P-Cadherin Expression Reduced in Squamous Cell Carcinoma of the Oral Cavity

An Indicator of Poor Prognosis

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BACKGROUND. The loss of cadherin expression has been shown to correlate to the invasion and metastasis of many types of carcinomas. The purpose of the current study was to evaluate whether the impaired expression of E-cadherin (E-cad) and P-cadherin (P-cad) correlated with the clinical evolution and prognosis of oral squamous cell carcinoma (OSCC).

METHODS. The authors used immunohistochemical methods to analyze the expression pattern of E-cad and P-cad in healthy oral mucosa, in oral carcinoma in situ (CIS), and in surgical samples of 50 patients with the early stages (Stages I–II) of OSCC.

RESULTS. E-cad showed weak expression in the basal layer of the healthy oral mucosa and reduced expression in patients with oral CIS. P-cad expression was conserved on the basal and suprabasal layers of the healthy mucosa and, also, in the CIS. In the group of patients with OSCC, univariate analysis demonstrated that reduced expression of E-cad or P-cad correlated significantly with locoregional disease recurrence in the follow-up ($P = 0.03$ and $P = 0.01$, respectively). However, only the reduction in the expression of P-cad emerged as an independent prognostic marker in the multivariate analysis ($P = 0.04$, hazard ratio = 8.06).

CONCLUSIONS. These findings suggested that a decrease in E-cad and/or P-cad expression may contribute to the invasive potential of early OSCC. According to the current data, P-cad expression may be a potential independent prognostic factor in patients with OSCC. ***Cancer* 2005;103:960–9.** © *2005 American Cancer Society.*

KEYWORDS: cadherin, cell adhesion molecule, oral squamous cell carcinoma, poor prognosis.

Oral squamous cell carcinoma (OSCC) is the most frequent malignant tumor of the oral cavity. In spite of improved therapeutic procedures, patients with advanced-stage OSCC have a generally poor prognosis, with an overall 5-year survival rate that ranges from 20% to 60%.[1–3] The cellular factors that underlie locoregional and distant metastasis of these neoplasms are not well understood. An extensive effort has started to identify features of the oral tumors that predict treatment response and prognosis.

Cell adhesion molecules are fundamental determinants of tissue organization during the development of adult organisms. The cadherins constitute a large family of glycoproteins that mediate cell-to-cell adhesion through calcium-dependent, homotypic interactions.[4] The best characterized and most widely distributed members of this family are the "classical" cadherins. Designated by their tissue distribution, several subclasses of classical cadherins exist, including the epithelial E-cadherin (E-cad; also named L-Cam, uvumorulin, Arc-1,

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and cell-CAM 120/80) and the placental P-cadherin (P-cad). These molecules are believed to be involved not only in mediating intercellular adhesion, but also in facilitating transduction of signals that influence several important biologic processes, including cellular motility, proliferative activity, and apoptosis.[5,6]

E-cad is the predominant cadherin family member that is expressed in epithelial tissue. The expression of this cadherin in cancer cells has been studied extensively in many forms of human tumors including OSCC, showing that its down-regulation is related to tumor progression and acquisition of an invasive phenotype.[7–11] However, the expression of P-cad has not been studied as extensively and little is known about the role of this molecule in oral carcinogenesis.

The current study was undertaken to examine tissue samples from a series of patients with early-stage OSCC for the expression of the classic adhesion molecules E-cad and P-cad, to establish the relation with clinicopathologic parameters, and to assess the potential prognostic role in predicting disease recurrence, disease-free interval, and survival.

MATERIALS AND METHODS

Patient Material

Routinely formalin-fixed and paraffin-embedded material from 50 patients with previously untreated primary OSCC was obtained from the files of the Department of Pathology, Hospital de la Princesa (Madrid, Spain). All specimens correspond to patients who underwent local surgery and neck dissection at our hospital (Department of Oral and Maxillofacial Surgery) between 1987 and 2000. The neck was routinely treated by modified radical neck dissection, ipsilateral ($n = 33$) or bilateral ($n = 17$). Postsurgery, all patients had histologically confirmed negative lymph nodes and were classified as having intraoral-confined disease. The site of primary origin of the tumors was the tongue in 27 patients (54%) and the floor of the mouth in 23 patients (46%). This series comprised patients with early-stage OSCC (Stage I [$n = 18$] and Stage II [$n = 32$]). All pathology slides were reviewed to determine histologic grade, status of surgical margins, perineural metastasis, and tumor thickness using an optical micrometer. The patients were classified in 2 groups according to their tumor thickness: ≤ 5 mm and > 5 mm. The evaluation of histopathologic differentiation was performed according to the World Health Organization grading system. Sixteen patients had well differentiated carcinoma, 28 had moderately differentiated carcinoma, and 6 had poorly differentiated carcinoma. Nine patients received postoperative radiotherapy according to the protocol of our hospital. Five patients whose surgical margins involved tumor received postsurgical radiotherapy. Postsurgical radiotherapy was received by another 4 patients who had evidence of perineural invasion, tumor thickness > 10 mm, and moderately or poorly differentiated tumors as determined by histopathologic grading of the lesion.

Complete clinical follow-up data were retrieved from the oral cancer database of the Department of Oral and Maxillofacial Surgery, including disease-specific and disease recurrence-free survival of the patients. The minimum postoperative follow-up of patients alive without evidence of disease was 36 months.

Immunohistochemistry

E-cad and P-cad immunostaining was performed directly in representative blocks using the Envision method in the absence or presence of horseradish peroxidase (HRP; Dako A/S, Glostrup, Denmark) with heat-induced antigen retrieval. Briefly, 3 μM paraffin-embedded tissue sections were deparaffined in xylene, rehydrated through a series of graded alcohols, and washed in phosphate-buffered saline. Before endogenous peroxidase blocking, antigen was retrieved by heating the slides in 1 mM ethylenediaminetetraacetic acid (pH 8) for 2 minutes in a pressure cooker. P-cad mouse monoclonal antibody (MoAb; Novocastra Laboratories Ltd., Newcastle, U.K.) and E-cad MoAb (Transduction Laboratories, Lexington, KY) were used as primary antibodies at dilutions of 1:200 and 1:800, respectively. Envision+-peroxidase anti-mouse polymer labeled with HRP (Dako Corporation, Carpinteria, CA) was used as the secondary antibody. The reaction product was developed with a diaminobenzidine substrate-chromogen solution (Dako, Glostrup, Denmark).

Evaluation of Immunohistochemical Staining

For use as a control for immunostaining, three samples of clinically healthy oral mucosa were obtained. Also, three specimens of oral carcinoma in situ (CIS) were morphologically analyzed. In relation to the staining pattern of oral carcinomas, a semiquantitative estimation based on the staining intensity and relative abundance of cadherin-immunoreactive cells towards the invasive front of the tumor tissue was performed independently by two investigators (C.G. and E.G.M.). The determination was performed blindly without knowledge of the eventual clinical outcome. When differences between the observers occurred, the respective slides were reinvestigated jointly by both investigators.

The semiquantitative method of estimation of P-cad and E-cad expression has been reported previ-

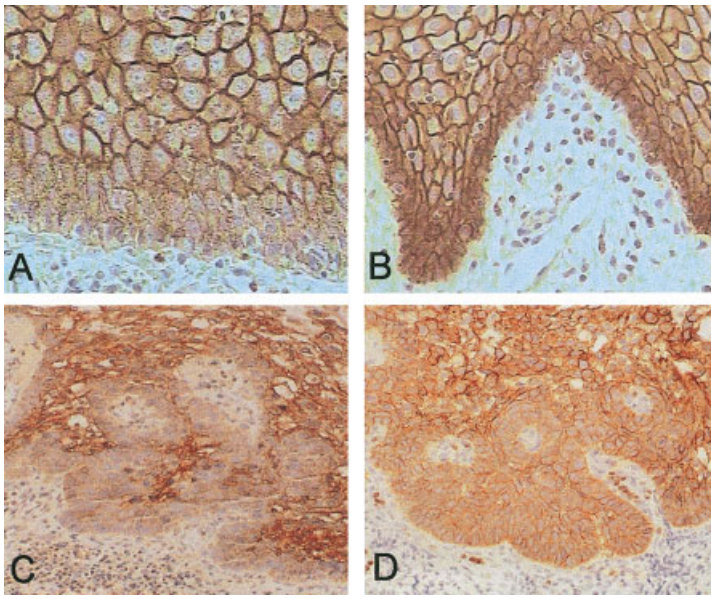

FIGURE 1. Cadherin expression in normal healthy oral mucosa and in oral carcinoma in situ (CIS). (A) E-cadherin immunostaining in normal pluristratified epithelium of the oral cavity, showing a membranous pattern of staining in the suprabasal layer and in the stratum spinosum. (B) P-cadherin immunostaining in normal pluristratified epithelium of the oral cavity, showing a homogeneous expression from the basal cells to the stratum spinosum. (C) E-cadherin immunostaining in oral CIS. Note cytoplasmic expression fundamentally in the basal and suprabasal layers. (D) P-cadherin immunostaining in oral CIS. The basal cells show a strong membranous expression of this cadherin. Original magnification $\times$ 20 (C,D); $\times$ 40 (A,B).

ously by our group.[12–14] In the invasive front, the intensity of the stain was the intensity was graded from 0 (equivalent to background staining of the acellular stroma) to +3 (intense stain equivalent to normal oral epithelium). The abundance of cadherin-positive cells was graded from 0 to 4 by counting $\geq$ 100 cancer cells in areas of heterogeneous cadherin expression (0, $<$ 5% of positive cells; 1, 5–25%; 2, 26–50%; 3, 51–75%; 4, 76–100%). With these data, a composite score was obtained by adding the values of the immunoreaction intensity and relative abundance. Conserved cadherin expression (EC) was estimated when the composite score was 6 or 7. Scores of 0–5 indicated reduced cadherin expression (ER).

Statistical Analysis

The primary location of the lesion, the degree of histologic differentiation, the pT stage, perineural metastasis, tumor thickness, the disease-specific survival, and disease recurrence in the follow-up were analyzed in relation to E-cad and P-cad expression using the Pearson chi-square test with Yates correction where applicable. Disease-specific survival was considered as the period from receipt of primary surgery to death of the patient of oral carcinoma or last contact. As another measure of prognosis, we examined disease recurrence- free survival, defined as the time from initial surgical treatment to the date of first disease recurrence or last contact. Disease-specific survival and disease recurrence-free survival were estimated according to the product-limit method of Kaplan and Meier.[15] The statistical significance of differences between distributions was examined by the log-rank test (Mantel–Haenszel method).[16] The prognostic importance of tumor location, histologic grading, tumor size, surgical margin status, postoperative adjuvant radiotherapy, tumor thickness, perineural metastasis, and expression of E-cad and P-cad were analyzed using the Cox proportional hazards model.[17] P values < 0.05 were considered significant. Statistical analysis was conducted using SPSS, Version 8.0 (SPSS Inc., Chicago, IL).

RESULTS

Patterns of Cadherin Expression

Healthy oral mucosa

In the normal mucosa, both E-cad and P-cad showed a strong membranous pattern of staining. There was no expression of cadherins at the stromal surface of basal epithelial cells facing the basement membrane. The cells of the parabasal layer and stratum spinosum showed uniform and strong expression of E-cad along the entire circumference of each cell but there was only weak E-cad expression in the basal layer (Fig. 1A). The basal and suprabasal layers as well as the stratum spinosum displayed expression of P-cad (Fig. 1B).

Oral carcinoma in situ

These samples showed reduced expression of E-cad in the basal and suprabasal layers (Fig. 1C). However, membranous immunoreactivity of P-cad was observed in all epithelial layers (Fig. 1D). The expression of this cadherin in the basal cells suggests a relation with the maintenance of the cellular cohesion of these cells.

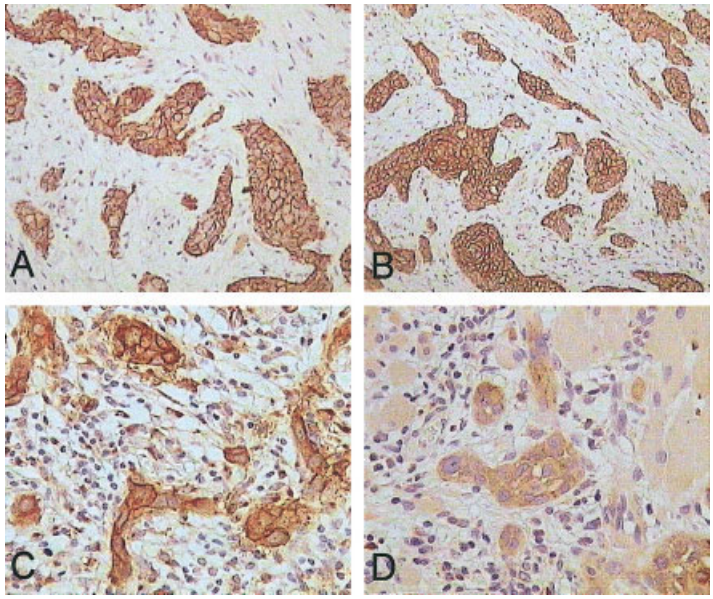

FIGURE 2. Two different groups of cadherin expression in oral squamous cell carcinoma. (A) Group 1: E-cadherin expression conserved. (B) Group 1: P-cadherin expression conserved. (C) Group 2: E-cadherin expression conserved. (D) Group 2: P-cadherin expression reduced. Original magnification × 20 (A,B); × 40 (C,D).

Oral squamous cell carcinoma

According to the expression of E-cad and P-cad, the patients were classified into four groups: 1) E-cad and P-cad EC (Fig. 2A and B), 2) E-cad EC and P-cad ER (Fig. 2C and D), 3) E-cad ER and P-cad EC, and 4)E-cad and P-cad ER. There were 9 patients in Group 1, 9 patients in Group 2, 12 patients in Group 3, and 20 patients in Group 4.

Relationship among Cadherin Expression, Clinicopathologic Variables, and Prognosis

The correlation between E-cad expression and P-cad expression, as well as clinicopathologic parameters and prognosis is summarized in Table 1. The expression of E-cad and P-cad did not correlate with the primary location of the tumor, perineural metastais, or tumor size (pT). Also, no significant difference was found with the degree of differentiation ($P > 0.05$). However, a statistical relation was observed between loss of E-cad expression and tumor thickness > 5 mm (chi-square = 4.607, $P = 0.03$). In the follow-up, 11 patients developed disease recurrence. The characteristics of these patients are summarized in Table 2. The loss of P-cad expression significantly correlated with locoregional disease recurrence in the follow-up (chi-square = 6.975, $P = 0.01$) and, in relation to E-cad expression, a lower but still significant correlation was found between loss of this cadherin and disease recurrence (chi-square^2 = 5.003, $P = 0.03$). The rate of disease recurrence in the 4 groups defined earlier in the text was 0% in Group 1 (0 of 12 patients), 11.1% in Group 2 (1 of 9 patients), 11.1% in Group 3 (1 of 9 patients), and 45% in Group 4 (9 of 20 patients). In the global series, 5 patients died of disease during the follow-up, 1 from Group 3 and 4 from Group 4.

Perineural invasion was observed in 7 samples (14%). A tumor thickness < 5mm was observed in 26 patients, whereas 24 patients showed a tumor thickness ≥ 5 mm. Perineural invasion or tumor thickness was not correlated with disease recurrence during follow-up (only one patient with perineural invasion had disease recurrence). Five samples showed involved surgical margins (all cases pT2), but no relation was observed between margin status and disease recurrence. None of the samples that showed invasion of surgical margins was associated with disease recurrence during the follow-up. In the global group of 50 patients, the 2-year disease-specific survival rate was 91.7%, whereas the 2-year disease recurrence-free survival rate was 83.8%. Figure 3 shows the probability of disease recurrence-free survival obtained with the Kaplan–Meier method for the groups E-cad EC and E-cad ER. Figure 4 shows the same curves for P-cad expression. The P values for disease recurrence-free survival were significantly lower for patients with E-cad ER ($P = 0.03$) or P-cad ER ($P = 0.01$).

When cadherin expression, primary location, tumor stage, grade of differentiation, surgical margin status, postsurgical radiotherapy, tumor thickness, and perineural invasion were subjected to Cox multivariate regression analysis, P-cad expression was selected as the independent predictor of disease recurrence-free survival. The relative risk of disease recurrence was eight times higher in the group of patients with tumors showing P-cad ER than in the

TABLE 1
Correlations among Cadherin Expression, Clinicopathologic Parameters, and Prognosis in 50 Patients with Oral Squamous Cell Carcinomas

	E-cadherin expression (%)			P-cadherin expression (%)		
Characteristics	**ER**	**EC**	***P* value**	**ER**	**EC**	***P* value**
Location			0.3			0.34
Tongue	19 (59.4)	8 (44.4)		14 (48.3)	13 (61.9)	
Floor of the mouth	13 (40.6)	10 (55.6)		15 (51.7)	8 (38.1)	
Tumoral stage			0.12			0.12
Stage I	9 (28.1)	9 (50)		13 (44.8)	5 (23.8)	
Stage II	23 (71.9)	9 (50)		16 (55.2)	16 (76.2)	
Grade			0.06			0.3
Well differentiated	13 (40.6)	3 (16.7)		9 (31)	7 (33.3)	
Moderately differentiated	14 (43.8)	14 (77.8)		15 (51.7)	13 (61.9)	
Poorly differentiated	5 (15.6)	1 (5.6)		5 (17.1)	1 (4.8)	
Perineural spread			0.65			0.08
Yes	5 (15.6)	2 (11.1)		2 (6.9)	5 (23.8)	
No	27 (84.4)	16 (88.9)		27 (93.1)	16 (76.2)	
Tumor thickness (mm)			0.03			0.96
< 5	13 (40.6)	13 (72.2)		15 (51.7)	11 (52.4)	
≥ 5	19 (59.4)	5 (27.8)		14 (48.3)	10 (47.6)	
Survival			0.14			0.38
Dead of tumor	5 (15.6)	0 (0)		4 (13.8)	1 (4.8)	
Alive or dead, NED	27 (84.4)	18 (100)		25 (86.2)	20 (95.2)	
Disease recurrence			0.03			0.01
No	22 (68.8)	17 (94.4)		19 (65.5)	20 (95.2)	
Yes	10 (31.3)	1 (5.6)		10 (34.5)	1 (4.8)	

ER: cadherin expression reduced; EC: cadherin expression conserved; NED: no evidence of disease.

TABLE 2
Summary of Patients who Developed Disease Recurrence during the Follow-Up

Patient no.	Location of primary tumor	pT	Neck dissection	Postoperative RT	Surgical margin status	Location of disease recurrence	Treatment of disease recurrence	E-cadherin expression	P-cadherin Expression
1	Tongue	T2	Ipsilateral	No	Free	Locoregional	CT	Reduced	Reduced
2	Tongue	T2	Bilateral	No	Free	Locoregional	RT	Reduced	Reduced
3	Floor of the mouth	T1	Bilateral	No	Free	Local	SR + RT	Reduced	Reduced
4	Floor of the mouth	T2	Ipsilateral	Yes	Free	Locoregional	SR + RT	Reduced	Reduced
5	Floor of the mouth	T2	Bilateral	No	Free	Local	SR + RT	Conserved	Reduced
6	Floor of the mouth	T2	Ipsilateral	No	Free	Local	SR	Reduced	Reduced
7	Tongue	T2	Ipsilateral	No	Free	Contralateral neck	SR	Reduced	Reduced
8	Floor of the mouth	T1	Ipsilateral	No	Free	Contralateral neck	SR + RT	Reduced	Reduced
9	Tongue	T2	Ipsilateral	No	Free	Contralateral neck	SR	Reduced	Reduced
10	Floor of the mouth	T1	Ipsilateral	No	Free	Local	SR	Reduced	Reduced
11	Tongue	T1	Ipsilateral	No	Free	Ipsilateral neck	RT	Reduced	Conserved

CT: chemotherapy; RT: radiotherapy; SR: surgical resection.

group of patients with tumors showing P-cad EC (Table 3).

DISCUSSION

Invasion and metastasis require multiple cellular events including cytoskeletal alterations, disruption of cell-to-cell adhesive contacts, matrix protein proteolysis, and migration.[18] Alterations of intercellular adhesion have been demonstrated extensively in malignant epithelial neoplasia and are considered to be crucial for the invasiveness and metastatic behavior of such tumors. E-cad is the major representative of the classic cadherins and its expression is often altered in neoplasia. This adhesion molecule is down-regulated or heterogeneously expressed in various invasive carcinomas compared with noninvasive tumors that

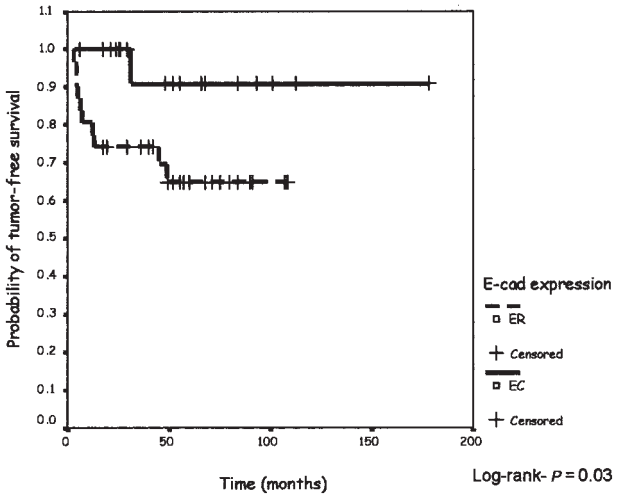

FIGURE 3. Kaplan–Meier curves show probability of disease recurrence-free survival for patients with E-cadherin (E-cad) expression conserved (EC) versus patients with E-cad expression reduced (ER; log-rank test).

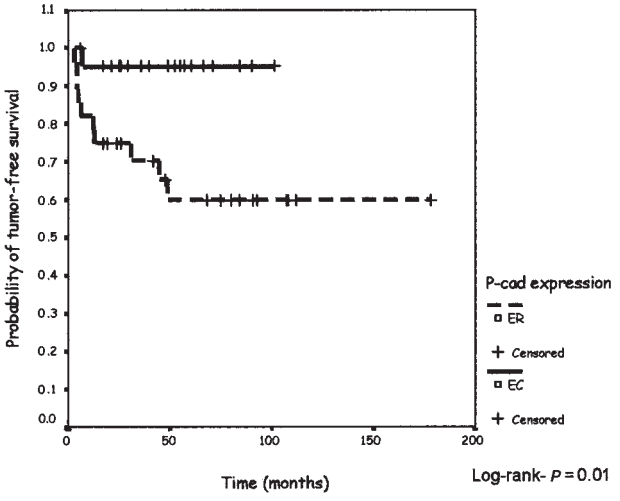

FIGURE 4. Kaplan–Meier curves show probability of disease recurrence-free survival for patients with P-cadherin (P-cad) expression conserved (EC) versus patients with P-cad expression reduced (ER; log-rank test).

show strong expression, such as it was observed in basal cell carcinomas.[19] Decreased levels of E-cad protein expression have been observed frequently in human tumors, including squamous cell carcinoma of the head and neck.[20] Several investigators have proposed that, in carcinomas, E-cad functions as an invasion-supressor molecule such that its loss permits or enhances the invasion of adjacent normal tissues.[21,22]

In the group of classic cadherins, E-cad has been well characterized and studied, whereas P-cad was identified by Nose and Takeichi [23] but its expression and role in most of carcinomas are still understood

TABLE 3
Multivariate Analysis of Disease-Free Survival (Cox Regression Analysis)

Characteristics	*P* value
Primary location	0.87
Histologic grading	0.06
pT stage	0.47
Surgical margin	0.17
Postsurgical radiotherapy	0.24
Tumor thickness	0.16
Perineural metastasis	0.86
E-cadherin expression	0.05
P-cadherin expression	0.04[a]

[a] A relative risk of 8.06 with a 95% confidence interval of 1.03–62.98.

poorly. P-cad was described originally in mouse placenta but has also been found in epithelial tissues, including lung epithelia,[24] basal cells of the skin,[25] and myoepithelial cells of the mammary gland.[26] Shimoyama et al.[27] have suggested that the expression of P-cad in the lower layers of stratified epithelia is related to a high cell turnover, whereas E-cad expression may be associated with general differentiation features. Recently, Tamura et al.[28] showed that the expression of P-cad was associated with proliferation, because its expression was observed in the proliferating epithelial cells of cancerous rat tongue tissue. In our study, the expression of E-cad and P-cad molecules was demonstrated in the tissue architecture of the normal oral mucosa. In the suprabasal layer and in the stratum spinosum, the expression of E-cad was similar to that of P-cad, whereas only P-cad was expressed in the basal cell layer (which is believed to be the proliferative zone of oral epithelium). Also, the samples of oral CIS showed weak expression of E-cad, whereas basal expression of P-cad was maintained, suggesting a function of this cadherin in the maintenance of cellular cohesion of this zone and, probably, avoiding the rupture of the basal membrane by the tumor cells.

Although P-cad expression is observed in tissues with a high turnover such as stratified epithelia, normal simple epithelia show no expression of this cadherin. P-cad expression has been associated with unfavorable prognosis in patients with breast,[29] endometrial,[30] or ovarian carcinoma.[31] In lung carcinomas, up-regulation of P-cad expression has been related to poorly differentiated tumors.[27] In contrast, reduced P-cad expression seems to correlate with tumor progression in gastric carcinoma.[32] In basal cell carcinoma, P-cad expression was related to infiltrative growth. However, it has been suggested that this cadherin may contribute to the maintenance of the

basaloid epithelial phenotype in cells with reduced E-cad expression.[33] Sakaki et al.[34] reported in a study of gingival carcinoma that the occurrence of reduced-type expression of P-cad increases significantly with the grade of differentiation, culminating in a complete loss of expression in poorly differentiated carcinomas. Williams et al.[35] observed a reduction or loss of P-cad expression in the deep invasive margin of oral carcinomas and in poorly differentiated tumors. Our data are in line with these previous works and we relate P-cad expression to prognosis in oral carcinoma as well. These conflicting results depending on tumor types can be associated, at least partially, with the type of tissue origin of the neoplasms.

The findings of the current study suggest that down-regulation of E-cad and P-cad is a common malignant event in OSCC progression, and correlates closely with the prognosis. The reduced expression of these adhesion molecules in tumor invasion suggests that these cells can detach easily. The dissociation of cancer cells from each other may enable the carcinoma cells to invade the stroma and cause metastasis. Down-regulation of cadherin expression allows malignant cells to escape from their site of origin, degrade the extracellular matrix, acquire a more motile and invasive phenotype, intravasate into blood vessels, and, finally, invade and metastasize.[36] It seems that the entrance of malignant cells into the intravascular compartment is associated with up-regulated cadherin expression, whereas the subsequent exit into extravascular tissues is associated with lower cadherin levels, as has been suggested recently in a study about E-cad expression and tumor emboli in breast carcinoma.[37] Immunohistochemical studies on lymph node and distant metastases from a variety of primary tumors have shown that the low expression of E-cad in poorly differentiated tumors is sometimes transient.[21] In a previous study focused on gastric carcinoma, it was observed that down-regulation of E-cad expression in the primary tumor may allow cell dispersal into the circulation, whereas re-expression of this cadherin may allow tumor-host adhesion at the metastatic site.[38] These changes in the expression of E-cad are feasible because epigenetic silencing of gene expression by promoter CpG hypermethylation has been considered an important alternative mechanism in inactivating tumor suppressor genes and tumor-associated genes in cancers.[39]

It is difficult to find another report in the literature that is completely comparable with ours. The current study was limited to a population of patients with negative lymph nodes. It is important to study prognostic factors in a group of patients with small tumors, because patients with unfavorable histologic prognostic markers would eventually be candidates for a more aggressive initial treatment regimen or adjuvant therapies. For this reason, we selected 50 patients with oral carcinoma tumors that did not exceed a dimension of 4 cm and who had received a complete clinical follow-up.

It has been reported previously that tumor thickness is an important factor in the prognosis of patients with oral carcinoma. In a retrospective study, O-Charoenrat et al.[40] showed that a tumor thickness > 5 mm significantly correlated with neck metastasis. The prognostic significance of this factor in head and neck carcinomas had been reported consistently in the literature.[41–44] In fact, only one study found no relation between tumor thickness and locoregional disease recurrence.[45] Several reports[46, 47] have shown a strong correlation between perineural invasion at the primary tumor site and neck metastasis but other authors have denied this relation.[48,49] In the current series, neither tumor thickness nor perineural invasion were found to affect the locoregional control. We believe that this special result is related to the small sample of patients studied and their characteristics (T1, T2 tumors without neck metastasis). However, the current study demonstrates that patients with free surgical margins or with P-cad EC had a remarkably favorable prognosis.

In general, patients with early-stage oral carcinoma have a good prognosis. However, in 1996 Bundgaard et al.[50] demonstrated that 25% of these patients had a poor prognosis despite the smallness of the tumor. In the follow-up, our study shows 3 patients (6% of the global group) with disease recurrence in the contralateral neck, which was related to E-cad and P-cad ER. In a series of 50 patients with early-stage tongue carcinoma treated with local resection and neck dissection, Yuen et al.[51] described subclinical contralateral neck metastasis in 2 patients (4%) and 3% of patients with pN0 who did not receive adjuvant radiotherapy developed regional disease recurrence on the contralateral neck. In addition, we observed a patient treated for a T1 tongue tumor who developed ipsilateral neck disease recurrence. This feature cannot be considered exceptional, as was observed by Kurita et al.[52] in a group of patients treated with local radium needle implants and radical neck dissection. Ambrosch et al.[53] reported in a series of oral and pharyngeal tumors treated by laser local resection and selective neck dissection that 4% of patients (3 of 73) with pN0 tumors had ipsilateral disease recurrence. Al-Rajhi et al.[54] reported a 19% rate of ipsilateral disease recurrence in patients with early-stage squamous cell carcinoma of the tongue. The current series reports an incidence of 14% of local or

locoregional disease recurrence (7 of 50). In these seven patients, reduced P-cad expression was appreciated. Kligerman et al.[55] performed a study of patients who received supraomohyoid neck dissection for early-stage OSCC. Their work showed that 24% of patients had disease recurrence in the follow-up. In addition, a similar study performed by Jones et al.[56] demonstrated that of 49 patients treated surgically (39% with postsurgical radiotherapy), 9 (18%) developed disease recurrence during the follow-up period.

In the current study, univariate analysis demonstrated that reduced expression of P-cad significantly influenced disease recurrence-free survival, and that low expression of E-cad and P-cad was a better prognostic factor than either cadherin alone. Regression analysis was used to evaluate a group of parameters and to determine their relative contributions to predicting either disease-specific or disease recurrence-free survival. This analysis of multiple clinical and histologic parameters demonstrated that P-cad expression was the single most important variable in predicting disease recurrence-free survival. Although histologic grading and E-cad expression contribute to the determination of prognosis as single parameters, they appear to have less value than P-cad expression in predicting disease recurrence-free survival. The analysis of P-cad expression in OSCC might be useful in identifying tumor areas with more aggressive potential, in addition to the study of E-cad expression. The current study data indicate that early-stage OSCC tumors may or may not express E-cad and P-cad and the prognosis of these patients depends on the loss of expression of these adhesion molecules.

Recent studies suppose a role for P-cad in human cancers[57] but to our knowledge to date none has reported its expression in OSCC. As it has been suggested for E-cad expression,[58] immunohistochemical detection of P-cad expression might be useful in predicting disease recurrence-free survival and in identifying patients with clinically negative lymph nodes who are at risk of occult metastases and who would benefit from more extensive lymph node dissection. The weak expression of E-cad and/or P-cad in conjunction with other clinicopathologic parameters can be used in the decision-making process to determine whether more aggressive patient management is required. In fact, four patients in the current study (with E-cad and P-cad ER) had an unfavorable outcome. A wider surgical resection margin or the addition of adjuvant radiotherapy may be other options for patients with poor prognosis.

We conclude that in the early stages of oral carcinogenesis, the reduction of P-cad expression is a prognostic marker for a disease recurrence-free interval. We also believe that in OSCC, changes in E-cad and P-cad expression at the invasive tumor front may promote invasion and metastasis. In addition, the findings of the current study confirm previous reports that have shown an association between the reduction of membranous E-cad expression and disease progression. P-cad is a better prognostic indicator than the altered expression of other cadherins, including the E-cad ER. However, these findings warrant further studies to examine the role of P-cad expression as a prognostic factor in larger patient samples with clinical follow-up data. Up-regulation of classical cadherins may serve as a novel antitumor therapeutic strategy by stimulating cells to change to a benign behavior.

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