin, beta–catenin, gamma–catenin and cyclin D1 expression in human esophageal squamous cell carcinoma. World J Gastroenterol 2004 15;10:3235–3239.

16 Nakanishi Y, Akimoto S, Sato Y, et al. Hirohashi S Prognostic significance of dysadherin expression in tongue cancer: immunohistochemical analysis of 91 cases. Appl Immunohistochem Mol Morphol. 2004;12:323–328.