[原著] Distribution of Reduced Expression of E-Cadherin and Catenins in Squamous Cell Carcinoma of the Tongue with Special Reference to Its Clinical Significance

Distribution of Reduced Expression of E-Cadherin and Catenins in Squamous Cell Carcinoma of the Tongue with Special Reference to Its Clinical Significance

Junko Kiyuna, Hiroyoshi Hiratsuka, Kenji Nakamori and Hajime Sunakawa

Department of Oral and Maxillofacial Surgery, Faculty of Medicine, University of the Ryukyus, 207 Uehara, Nishihara, Okinawa 903-0215, Japan

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ABSTRACT

The biologic aggressiveness of squamous cell carcinoma of the tongue is reflected in its ability to metastasize to cervical lymph nodes. Aberrant expression of E-cadherin and/or catenins is known to be associated with invasiveness and metastasis in a number of different cancer types but distributions of E-cadherin and catenins expression are unclear. Furthermore concerning squamous cell carcinoma of the tongue, the clinical significance of expression patterns has not been adequately investigated. In the current study, membranous expression of E-cadherin and catenins (α, β, and γ) at the superficial to middle part and the peripheral invading front of the tumor were evaluated in 79 patients with squamous cell carcinoma of the tongue by immunohistochemical methods using monoclonal antibodies. Both E-cadherin and catenins membranous expression was more preserved in the superficial to middle part of the tumor than in the peripheral invading front ($p=0.0007$, $=0.0001$, <0.0001, and <0.0001, respectively). Univariate analysis revealed that reduced membranous expression of E-cadherin, β-catenin, and γ-catenin at the invading front of the tumor showed significant reverse correlation with the T classification of the primary tumors ($p=0.02$, $=0.02$, and $=0.03$, respectively), and that reduced membranous expression of E-cadherin and γ-catenin was correlated with the clinical stage ($p=0.01$ and $=0.04$, respectively). In addition, reduced membranous expression of E-cadherin, α-catenin, β-catenin, and γ-catenin also showed a significant correlation with the mode of carcinoma invasion ($p=0.008$, $=0.01$, $=0.02$, and, $=0.02$, respectively). Moreover, reduced expression of E-cadherin, α-catenin, β-catenin, and γ-catenin was significantly associated with postsurgical lymph node metastasis ($p<0.0001$, $=0.007$, $=0.02$, and $=0.001$, respectively). Multivariate analysis revealed that the combined evaluation of reduction or loss of E-cadherin and/or γ-catenin membranous expression was the independent predictor for cervical lymph node metastasis (relative risk; 13.5) in patients with squamous cell carcinoma of the tongue. These results suggested that the assessment of E-cadherin and γ-catenin membranous expression at the peripheral invading front of the tumor could be clinically useful, especially for selecting patients who require neck dissection. *Ryukyu Med. J., 21(1) 19～27, 2002*

Key words: E-cadherin, Catenins, Lymph node metastasis, Squamous cell carcinoma of the tongue

INTRODUCTION

The treatment of squamous cell carcinoma of the tongue is usually surgery and radiation with or without concomitant chemotherapy and/or immunotherapy, used either singly or in combination. However, in spite of improved therapeutic advances, invasive and metastatic squamous cell carcinoma of the tongue shows a generally poor prognosis. It has been reported that determinate or disease-specific survival at 5 years was 65%[1] and 64%[2]. Although many biologic prognostic parameters have been investigated for their accuracy in predicting the prognosis of squamous cell carcinoma of the tongue[3-5], the results have often been conflicting. Currently, the TNM staging system continues to represent the most accurate prognostic parameter in patients with squamous cell carcinoma of the tongue[6]. It has been particularly emphasized that patients with squamous cell carcinoma of the tongue present a high risk for cervical lymph node metastasis, which reduces the probability of regional control and survival.

It is especially important to detect early cancer development in the lymph node since treatment of the disease in

its early stages results in a better clinical outcome. In spite of recent advances in the evaluation of lymph node metastasis, which include imaging techniques such as the computed tomography scan, magnetic resonance imaging, and ultrasonography, 28 to 41% of patients with squamous cell carcinoma of the tongue go unidentified[7-9]. If a diagnostic indicator of biologic aggressiveness in patients with squamous cell carcinoma of the tongue could be identified reliably at the time of initial evaluation or before the definitive treatment, it would permit an expanded classification. In addition to allowing for a comparison between treatment modalities, this indicator could ultimately allow for changes in treatment decisions or aid clinicians in deciding whether there is a need for a close follow-up, based on the relationship between this indicator and the patient's outcome.

With the advent of molecular techniques, an experimental model system shows metastasis to be a coordinated multi-step process encompassing the detachment of cells from the primary tumor to the development of a tumorigenic lesion at a distant site[10]. The process of metastasis appears to be regulated by a variety of gene products. Of these, cell to cell adhesion molecules play an important role in cancer invasion and metastasis.

E-cadherin, which is the prime mediator of calcium-dependent transmembrane glycoprotein, plays a major role in the establishment and maintenance of intercellular adhesion, cell polarity, and tissue architecture[11]. E-cadherin, which is composed of an extracellular domain, a transmembrane domain, and an intercellular domain, binds to catenins, and transduction of the cell to cell contact signals into cellular organization is mediated by the catenins. The catenin family comprises α-catenin, β-catenin, and γ-catenin and these link the cytoplasmic terminal tail of E-cadherin to the actin cytoskeleton[12]. β-catenin and γ-catenin bind directly to the cytoplasmic tail of E-cadherin, whereas α-catenin links the bound β-catenin to the actin microfilament network of the cellular cytoskeleton.

It has been suggested that aberrant expression of E-cadherin and catenins in human epithelial tumors in vivo might be directly related to decreased adhesion of tumor cells to each other, to the invasion into the surrounding tissues, and to the development of metastasis[13, 14]. Of particular interest, the role of E-cadherin and catenins in cancer metastasis has become topical in the past few years due to its apparent promise as a prognostic indicator, with loss or reduction of expression correlating with enhanced aggressiveness in various types of carcinomas[15-20]. Thus far, none of the studies on E-cadherin and catenins expression in squamous cell carcinoma of the tongue have been carried out in relation to the clinicopathologic features of the disease.

In the current study, we retrospectively investigated the patterns and distribution of E-cadherin, α-catenin, β-catenin, and γ-catenin expression by immunohistochemical techniques in untreated primary squamous cell carcinoma of the tongue in 79 patients. Individual patients were assessed in relation to clinical features (T classification and TNM stage) and in relation to pathologic features (tumor differentiation and histologic grading of mode of invasion after the method of Yamamoto *et al.*[21, 22]).

MATERIALS AND METHODS

Tissue samples

Formalin fixed, paraffin embedded incisional and excisional biopsied tissue samples were available from 79 patients with squamous cell carcinoma of the tongue who were treated surgically from 1990 to 1999 at the Oral and Maxillofacial Surgery Clinic, University of the Ryukyus Hospital. No patients with any form of antecedent therapy were included.

Histopathology and Immunohistochemistry

Serial sections of 4 μm thick were cut from the tissue blocks and mounted on silanied glass slides. One of the serial sections was stained with hematoxylin and eosin for verification of the original histopathologic diagnosis and for comparison to immunohistochemical staining. Every tissue specimen contained sufficient peripheral regions of the peripheral invading front of the cancer mass. After deparaffinization and rehydration of the sections, retrieval of the antigen in a citrate buffer (pH6.0) was done by the autoclave method[23], and followed by staining which was performed by the methods of ENVISION^{+TM} (DAKO Co., CA, USA)[24].

The monoclonal antibodies used in the current study were as follows: 1/100 diluted anti-E-cadherin (mouse IgG2a, clone 36, Transduction Laboratories, Lexington, KY), 1/50 diluted anti-α-catenin (mouse IgG1, clone 5, Transduction Laboratories), 1/250 diluted anti-β-catenin (mouse IgG1, clone 14, Transduction Laboratories), and 1/50 diluted anti-γ-catenin (mouse IgG2a, clone 15, Transduction Laboratories). Sections were incubated with each monoclonal antibody overnight at 4℃. Tris-HCl buffer (pH 7.6) was used as a rinse for each chemical reagent. After the reaction, products were visualized by 3,3-diaminobenzidine tetrahydrochloride solution containing 0.01% hydrogen peroxide, the sections were counter stained with hematoxylin. As positive controls, the immunoreactivity of perilesional non-cancerous squamous epithelium was evaluated for each monoclonal antibody. A negative control was also performed in each run by substituting primary antibodies with Tris-HCl buffer.

Evaluation of Immunostaining

Evaluation of E-cadherin, α-, β-, and γ-catenins expression was referenced to classification by Shiozaki *et al.*[25]. Namely, if the membranous staining of each protein in tumor cells shows approximately as much staining as is shown in perilesional non-cancerous epithelium it is evaluated as preserved expression. When the membranous expression of

Table 1 Expression of E-cadherin, α-, β-, and γ-catenins at the superficial to middle part and the invasive front of the squamous cell carcinoma of the tongue

	Superficial to middle part (%)		Invading front (%)		P value
	Preserved	Reduced	Preserved	Reduced	(χ^2-test)
E-cadherin	65 (82.3)	14 (17.7)	38 (48.1)	41 (51.9)	p=0.0007
α-catenin	55 (69.6)	24 (30.4)	28 (35.4)	51 (64.6)	p=0.0001
β-catenin	53 (67.1)	26 (32.9)	32 (40.5)	47 (59.5)	p<0.0001
γ-catenin	54 (68.4)	25 (31.6)	35 (44.3)	44 (55.7)	p<0.0001

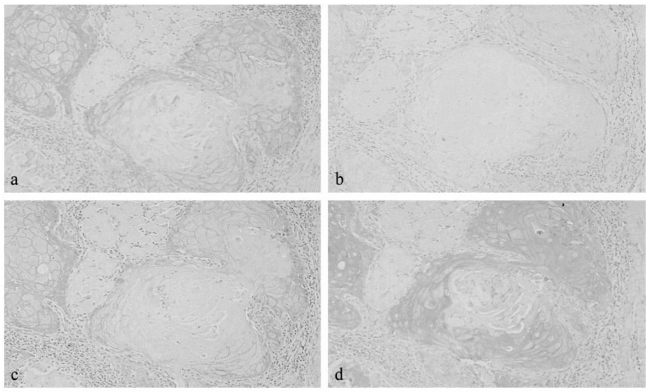

Fig. 1 E-cadherin, α-, β-, and γ-catenins expression at the superficial to middle part of well differentiated squamous cell carcinoma of the tongue. E-cadherin (a), β-catenin (c), and γ-catenin (d) are expressed at the cell boundaries but not cancer pearl. α-catenin (b) are weakly expressed compared with other proteins. (Original magnification × 400)

each protein in tumor cells was completely lost, they were evaluated as reduced expression. Where both positive mem branous staining and loss of each protein expression were observed, at least 500 tumor cells per tumor were counted, and cases with membranous staining of over 50% were evaluated as preserved expression. In addition, cytoplasmic and/or nuclear staining was evaluated as reduced expression. The sections were examined microscopically by two of the authors (K.J. and H.H.) and evaluated according to the staining pattern (membranous, cytoplasmatic, or nuclear).

The authors examined the entire tumor area in each section, and recorded two distinguishable parts, the superficial to middle part and the tumor-host borderline, the latter being the area of the peripheral deep invading front of the cancer, as previously described[21]. Occasional disagreement regarding the staining pattern and staining intensity were discussed and a consensus reached.

Statistical methods

Correlation between clinicopathologic findings and the expression pattern of each protein were evaluated by the chi-square test. Univariate and multivariate analysis of risk of histologically proven lymph node metastasis and immunohistopathologic variables were evaluated for the predictive value. Multivariate analysis was performed by multiple logistic regression using unconditional maximum likelihood estimation. Statistical analyses were performed using the StatView software package (SAS Institute Inc., Cary, NC). P value less than 0.05 was regarded as statistically significant.

RESULTS

Characteristics of patients

Clinical and pathologic data were obtained from the medical records of each patient, after tabulation of the immunohistologic analysis. The age of the patients ranged from 23 to 86, with an average age of 57 years; 50 patients were male and 29 were female. The clinical stages of the 79 patients were as follows: stage I (18), stage II (35), stage III (13) and stage IVA (13). With a median follow-up of 42.3 months, 77.2% of the patients were alive without disease at the time of analysis. Of the remaining patients, 2 patients died from other diseases, and 16 patients died from cancer.

Microscopically identified metastatic lymph nodes occurred in 29 (36.7%) of the 79 patients. Of those 29, 34% underwent therapeutic node dissection, 28% under went elective neck dissection, and 38% underwent required subsequent therapeutic lymph node dissections.

Expression pattern of E-cadherin and α-, β-, and γ-catenins in non-cancerous squamous epithelium

In the perilesional squamous epithelium, the expression pattern of E-cadherin and α-, β-, and γ-catenins showed strong cell membranous staining ranging over the basal cell to the prickle cell layer. No membranous staining for E-cadherin and catenins was observed in the granular and corneal cell layers. Also, no membranous staining in basal cells bounded by the submucosa was found. A diffuse cytoplasmic staining for E-cadherin and catenins was found in parabasal layers. E-cadherin, β-catenin and γ-catenin showed about the same staining intensity, but α-catenin showed slightly less when compared with the rest.

Expression patterns of E-cadherin and α-, β-, and γ-catenins in squamous cell carcinoma of the tongue

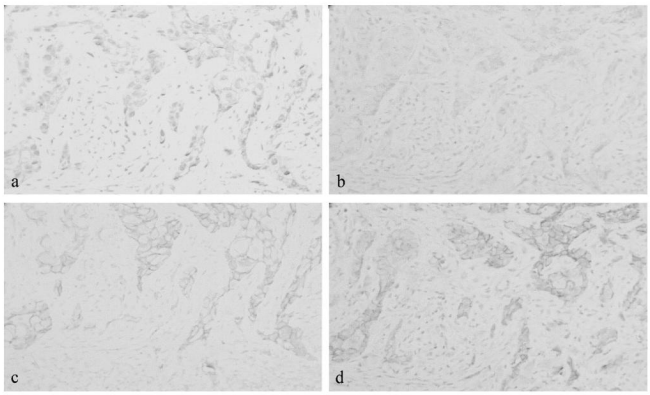

Fig. 2 E-cadherin, α-, β-, and γ-catenins expression at the invading front of the tumor. Loss of membranous staining for E-cadherin (a) and weak heterogeneous immunostaining for α-catenins (b) are apparent. Preserved membranous expression of β- (c) and γ-catenins (d) are also observed. Note the pattern of expression of E-cadherin is prevalently nuclear. (Original magnification × 400)

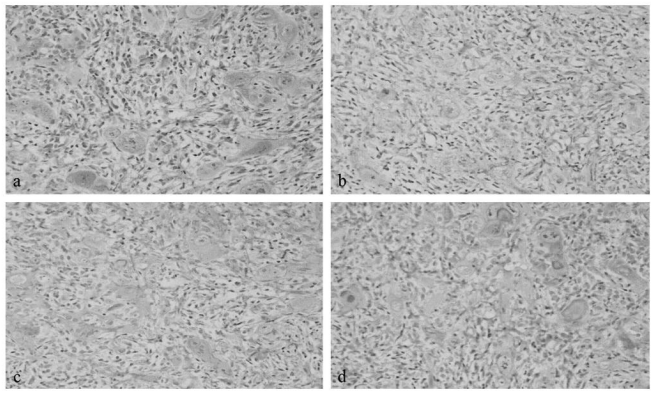

Fig. 3 Aberrant expression of E-cadherin, α-, β-, and γ-catenins at invading front of the tumor in invasive squamous cell carcinoma (grade 4 D) of the tongue. Although cytoplasmic expression is seen in E-cadherin (a), β-, and γ-catenins, and nuclear expression is observed in E-cadherin (a) and γ-catenins (d), loss of membranous expression of E-cadherin (a), α-catenin (b), β-catenin (c), and γ-catenin (d) is evident. (Original magnification × 400)

The intensity of membranous immunoreactivity was equally strong in E-cadherin, β-catenin and γ-catenin, but mostly weak in α-catenin, as was also observed in the perilesional squamous epithelium. In well differentiated cancer nests, neither keratin pearls nor keratinizing cells stained positively for E-cadherin, α-catenin, β-catenin, and γ-catenin. Similar results are observed in the perilesional squamous epithelium (Fig. 1). Some tissue samples showed heterogeneous immunoreactivity by each antibody (Fig. 2 and 3). When the samples were examined as a whole, the expression of E- cadherin, α-, β-, and γ-catenins was preserved in 38 tumors (48.1%), 28 tumors (35.4%), 32 tumors (40.5%), and 35 tumors (44.3%), respectively. There was heterogeneous or weak homogeneous membranous expression in 22 tumors (27.9%), 41 tumors (51.9%), 32 tumors (40.5%), and 31 tumors (39.2%), respectively. The loss of expression of E-cadherin, α-, β-, and γ-catenins was also observed in 19 tumors (24.0%), 10 tumors (12.7%), 15 tumors (19.0%), and 13 tumors (16.5%), respectively. On the other hand, various levels of intensity of cytoplasmic expression of E-cadherin, α-, β-, and γ- catenins was seen in 30 tumors (38.0%), 5 tumors (6.3%), 9 tumors (11.4%), and 39 tumors (49.4%), respectively. In the assessment of the tumor samples as a whole, the greatest intensity of nuclear expression of E-cadherin and γ-catenin appeared predominantly in 39 tumors (49.4%), and 40 tumors (50.6%), respectively.

With respect to site distribution, reduced expression of E-cadherin, α-, β-, and γ-catenins in the surface to middle part of the tumor was observed in 14 tumors (17.7%), 24 tumors (30.4%), 26 tumors (32.9%), and 25 tumors (31.6%), respectively. On the contrary, reduced expression of E-cadherin, α-, β-, and γ-catenins in the peripheral invading front of the tumor appeared in 41 tumors (51.9%), 51 tumors (64.6%), 47 tumors (59.5%), and 44 tumors (55.7%), respectively. The reverse correlation of the distribution of the reduced expression of E-cadherin, α-, β-, and γ-catenin showed statistically significant differences ($p=0.0007$, $=0.0001$, <0.0001, and <0.0001, respectively) (Table 1).

Relationship between the distribution of E- cadherin and α-, β-, and γ-catenins expression and clinicopathologic features

In the assessment of patterns of immunoreactivity to E-cadherin and catenins at the superficial to middle part of the tumor, reduced membranous E-cadherin expression was significantly associated with the tumor stage ($p=0.005$), and similar immunoreactivity of α-catenin correlated significantly with the mode of carcinoma invasion ($p=0.03$) (Table 2). On the other hand, in the immunohistochemical evaluation of membranous expression patterns of the peripheral invading front of the tumor sample, reduced expression of E-cadherin, β-, and γ-catenins correlated with the T classification of the primary tumor ($p=0.02$, $=0.02$, and $=0.03$, respectively). Reduced membranous expression of E-cadherin and γ-catenin also correlated with the tumor stage ($p=0.01$ and $=0.04$, respectively). In addition, reduced expression of E-cadherin, α-, β-, and γ-catenins was associated with the mode of carcinoma invasion ($p=0.008$, $=0.01$, $=0.02$, and $=0.02$, respectively) (Table 3).

With respect to histologically proven lymph node metastasis, reduced expression of E-cadherin, α-, β-, and γ-catenins at the invading front of the tumor was closely associated with pathologic lymph node metastasis (Table 4). Based on this result, the authors tried to evaluate the combined expression pattern of E-cadherin and γ-catenin since each had a smaller p-value than those of α-catenin

Table 2 Relationships between clinical, histopathologic variables and expression of E-cadherin, α-, β-, and γ-catenins at the superficial to middle part of the squamous cell carcinoma of the tongue

		E-cadherin			α-catenin			β-catenin			γ-catenin		
		Preserved	Reduced	P value	Preserved	Reduced	P value	Preserved	Reduced	P value	Preserved	Reduced	P value
T classification				NS#			NS			NS			NS
T1	(n=19)	19	0		16	3		16	3		15	4	
T2	(n=42)	34	8		29	13		25	17		27	15	
T3	(n= 9)	6	3		5	4		7	2		6	3	
T4	(n= 9)	6	3		5	4		5	4		6	3	
Stage				0.005			NS			NS			NS
I	(n=18)	18	0		15	3		15	3		14	4	
II	(n=35)	31	4		26	9		24	11		24	11	
III	(n=13)	8	5		8	5		8	5		8	5	
IV	(n=13)	8	5		6	7		6	7		8	5	
Tumour differenciation				NS			NS			NS			NS
Well	(n=37)	30	7		29	8		29	8		28	9	
Moderate	(n=35)	29	6		21	14		21	14		21	14	
Poor	(n= 7)	6	1		5	2		3	4		5	2	
Mode of carcinoma invasion				NS			0.03			NS			NS
Type 1,2	(n=23)	21	2		20	3		20	3		17	6	
Type 3	(n=32)	26	6		23	9		21	11		22	10	
Type 4C	(n= 8)	7	1		3	5		4	4		5	3	
Type 4D	(n=16)	11	5		9	7		8	8		10	6	
Pathologic lymph node metastasis				NS			NS			NS			NS
N0, pN0	(n=50)	44	6		39	11		36	14		37	13	
pN+	(n=29)	21	8		16	13		17	12		17	12	

NS: Not significant

Table 3 Relationships between clinical, histopathologic variables and expression of E-cadherin, α-, β-, and γ-catenins at the invading front of the squamous cell carcinoma of the tongue

		E-cadherin			α-catenin			β-catenin			γ-catenin		
		Preserved	Reduced	P value	Preserved	Reduced	P value	Preserved	Reduced	P value	Preserved	Reduced	P value
T classification				0.02			NS			0.02			0.03
T1	(n=19)	14	5		6	13		9	10		11	8	
T2	(n=42)	15	27		15	27		15	27		15	27	
T3	(n= 9)	6	3		5	4		7	2		7	2	
T4	(n= 9)	3	6		2	7		1	8		2	7	
Stage				0.01			NS			NS			0.04
I	(n=18)	14	4		6	12		9	9		11	7	
II	(n=35)	14	21		14	21		15	20		14	21	
III	(n=13)	7	6		5	7		7	6		8	5	
III	(n=13)	3	10		2	11		1	12		2	11	
Tumour differenciation				NS#			NS			NS			NS
Well	(n=37)	20	17		15	22		18	19		18	19	
Moderate	(n=35)	15	20		10	25		12	23		15	20	
Poor	(n= 7)	3	4		3	4		2	5		2	5	
Mode of carcinoma invasion				0.008			0.01			0.02			0.02
Type 1,2	(n=23)	16	7		13	10		14	9		15	8	
Type 3	(n=32)	17	15		12	20		13	19		15	17	
Type 4C	(n= 8)	2	6		1	7		3	5		2	6	
Type 4D	(n=16)	3	13		2	14		2	14		3	13	
Pathologic lymph node metastasis					<0.0001			0.01			0.001		
N0, pN0	(n=50)	33	17		23	27		25	25		29	21	
pN+	(n=29)	5	24		5	24		7	22		6	23	

NS: Not significant

and β-catenin. Multivariate analysis showed that combined evaluation of reduced membranous expression of E-cadherin and/or γ-catenin at the invading front of the tumor was the only variable that more closely correlated with the risk of cervical lymph node metastasis. Patients with reduced membranous expression of E-cadherin and /or γ-catenin had a risk 13.5 times higher than those with preserved membranous expression of E-cadherin and γ-catenin (Table 5). Although clinical assessment of lymph node metastasis (N classification) and the mode of the carcinoma invasion were similarly correlated to the development of lymph node metastasis, when these variables

Table 4 Relative risk of pathologic lymph node metastasis as indicated in cases surveyed by expression pattern of E-cadherin, α-, β-, and γ-catenins at the invading front of the quamous cell carcinoma of the tongue (univariate analysis)

Expression pattern at invading front of the tumor		Lymph node metastasis Yes	No	Relative risk	95% CI#	P-value###
E-cadherin	preserved	5	33	1.0	Reference##	<0.0001
	reduced	24	17	9.3	3.0-28.7	
α-catenin	preserved	5	23	1.0	Reference	0.007
	reduced	24	27	4.0	1.3-12.4	
β-catenin	preserved	7	25	1.0	Reference	0.02
	reduced	22	25	3.1	1.1-8.6	
γ-catenin	preserved	6	29	1.0	Reference	0.001
	reduced	23	21	5.2	1.8-15.2	

Confidence interval
Reference category
Value of P according to the maximum likelihood statistics

Table 5 Multivariate models of risk of pathologic lymph node metastasis in assessment of expression pattern of α-, β-catenins, and combined evaluation of E-cadherin and γ-catenin at the invading front of the squamous cell carcinoma of the tongue

Expression pattern at invading front of the tumor		Lymph node metastasis Yes	No	Relative risk	95% CI#	P-value###
α-catenin	preserved	5	23	1.0	Reference ##	0.2
	reduced	24	17	2.1	0.5- 8.3	
β-catenin	preserved	7	25	1.0	Reference	0.4
	reduced	22	25	0.5	0.1-2.6	
E-cadherin	preserved	7	40	1.0	Reference	<0.0001
and/or γ-catenin	reduced	22	10	13.5	3.3-55.0	

Confidence interval
Reference category
Value of P according to the maximum likelihood statistics

were added to the immunohistochemical assessment of the pattern of membranous expression of E-cadherin and γ-catenin, multivariate logistic models revealed that these differences were not statistically significant.

DISCUSSION

One of the striking findings of the current study was that the reduced membranous expressions of E-cadherin, α-, β-, and γ-catenins showed a reverse correlation in sites distinct from the superficial to middle part of the tumor to the peripheral invading front of the tumor. Kikuchi *et al.*[26] have already pointed out that if cancer patients had specific immune resistance to their own cancer, a battlefield would be at the site of cancer invasion and the area of its spread and that one might see the extent of the host defense force against cancer by observing its histology. Indeed, the authors' previous studies have also revealed that attention should be paid to the area of tumor-host interaction in the deep invading front when evaluating the biologic characteristics of the individual tumor[21, 27, 28].

During the past decade, some authors have underscored the need to assess the advancing edge of the neoplastic process to determine the biologic aggressiveness of disease as well[29, 30]. Cano *et al.*[31], by means of in situ hybridization, showed that Snail protein is a strong repressor of E-cadherin transcription. Snail specifically interacts with the E-pal element of the mouse E-cadherin promoter through its E2-box sequence. This was expressed by an E-cadherin-deficient murine, in human carcinoma cell lines, in tumors and in dedifferentiated and invasive regions of carcinomas. However, such expression was absent from well differentiated, non-invasive mouse and human carcinomas.

In the current study, an evaluation of the expression patterns of the membranous protein molecules of E-cadherin

and α-, β-, and γ-catenins at the peripheral invading front was correlate with the T classification of the primary tumors, the clinical stage, the mode of carcinoma invasion, and pathologic lymph node metastasis in patients with squamous cell carcinoma of the tongue. Therefore, the authors believe that the biologic potential of squamous cell carcinoma of the tongue will likely be re flected in cells at the periphery of an invasive tumor rather than at its center.

The results from the current study indicate that aberrations of E-cadherin and catenins membranous expression are a common feature in squamous cell carcinoma of the tongue. These findings have also been evidenced in various types of epithelial tumors such as colorectal[18, 32, 33], prostate[34], liver[19], breast[17], bladder[35], stomach[16], thyroid[36] and esophagus[20].

With respect to clinical implications, results have been controversial. There have been studies showing that abnormal expression of β-catenin was closely related to survival in colorectal cancer[33] and in squamous cell carcinoma of the esophagus[20], and that the underexpression of E-cadherin, α-catenin, and γ-catenin as well as an overexpression of β-catenin were correlated with survival in hepatocellular carcinoma[19].

In regard to squamous cell carcinoma of the head and neck, including oral cavity cancer, a few investigations have been published on the relationship between decreased expression of E-cadherin, but none have been published on catenins, and cervical lymph node metastasis[39, 40] and pattern of carcinoma invasion[40-42] in a small series. Recently, Chow *et al.*[43] reported that a reduced expression of γ-catenin correlated with cervical lymph node metastasis. It has been supported that the pattern of carcinoma invasion is reflected in the aggressive behavior of the tumor[21, 22, 27-30]. Of particular interest for further study might be an investigation of whether or not diffuse invasive carcinoma, which is the most aggressive morphologic characteristic of the squamous cell carcinoma of the oral cavity, could be subdivided by assessment of the expression pattern of E-cadherin and catenins by categories as a prognostic parameter.

Some authors reported that the combined evaluation of E-cadherin and/or catenins is of greater prognostic value than evaluation of the expression patterns of the individual proteins[16, 35]. Bukholm *et al.*[17] have described a reduced expression in either E-cadherin, α-catenin, β-catenin, or γ-catenin associated with lymph node metastasis in 90 patients with breast carcinoma.

On the contrary, some studies have reported that reduced E-cadherin and catenins expression in primary cancer have no significant predictive value regarding clinicopathologic features and metastasis[37, 38]. Thus the clinical relevance of aberrant expression of E-cadherin and catenins will differ from the primary site of the tumors and the histological types in relation to the number of tumors examined, selection of stage and grade of the tumors, and probably to the assessment of staining patterns of immunoreactivity of the proteins.

It is important to note that alterations in any component may lead to disrupting the function of the complex. The mechanisms of inactivation of the E-cadherin-mediated cell adhesion system are multiple: mutations of the genes for E-cadherin and catenins (Oyama *et al.*)[44], reduced ex pression of E-cadherin due to CpG methylation around the promoter region of the E-cadherin gene (Saito *et al.*)[45], aberrant tyrosine phosphorylation of β-catenin initiated epidermal growth factor (Shibamoto *et al.*)[46], and so on. These results indicated that E-cadherin immunoreactivity does not always imply the presence of a functionally normal E-cadherin-mediated cell adhesion system. Indeed, the results of the present study through multivariate analysis showed close correlation between combined evaluation of reduced expression of E-cadherin and/or γ-catenin and occult cervical lymph node metastasis. Thus, to predict tumor invasion and metastasis in squamous cell carcinoma of the tongue, it is useful to investigate not only the expression of E-cadherin but also the expression of the catenins.

In the current study, the significant correlation between reduced membranous E-cadherin, α-, β-, and γ-catenins expression at the peripheral invading front of the tumor and the pathologic lymph node metastasis supports the theoretical assumption of the biological importance of the molecules. Multivariate analysis revealed that reduced membranous E-cadherin and γ-catenin expression at the peripheral invading front of the tumor was the only independent predictor for lymph node metastasis. These results suggest that an assessment of membranous E-cadherin and γ-catenin expression for clinical use might be useful for patients with squamous cell carcinoma of the tongue.

In conclusion, the assessment of E-cadherin and catenins expression patterns at the peripheral invading front of the tumor is clinically useful, especially for selecting patients who require neck dissection.

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