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Prognostic significance of E-cadherin and β-catenin expression in HPV-negative oropharyngeal squamous cell carcinomas

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Abstract

Background: The purpose of this work was to investigate the prognostic significance of E-cadherin and β-catenin expression in surgically treated human papillomavirus (HPV)-negative patients with oropharyngeal squamous cell carcinoma (SCC).

Methods: Consecutive patients with oropharyngeal SCC who underwent surgical treatment between 1990 and 2009 were retrospectively collected. Immunohistochemical analysis of E-cadherin and β-catenin expression was performed on tissue microarrays.

Results: E-cadherin and β-catenin expression was evaluable in 232 cases. Low membranous E-cadherin, low membranous β-catenin expression, and nuclear β-catenin expression were associated with a poorer disease-specific and overall survival, although the differences were only significant for β-catenin membranous expression ($P = .024$ and $P = .016$, respectively). In multivariate analysis, nodal metastasis and low membranous β-catenin expression were significant independent predictors of reduced disease-specific and overall survival.

Conclusion: Low membranous β-catenin expression is a significant independent predictor of both reduced disease-specific and overall survival in patients with HPV-negative oropharyngeal SCC.

KEYWORDS
β-catenin, E-cadherin, human papillomavirus (HPV), oropharynx, squamous cell carcinoma

1 | INTRODUCTION

The incidence of oropharyngeal squamous cell carcinoma (SCC) is increasing, whereas decreasing tumors are arising in other subsites within the head and neck area.[1–3] This phenomenon has been observed worldwide and also in Spain.[4] The opposite incidence trends for head and neck squamous cell carcinoma (HNSCC) and oropharyngeal SCC are probably related to the 2 major etiological factors in these cancers. Although the habit of smoking tobacco is generally slowly decreasing, mounting evidence shows an increasing prevalence of human papillomavirus (HPV) in HNSCC, particularly in oropharyngeal SCC.[2] In addition, HPV infection has not only an etiological role but also a prognostic significance: the HPV-positive cases had a much better survival than the HPV-negative ones.[5,6]

Among many predictive factors already identified in oropharyngeal SCC, there are some that are particularly important and universally accepted, such as the HPV status, whereas other molecular markers, such as cadherin and catenin expression, remain controversial.

Dysfunction of E-cadherin/catenin complex is directly involved in carcinogenesis. The E-cadherin glycoprotein (encoded by the *CDH1* gene, located on chromosome region

16q22.1) is a Ca^{2+}-dependent intercellular adhesion molecule in epithelial cells, which plays a key role in establishing and maintaining intercellular connections and morphogenesis. The cytoplasmic terminus of the E-cadherin molecule has been linked to the actin cytoskeleton via α-catenin and β-catenin.[7] *CDH1* is a tumor suppressor gene, whose loss has been found to promote tumor invasion and metastasis.

It has also been reported in various cancer models[8] that downregulation of α-catenin and β-catenin is associated with dysfunction of E-cadherin-mediated cell adhesion, and increased metastatic potential.[9] Changes or alterations in the expression and/or function of these cell-to-cell adhesion molecules have been postulated to be an early event in the multistep process of tumor development.[10]

β-catenin is particularly interesting because it functions as both a component of cadherin-catenin adhesion system and a signaling molecule.[10] Wnt signaling inhibits β-catenin proteasomal degradation leading to β-catenin stabilization and accumulation in the cytoplasm.[11] Subsequent nuclear translocation allows it to bind to T-cell factor/lymphoid enhancer factor family of transcription factors thereby inducing the expression of target genes, such as cyclin D1.[11] Depending on its subcellular localization, β-catenin plays a dual role in carcinogenesis: as an adhesion molecule (in cell membrane) and as a signaling factor (in the nucleus). β-catenin mutations that result in β-catenin stabilization and cytoplasmic accumulation have been described.[12]

Decreased expression of E-cadherin/catenin complex components has been linked to reduced cell adhesion and increased invasiveness of cancers cells.[13] Low E-cadherin expression has been associated with a higher development of distant metastasis and a poor prognosis in patients with HNSCC.[14–19] Similarly, decreased expression of β-catenin has also been associated with poor prognosis in these patients.[19–22] Nevertheless, most studies published so far included tumors from different head and neck sites, and only very few have focused specifically in oropharyngeal SCC; however, none addressed the prognostic significance of these 2 proteins in relation to HPV status and clinical outcome.

This prompted us to investigate the prognostic significance of E-cadherin and β-catenin expression using a large homogeneous series of HPV-negative patients with oropharyngeal SCC surgically treated at a single institution.

2 | MATERIALS AND METHODS

2.1 | Patients and tissue specimens

Surgical tissue specimens from 241 consecutive patients with oropharyngeal SCC (135 of tonsillar and 106 of base of tongue origin; we did not include cases originating from the soft palate) who underwent surgical treatment at the Hospital Universitario Central de Asturias between 1990 and 2009 were retrospectively collected, after institutional review board guidelines. Written informed consent was obtained from all patients. All patients included in this retrospective study underwent surgical treatment of the primary oropharyngeal SCC and postoperative radiotherapy when indicated. The inclusion criteria were: (1) diagnosis of SCC of the tonsil or base of tongue, which was confirmed by biopsy; (2) patients not previously treated for any neoplasm; (3) absence of detectable HPV infection (following the algorithm for HPV detection described below); and (4) curative surgery as the first treatment. Exclusion criteria were: (1) inoperable disease or unresectable tumors; (2) distant metastases at the time of admission; (3) presence of other primary tumors; and (4) patients who refused surgical treatment. The patients' data were collected from the medical records and included sex, age, tobacco and/or alcohol consumption, tumor location and size, pT and pN classification, treatment (surgery and postoperative radiotherapy), and clinical follow-up (recurrence, occurrence of a second primary tumor, and death).

Representative tissue sections were obtained from archival, formalin-fixed paraffin-embedded blocks and the histological diagnosis was confirmed by an experienced pathologist. The series included 110 well, 90 moderately, and 41 poorly differentiated tumors, the histological grade was determined according to the degree of differentiation of the tumor.

All patients had a single primary tumor and received no treatment before surgery. Only 10 patients were women, and the mean age was 58 years (range 30-85 years). All but 5 patients were habitual tobacco smokers, 126 moderate (1-50 pack-years) and 110 heavy (>50 pack-years), and 225 were habitual alcohol drinkers, 70 moderate (<40 gr/day) and 155 heavy (>41 gr/day). The stage of the tumors was determined according to the TNM system of the International Union Against Cancer (seventh edition): 6 tumors were stage I, 19 were stage II, 40 were stage III, 149 were stage IVa, and 27 were stage IVb. No patient had distant metastases at the time of diagnosis. One hundred sixty-three of 241 patients (68%) received postoperative radiotherapy. As a general rule, postoperative radiotherapy was indicated in all patients with stage IV disease, and in patients with stages II-III with adverse pathological features (affected margins or extranodal invasion). Some of these patients did not receive postoperative radiotherapy due to postoperative complications that extensively delayed its administration or patient refusal.

2.2 | Tissue microarray construction

Morphologically representative areas were selected from each individual formalin-fixed paraffin-embedded tumor block for the construction of a tissue microarray (TMA). Three 1-mm cylinders were taken to construct TMA blocks, as described previously.[23] A total of 10 TMAs were created,

containing 3 tissue cores of each of the 241 oropharyngeal SCCs. In addition, each TMA included 3 cores of normal epithelium (tonsil) as an internal control.

2.3 | Immunohistochemistry

The TMAs were cut into 3-μm sections and dried on Flex IHC microscope slides (Dako Cytomation, Glostrup, Denmark). Immunohistochemistry was performed using an automatic staining workstation (Dako Autostainer; Dako Cytomation) with the Envision system and diaminobenzidine chromogen as substrate. The following primary antibodies were used: anti-E-Cadherin (BD Biosciences) at 1:4000 dilution, and anti-β-Catenin (BD Biosciences) at 1:200 dilution.

Samples were evaluated by 2 independent observers without knowledge of clinical data. Immunostaining was quantified as follows: quantity scores from 0 to 3 were respectively assigned if 0%, 1%-10%, 11%-50%, and 51%-100% of the tumor cells showed membranous staining. The staining intensity was rated on a scale of 0-2 (0 = negative, 1 = weak, and 2 = strong). The raw data were then converted to a German immunoreactive score (IRS) by multiplying the quantity and staining intensity scores. Theoretically, the scores could range from 0-6. An IRS score equal or above the median (4 or higher) was considered strong expression and 0-3 was a weak expression. In the case of β-catenin, the presence of nuclear staining in >10% of cells was recorded as positive nuclear staining.

2.4 | Human papillomavirus detection

The algorithm used to discard the presence of HPV in these patients was previously described in detail.[24] Briefly, the presence of HPV was assessed by p16-immunohistochemistry, and those cases showing p16-positive immunostaining (70% or more tumor cells with intense nuclear and cytoplasmic staining) were subjected to high-risk HPV DNA detection and genotyping by GP5+/6+-polymerase chain reaction with an enzyme immunoassay readout for detection of 14 high-risk HPV types. Subsequent genotyping of enzyme immunoassay-positive cases was performed by bead-based array on the Luminex platform. In addition, in situ hybridization with biotinylated HPV DNA probes considered to react with HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68 (Y1443; Dako Cytomation) was performed on all the carcinomas using 3 μm formalin-fixed paraffin-embedded tissue sections of the TMAs, in accord with the manufacturer's instructions.

2.5 | Statistical analysis

Chi-square and Fisher's exact tests were used for comparison between categorical variables. For time-to-event analysis, Kaplan-Meier curves were plotted. Cox proportional hazards models were utilized for univariate and multivariate analyses. The hazard ratios (HRs) with 95% confidence interval (CIs) and P values were reported. All tests were 2-sided. P values of $\leq .05$ were considered statistically significant.

3 | RESULTS

3.1 | Patterns of expression in normal epithelium and primary carcinomas

Both E-cadherin and β-catenin showed strong membranous labeling in all the cells (IRS = 6) in the normal tonsillar squamous epithelium used as a positive control (Figure 1A).

E-cadherin and β-catenin expression were evaluable in 232 cases. All but 5 carcinomas showed some E-cadherin staining. Expression was generally weaker than in normal epithelium; the mean IRS score (±SD) for carcinomas was 4.2 ± 1.8 (median 4). Examples of negative and positive E-cadherin staining are shown in Figure 1.

Membranous β-catenin expression was present in 223 cases. Similar to E-cadherin, membranous β-catenin staining was weaker than in normal epithelium, with a mean IRS score of 3.9 ± 1.9 (median 4). Nuclear β-catenin expression was observed in 40 cases (17%), and, in general, these cases showed negative or very weak membranous expression (see Figure 1).

There was a close correlation between E-cadherin and membranous β-catenin expression (Pearson correlation coefficient 0.63; $P < .001$); and an inverse correlation between E-cadherin and membranous β-catenin expression and nuclear β-catenin expression (Pearson correlation coefficient −0.259; $P < .001$ for E-cadherin, and −0.304; $P < .001$ for β-catenin).

3.2 | Associations of E-cadherin and β-catenin expression with clinicopathological parameters

Table 1 summarizes the correlations of E-cadherin and β-catenin (membranous/nuclear) expression with tobacco consumption, pT classification, nodal metastases, disease stage, pathologic grading, and tumor site. Only tumor location in the base of the tongue was significantly associated with a lower E-cadherin and membranous β-catenin expression. None of the other parameters were significantly associated with E-cadherin, membranous β-catenin, or nuclear β-catenin expression, although lower E-cadherin expression was more frequent in more advanced tumors (T3-T4, N+, and stages III-IV tumors).

3.3 | Association of E-cadherin and β-catenin expression with survival

Univariate Cox regression analysis showed that patients with tumors with low E-cadherin expression had a poorer disease-

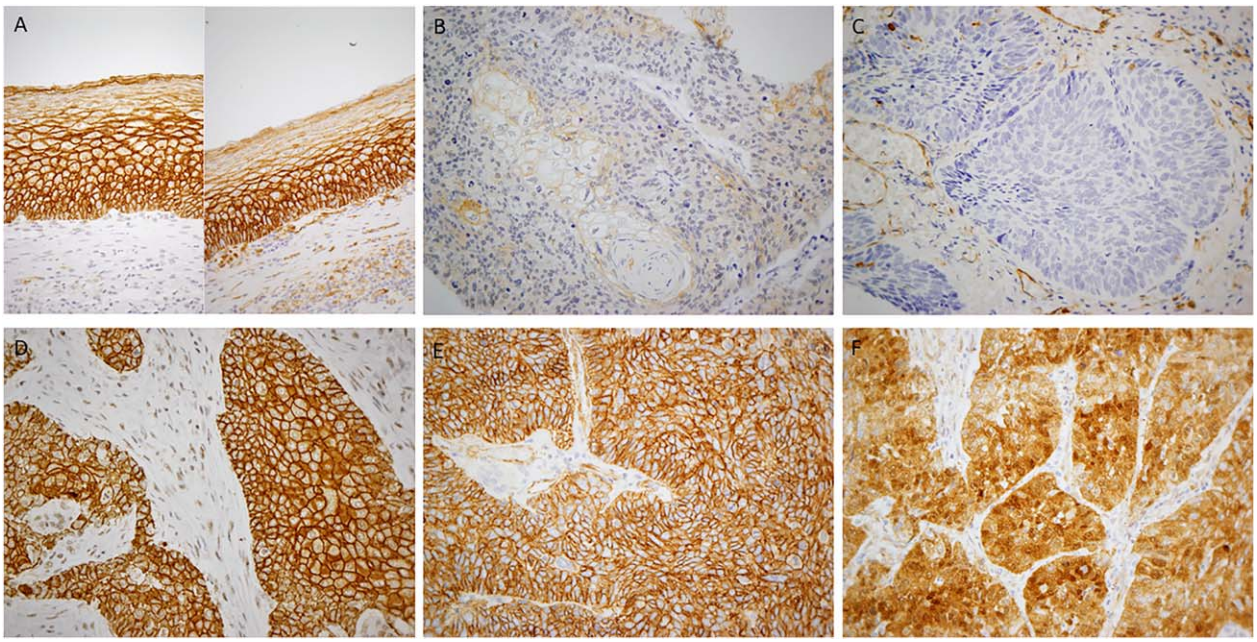

FIGURE 1 A, Expression of E-cadherin (left) and β-catenin (right) in normal epithelium, and representative examples of B, negative E-cadherin, and C, β-catenin staining, and D, positive E-cadherin, E, membranous β-catenin, and F, nuclear β-catenin staining (original magnification × 400) [Color figure can be viewed at wileyonlinelibrary.com]

specific and overall survival, although the differences were only statistically significant for the overall survival (HR 1.3; 95% CI 0.95-1.9; $P = .096$; and HR 1.44; 95% CI 1.07-1.94; $P = .017$, respectively; Figure 2A,B). Low β-catenin expression was significantly associated with both poorer disease-specific and overall survival (HR 1.5; 95% CI 1.05-2.14; $P = .024$; and HR 1.45; 95% CI 1.07-1.96; $P = .016$, respectively; Figure 2C,D). Nuclear β-catenin expression was significantly associated with lower disease-specific survival (HR 1.6; 95% CI 1.04-2.49; $P = .033$; Figure 2E), and a trend was also observed with lower overall survival (HR 1.35; 95% CI 0.91-2.0; $P = .14$; Figure 2F). Furthermore, multivariate Cox analysis was performed, including tumor size (dichotomized as T1-T2 vs T3-T4), localization (tonsil vs tongue base), lymph node metastasis (N0 vs N+), E-cadherin expression, membranous β-catenin expression, and nuclear β-catenin expression. This model showed that, in the case of disease-specific survival, only the presence of nodal metastasis and low membranous β-catenin expression were significant independent predictors of reduced disease-specific survival, whereas in the case of overall survival, these predictors were T3-T4 classification, presence of nodal metastasis, and low membranous β-catenin expression (Table 2).

4 | DISCUSSION

This study aimed to investigate for the first time the prognostic significance of E-cadherin and β-catenin expression in a large homogeneous series of HPV-negative oropharyngeal SCC.

All but 5 oropharyngeal SCCs in our series expressed E-cadherin to some degree. Low E-cadherin expression levels were overall observed in tumors compared to normal epithelium, found to associate with poor prognostic features (T3-T4 tumors, nodal metastasis, and stages III-IV tumors), and reduced survival. Our data are consistent with other studies in patients with HNSCC that reported low E-cadherin expression to be correlated with invasion and metastasis and poor clinical outcome.[14–19,25] Together, these findings are in line with the proposed function of E-cadherin as an invasion-suppressor molecule, and as such its loss may facilitate or enhance invasion of adjacent normal tissues.[8,10] Nevertheless, studies focused on oropharyngeal SCC are scarce. Stenner et al,[26] studied a small series of 48 tonsillar carcinoma samples (23 HPV-negative), and found that E-cadherin expression in the HPV-unrelated carcinomas was significantly lower in the metastases than in the primary tumors, suggesting that E-cadherin downregulation might be a crucial step for disease progression and tumor spreading. In addition, a study by Wakisaka et al,[27] using a series of 53 oropharyngeal SCCs (31 HPV-negative), reported that E-cadherin-negative tumors correlated significantly with more advanced nodal status. In marked contrast to these data and ours, Ukpo et al,[28] failed to find any association of E-cadherin expression with nodal or distant metastasis and survival. It is worth mentioning that this study analyzed 154 patients with oropharyngeal SCC, which only included a small proportion of 55 HPV-negative cases. Hence, taking

TABLE 1 Expression of E-cadherin and β-catenin in relation to the clinicopathological characteristics of patients with oropharyngeal SCC

Characteristic	No. of cases	Low E-cadherin expression (%)	*P* value	Low β-catenin expression (%)	*P* value	Nuclear β-catenin expression (%)	*P* value
Tobacco consumption							
Never	5	3 (60)	.48	3 (60)	.89	2 (40)	.9
Mild (0-20 PY)	119	57 (48)		66 (55)		17 (14)	
Moderate (20-50 PY)	91	41 (45)		46 (50)		17 (19)	
Severe (>50 PY)	17	5 (29)		9 (53)		3 (17)	
pT classification							
T1-T2	71	27 (38)	.20	32 (45)	.12	13 (18)	.50
T3	78	41 (52)		41 (52)		10 (13)	
T4	83	38 (46)		51 (61)		16 (19)	
pN classification							
N0	58	21 (36)	.13	34 (58)	.45	8 (14)	.55
N1-3	174	85 (49)		90 (52)		31 (18)	
Disease stage							
I-II	24	7 (29)	.23	12 (50)	.94	4 (17)	.09
III	39	19 (49)		21 (54)		2 (5)	
IV	169	80 (47)		91 (54)		33 (19)	
Pathological grade							
Well	108	52 (48)	.69	63 (58)	.23	20 (18)	.46
Moderately	86	36 (42)		44 (51)		11 (13)	
Poorly	39	18 (46)		17 (43)		8 (20)	
Site							
Base of tongue	103	59 (57)	.002	64 (62)	.024	14 (14)	.29
Tonsil	129	47 (36)		60 (46)		25 (19)	
Tumor recurrence							
No	93	39 (42)	.42	45 (48)	.29	12 (13)	.21
Yes	139	67 (48)		79 (57)		27 (19)	
Total cases	232	106 (46)		124 (53)		39 (17)	

Abbreviation: PY, pack-years.

this into consideration, the dominant prognostic significance of HPV in this cohort (64%) could have influenced the results.

On the other hand, we have found that low β-catenin expression is an independent predictor of reduced disease-specific and overall survival in HPV-negative patients with oropharyngeal SCC. Accordingly, the loss of β-catenin has also been associated with aggressive phenotypes and lymph node metastasis in oral cancer.[21,22] In contrast to these findings, in a large study that included 374 oral/oropharyngeal carcinoma cases (although only 4 were from the oropharynx), the authors described that all carcinomas exhibited significant alterations of β-catenin expression.[29] β-catenin protein was mainly detected in the cytoplasm of cancer cells and only focal nuclear positivity was observed. In addition, higher cytoplasmic expression was correlated significantly with poor histological differentiation, advanced stage, and worst patient outcome, suggesting that intracellular translocation of β-catenin may be important in the activation of Wnt signaling in oral cancer, and subsequently support tumor growth and aggressive tumor behavior. Nevertheless, this study did not include assessment of HPV or p16 status. Yu et al[20] analyzed 94 patients with oropharyngeal SCC (although without HPV data) and found that patients with tumors classified as low β-catenin had a 3.5-fold increased risk of local recurrence compared with the high β-catenin group ($P = .0021$). Contrasting this, in another study in 208 patients with oropharyngeal SCC, of which 138 were p16-positive, low levels of membrane β-catenin were significantly correlated with better disease-free survival in both the whole patient series and also the p16-positive subgroup.[28] Interestingly, β-catenin expression was mainly localized at

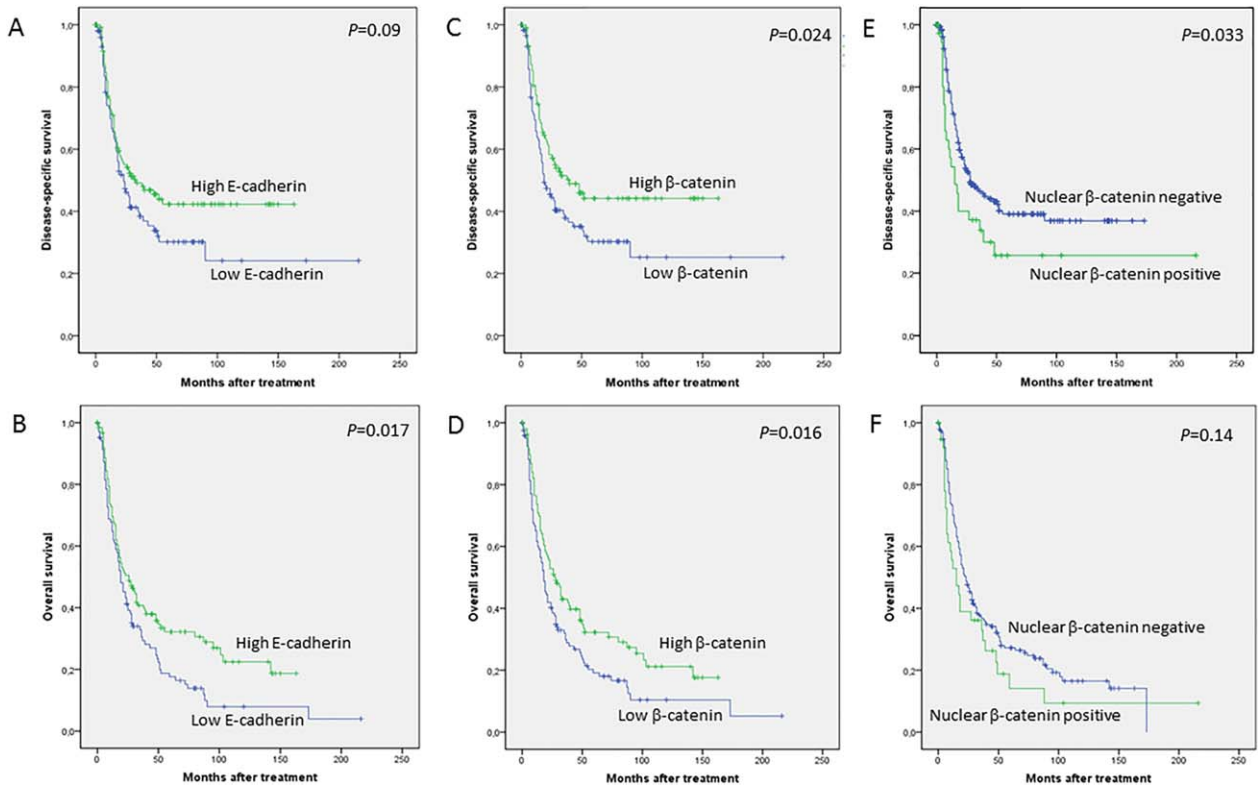

FIGURE 2 Disease-specific and overall survival curves by membranous E-cadherin expression A, B, membranous β-catenin expression C, D, and nuclear β-catenin expression E, F [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 2 Multivariate Cox regression analysis for the disease-specific survival and the overall survival

Parameter	HR (95% CI) DSS	*P* value	HR (95% CI) OS	*P* value
Tumor site				
Tonsil	1		1	
Base of tongue	1.03 (0.71-1.49)	.87	1.03 (0.75-1.42)	.84
pT classification				
T1-T2	1		1	
T3-T4	1.43 (0.097-2.18)	.068	1.42 (1.01-1.99)	.043
pN classification				
N0	1		1	
N1-3	2.25 (1.4-3.6)	.001	1.88 (1.29-2.73)	.001
E-cadherin expression				
High	1		1	
Low	1.2 (0.75-1.94)	.44	1.06 (0.70-1.61)	.78
β-catenin expression (membranous)				
High	1		1	
Low	1.55 (1.08-2.23)	.017	1.52 (1.11-2.07)	.009
β-catenin expression (nuclear)				
Negative	1		1	
Positive	1.41 (0.89-2.23)	.14	1.18 (0.78-1.78)	.43

Abbreviations: CI, confidence interval; DSS, disease-specific survival; HR, hazard ratio; OS, overall survival.

the cell membrane in p16-negative oropharyngeal SCCs, as in our study; however, in p16-positive cases, β-catenin levels decreased at the membrane. This result reflects the influence of p16-positive status on β-catenin expression, which could also explain the discrepancies between the studies on oropharyngeal SCC. Furthermore, given the close relationship between p16 expression and HPV status, these findings also highlight the importance of studying the prognostic significance of β-catenin expression in relation to HPV status, particularly in oropharyngeal SCC, as HPV infection represents a major etiological factor.

We also found that nuclear β-catenin expression in HPV-negative oropharyngeal SCC is rare (17%), and associated with a worse outcome. Comparable results have been reported in hypopharyngeal/oropharyngeal cancers.[30] However, Hu et al[31] found that nuclear β-catenin expression was common (54%) and associated with p16-positivity. Moreover, nuclear β-catenin levels were significantly associated with reduced disease-free survival only in the p16-positive subgroup, but not in the whole patient series.

To our knowledge, the present work is so far one of the largest studies to investigate the clinical significance of E-cadherin and β-catenin expression using a large homogeneous cohort of 241 patients with HPV-negative oropharyngeal SCC. By comparison, most reported data are based on studies with small number of patients, mixture of head and neck subsites, and unknown HPV status.

The weaknesses of our study are the retrospective design and the use of TMAs to evaluate protein expression. We are aware that variation of staining patterns may not be captured in TMA punches compared with whole tissue sections, and, consequently, that a small tissue punch may not be representative of the entire tumor. However, it is important to note that all TMA punches were obtained in triplicate selecting 3 different areas from each tumor (including the invasive front, center, and superficial areas), and most cases showed highly concordant staining patterns for the 3 cores, which indicate that possible biases due to tissue selection or any significant staining variation in our TMA punches were avoided. It is also worth mentioning that when analyzing diagnostic sections, tumor heterogeneity could also influence scoring.

In summary, our findings uncover that low membranous β-catenin expression is a significant independent predictor of reduced survival in HPV-negative oropharyngeal SCC.

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REFERENCES

[1] Chaturvedi AK, Engels EA, Anderson WF, Gillison ML. Incidence trends for human papillomavirus-related and -unrelated oral squamous cell carcinomas in the United States. *J Clin Oncol*. 2008;26(4):612-619.

[2] Marur S, D'Souza G, Westra WH, Forastiere AA. HPV-associated head and neck cancer: a virus-related cancer epidemic. *Lancet Oncol*. 2010;11(8):781-789.

[3] de Camargo Cancela M, de Souza DL, Curado MP. International incidence of oropharyngeal cancer: a population-based study. *Oral Oncol*. 2012;48(6):484-490.

[4] de Souza DL, de Camargo Cancela M, Pérez MM, Curado MP. Trends in the incidence of oral cavity and oropharyngeal cancers in Spain. *Head Neck*. 2012;34(5):649-654.

[5] Ang KK, Harris J, Wheeler R, et al. Human papillomavirus and survival of patients with oropharyngeal cancer. *N Engl J Med*. 2010;363(1):24-35.

[6] Ragin CC, Taioli E. Survival of squamous cell carcinoma of the head and neck in relation to human papillomavirus infection: review and meta-analysis. *Int J Cancer*. 2007;121(8):1813-1820.

[7] Cavallaro U, Christofori G. Cell adhesion and signalling by cadherins and Ig-CAMs in cancer. *Nat Rev Cancer*. 2004;4(2):118-132.

[8] Wijnhoven BP, Dinjens WN, Pignatelli M. E-cadherin-catenin cell-cell adhesion complex and human cancer. *Br J Surg*. 2000;87(8):992-1005.

[9] Andrews NA, Jones AS, Helliwell TR, Kinsella AR. Expression of the E-cadherin-catenin cell adhesion complex in primary squamous cell carcinomas of the head and neck and their nodal metastases. *Br J Cancer*. 1997;75(10):1474-1480.

[10] Conacci-Sorrell M, Zhurinsky J, Ben-Ze'ev A. The cadherin-catenin adhesion system in signaling and cancer. *J Clin Invest*. 2002;109(8):987-991.

[11] Polakis P. Wnt signaling and cancer. *Genes Dev*. 2000;14:1837-1851.

[12] Provost E, Yamamoto Y, Lizardi I, et al. Functional correlates of mutations in beta-catenin exon 3 phosphorylation sites. *J Biol Chem*. 2003;278(34):31781-31789.

[13] Frixen UH, Behrens J, Sachs M, et al. E-cadherin-mediated cell-cell adhesion prevents invasiveness of human carcinoma cells. *J Cell Biol*. 1991;113(1):173-185.

[14] Bosch FX, Andl C, Abel U, Kartenbeck J. 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(5):779-790.

[15] Rodrigo JP, Domínguez F, Alvarez C, Manrique C, Herrero A, Suárez C. Expression of E-cadherin in squamous cell carcinomas of the supraglottic larynx with correlations to clinicopathological features. *Eur J Cancer*. 2002;38(8):1059-1064.

[16] Nijkamp MM, Span PN, Hoogsteen IJ, van der Kogel AJ, Kaanders JH, Bussink J. Expression of E-cadherin and vimentin correlates with metastasis formation in head and neck squamous cell carcinoma patients. *Radiother Oncol*. 2011;99(3):344-348.

[17] Rodrigo JP, Martínez P, Allonca E, et al. Immunohistochemical markers of distant metastasis in laryngeal and hypopharyngeal squamous cell carcinomas. *Clin Exp Metastasis*. 2014;31(3):317-325.

[18] Zhao Z, Ge J, Sun Y, et al. Is E-cadherin immunoexpression a prognostic factor for head and neck squamous cell carcinoma (HNSCC)?. A systematic review and meta-analysis. *Oral Oncol*. 2012;48(9):761-767.

[19] Pectasides E, Rampias T, Sasaki C, et al. Markers of epithelial to mesenchymal transition in association with survival in head and neck squamous cell carcinoma (HNSCC). *PLoS One*. 2014; 9(4):e94273.

[20] Yu Z, Weinberger PM, Provost E, et al. beta-Catenin functions mainly as an adhesion molecule in patients with squamous cell cancer of the head and neck. *Clin Cancer Res*. 2005;11(7):2471-2477.

[21] Bánkfalvi A, Krabort M, Buchwalow IB, Végh A, Felszeghy E, Piffkó J. Gains and losses of adhesion molecules (CD44, E-cadherin, and beta-catenin) during oral carcinogenesis and tumour progression. *J Pathol*. 2002;198(3):343-351.

[22] Tanaka N, Odajima T, Ogi K, Ikeda T, Satoh M. Expression of E-cadherin, alpha-catenin, and beta-catenin in the process of lymph node metastasis in oral squamous cell carcinoma. *Br J Cancer*. 2003;89(3):557-563.

[23] Menéndez ST, Rodrigo JP, Alvarez-Teijeiro S, et al. Role of HERG1 potassium channel in both malignant transformation and disease progression in head and neck carcinomas. *Mod Pathol*. 2012;25(8):1069-1078.

[24] Rodrigo JP, Heideman DA, García-Pedrero JM, et al. Time trends in the prevalence of HPV in oropharyngeal squamous cell carcinomas in northern Spain (1990-2009). *Int J Cancer*. 2014; 134(2):487-492.

[25] Huber GF, Züllig L, Soltermann A, et al. Down regulation of E-Cadherin (ECAD) - a predictor for occult metastatic disease in sentinel node biopsy of early squamous cell carcinomas of the oral cavity and oropharynx. *BMC Cancer*. 2011;11(217):1-8.

[26] Stenner M, Yosef B, Huebbers CU, et al. Nuclear translocation of β-catenin and decreased expression of epithelial cadherin in human papillomavirus-positive tonsillar cancer: an early event in human papillomavirus-related tumour progression? *Histopathology*. 2011;58(7):1117-1126.

[27] Wakisaka N, Yoshida S, Kondo S, et al. Induction of epithelial-mesenchymal transition and loss of podoplanin expression are associated with progression of lymph node metastases in human papillomavirus-related oropharyngeal carcinoma. *Histopathology*. 2015;66(6):771-780.

[28] Ukpo OC, Thorstad WL, Zhang Q, Lewis JS Jr. Lack of association of cadherin expression and histopathologic type, metastasis, or patient outcome in oropharyngeal squamous cell carcinoma: a tissue microarray study. *Head Neck Pathol*. 2012;6(1):38-47.

[29] Santoro A, Pannone G, Papagerakis S, et al. Beta-catenin and epithelial tumors: a study based on 374 oropharyngeal cancers. *Biomed Res Int*. 2014;2014:948264.

[30] Pukkila MJ, Virtaniemi JA, Kumpulainen EJ, et al. Nuclear beta catenin expression is related to unfavourable outcome in oropharyngeal and hypopharyngeal squamous cell carcinoma. *J Clin Pathol*. 2001;54(1):42-47.

[31] Hu Z, Müller S, Qian G, et al. Human papillomavirus 16 oncoprotein regulates the translocation of β-catenin via the activation of epidermal growth factor receptor. *Cancer*. 2015;121(2):214-225.

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