The expression and prognostic relevance of CDH3 in tongue squamous cell carcinoma

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Key words: CDH3; tongue squamous cell carcinoma; lymph node metastasis; survival; immunohistochemistry.

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Abstract

P-cadherin (CDH3) is a cell-to-cell adhesion molecule that regulates several cellular homeostatic processes in normal tissues. Lack of CDH3 expression is associated with aggressive behavior in oral squamous cell carcinoma (OSCC). Previous studies have shown that CDH3 is downregulated in high-grade OSCC and its reduced expression is predictive for poorer survival. The aim of this study was to evaluate the expression and prognostic relevance of CDH3 in tongue squamous cell carcinoma (TSCC). A retrospective series of 211 TSCC and 50 lymph node samples were stained immunohistochemically with polyclonal antibody (anti-CDH3). CDH3 expression was assessed semi-quantitatively with light microscopy. Fisher's exact test was used to compare patient and tumor characteristics, and the correlations were tested by Spearman correlation. Survival curves were drawn by the Kaplan–Meier method and analyzed by the log-rank test. Univariate and multivariate Cox regression was used to estimate the association between CDH3 expression and survival. CDH3 expression did not affect TSCC patient's disease-specific survival or overall survival. Strong CDH3 expression in the primary tumor predicted poor disease-specific and overall survival in patients with recurrent disease. CDH3 expression in lymph nodes without metastasis was negative in all cases. CDH3 expression was positive in all lymph node metastases with extranodal extension. In contrast to previous report about the prognostic value of CDH3 in OSCC, we were not able to validate the result in TSCC.

Introduction

Oral squamous cell carcinoma (OSCC) is a subset of head and neck squamous cell carcinomas [1]. OSCC is the eighth most common cancer globally and it is usually located in the lateral border of the tongue [2, 3]. Tongue squamous cell carcinoma (TSCC) accounts for more than 50% of the total annual number of OSCC cases [4]. TSCC is often related to use of tobacco or alcohol [2, 5–7]. Inflammatory alterations of the mouth such as leukoplakia, erythroplakia, and lichen ruber planus have been linked to TSCC [2]. TSCC frequently metastasizes to the cervical lymph nodes (LNs) [8, 9]. The prognosis of TSCC has improved during the last years, but it still remains a disease with poor survival [10]. In a Finnish national study consisting of university hospital OSCC patients between 2005 and 2009, the disease-specific 5-year survival rate was 76% [11]. According to the Finnish Cancer Registry reports, the OSCC patients diagnosed between 2014 and 2016 had an age-adjusted relative 5-year survival rate of 67% for women and 61% for men [12].

Cell-cell adhesion is often disorganized in cancer, contributing to the unregulated behavior of tumor cells, such as invasion and metastasis [13]. Tumor invasion involves at least two major cell-cell adhesion-related processes: detachment of tumor cells from the primary tumor, and re-adhesion of cells at the metastasizing site [13]. Cadherins are a family of transmembrane glycoproteins that mediate calcium-dependent cell adhesion and have important functions in the maintenance of normal tissue architecture [14]. Cadherins mediate cell-cell adhesion by extracellular cadherin domains, whereas the intracellular cytoplasmic tail associates with a large number of adaptor and signaling proteins [14, 15]. The classic cadherins are single-span proteins that have five extracellular calcium-binding repeats located primarily within adherens junction [15]. Downregulation or loss of cadherins correlate with an increased metastatic potential of epithelial cancer cells. This is due to the loss of their adhesive properties [16].

The members of the cadherin family are found in different locations in human tissues. E-cadherin is expressed in all epithelial tissues [17, 18] whereas N-cadherin is found in neural tissues, fibroblasts, skeletal tissues, and endothelial tissues [17, 18]. A less well-known cadherin is P-cadherin (CDH3) that was firstly found in placenta [19]. It is a protein that in humans is encoded by the CDH3 gene. It participates in embryonic development by regulating several cellular homeostatic processes. CDH3 also maintains adult tissue architecture, structural integrity of epithelial tissues, and is important for cell differentiation, shape, polarity, growth, and migration [20–22]. The association between CDH3 overexpression and poor prognosis has been reported in many cancers, including endometrial, breast, ovarian, prostate, and gastric cancer [23]. In contrast, absence of CDH3 expression constitutes a hallmark of aggressive biological behavior in OSCC [24, 25]. CDH3 was found to be downregulated

in high-grade OSCC [25]. Hence, reduced or negative CDH3 membranous expression was related to poorer overall and disease-specific survival than CDH3-expressing tumors [25].

Healthy tissues usually express low levels of CDH3, and thus targeting CDH3 may be a good therapeutic approach [23]. A human monoclonal antibody against CDH3 has demonstrated significant anti-tumor and anti-metastatic activity in distinct CDH3-overexpressing tumor models. These included breast, gastric, lung, prostate, and colon carcinomas, and the antibody did not introduce any adverse secondary effects in mice [26]. In contrast, in a mouse model of human ovarian cancer metastasis, CDH3 silencing leads to induced tumor growth and involves the adhesion of metastatic cells to the peritoneum [27, 28]. To understand the role of CDH3 in cancer, this study aimed at evaluating CDH3 expression and its predictive role in TSCC. Based on available expression data in cancer data-bases that support general CDH3 overexpression particularly in TSCC, we hypothesized that CDH3 expression associates with TSCC survival.

Materials and methods

Database search

Database search was performed from Oncomine (https://www.oncomine.org). The CDH3 expression profiles were taken from The Cancer Genome Atlas head and neck cancer samples (http://tcga-data.nci.nih.gov/tcga/).

Clinical samples

This was a collaborative study between Departments of Otorhinolaryngology and Pathology, Tampere University Hospital (Tampere, Finland) and Haartman Institute, University of Helsinki (Helsinki, Finland). The study protocol was approved by the hospital's Research Ethics Committee in Tampere. The formalin-fixed paraffin-embedded samples were collected retrospectively. The samples had been obtained from 128 patients who had been treated for TSCC during the years 1999–2013. Hundred and six TSCC samples were from surgical resections, and 22 samples were diagnostic biopsies. Hundred and seventeen samples were from the primary tumor, 10 from the recurrent tumor and one sample was from a second primary TSCC. Lymph node samples were available from 50 TSCC patients. The control group consisted of 83 patients with no evidence of TSCC. Final pathology reports for the control group were as follows: hyperplasia (n = 44), hyperkeratosis (n = 21), parakeratosis (n = 4), hyperkeratosis-parakeratosis (n = 1), chronic inflammation (n = 1), and normal tissue or no diagnostic abnormality (n = 12). Sixty-one samples were diagnostic biopsies and 22 out

of these patients had the epithelial lesion radically resected. All samples were re-reviewed by a pathologist (TP). The demographic data of the patients are shown in Table 1. TNM classification was defined by the TNM classification that was effective at the timepoint of diagnosis.

Tissue handling and immunohistochemistry

CDH3 immunohistochemical staining was performed in 211 tongue cancer tissue samples and 50 LN samples. The samples were cut into 4 µm thick paraffin sections and placed on Superfrost Plus microscope slides (Menzel-Glaser, Braunschweig, Germany). Fully automated immunostaining was performed by Ventana BenchMark LT Automated IHC Stainer (Ventana Medical System, Tucson, AZ, USA). Ventana EZ Prep solution (catalog No 950-100, Ventana Medical System) was used for deparaffinization. For epitope retrieval CC1, Tris -EDTA buffer pH 8.0 (catalog No 950-124, Ventana Medical System) was used at 95 to 100 °C for 30 min with paraffin-embedded tissue sections. Endogenous peroxidase was blocked with UV-Inhibitor 3% H202 (Ventana Medical System) for 4 min at 37 °C. Tissue slides were rinsed between steps with Ventana Tris-based Reaction buffer (catalogue No. 950-300, Ventana Medical System). Slides were incubated at 37 °C for 32 min with pAb anti-CDH3 (1:100, catalogue No. HPA001767 Sigma-Aldrich Co. LLC, St. Louis, MO, USA) followed by application of Ventana Ultraview HRP Universal Multimer (4 min at 37 °C). 3,3′-diaminobenzidine (DAB) was used as a chromogen and hematoxylin as a nuclear stain. Known positive tissue samples from esophagus adenocarcinoma were used to confirm the reliability of the staining in all separate staining patches [29]. The specificity of immunohistochemistry was controlled by omitting the primary antibody or replacing it with irrelevant antisera.

Light microscopic evaluation

The CDH3 expression in the samples was evaluated semi-quantitatively with a Leica DM 2000 light microscopy (Leica Microsystems GmbH, Wetzlar, Germany) by two independent observers (LJ and MS) without knowledge of clinical status and outcome data. Cytoplasmic staining of the CDH3-positive cells was evaluated in basal part of epithelium of normal or benign tongue tissue and from the tumor area. In addition, the CDH3-positive cells were evaluated from the primary tumors' invasive front and from cervical LN tissue. CDH3 staining intensity was evaluated and graded as: 0 = no staining, 1 = weak staining, 2 = moderate staining, and 3 = strong staining. For statistical analysis, the TSCC samples were divided into two groups: low CDH3 immunoreactivity (scores 0–2) and high immunoreactivity (score 3). Due to different staining properties, the LN samples were divided into two different groups: negative CDH3 immunoreactivity (score 0) and positive CDH3 (scores 1–3) expression.

Data analysis

Fisher's exact test (two-tailed) and Mann–Whitney U were used in comparisons. Associations were assessed by Fisher's exact test (dichotomous), Kruskal–Wallis, and Mann–Whitney U tests (continuous). Survival curves were drawn by the Kaplan–Meier method, and associations were analyzed by the log-rank test. Overall survival was calculated from the date of diagnosis or operative therapy to death, while disease-specific survival was calculated from the date of diagnosis or operative therapy to death from TSCC, or December 2018, whichever came first. Univariate and multivariable Cox's proportional hazard models for survival were constructed with the following eight predictor variables: gender, age, CDH3 expression, smoking, tumor size, tumor grade, tumor depth, and tumor resection margin. The statistically significant variables were entered to the multivariable Cox's proportional hazard model. Two-tailed p-value of <0.05 was considered statistically significant.

Results

Patient and tumor characteristics

The patient demographics are summarized in Table 1. The average age of the patients in the control group was 55 years (range 9–85) and in the TSCC group 62 years (range 17–94) ($p = 0.011$, Table 1). The number of smokers and patients with previously diagnosed lichen ruber planus in the oral mucosa was higher in the TSCC group compared with the control group ($p = 0.001$, $p = 0.024$, respectively, Table 1). No significant differences in other demographic factors were found.

The characteristics of the tumors are shown in Table 2. Ninety-one samples were taken from the oral tongue, 31 from the base of tongue, and six samples with unknown origin from the tongue. One hundred and seventeen (91.4%) of the TSCC samples were from the primary tumor, 10 (7.8%) were from the recurrent tumor, and 1 (0.8%) was from a second primary tumor. Thirty-seven (28.9%) of TSCC patients presented with a tumor size of 4 cm or more and 27 (21.2%) of the tumors were poorly differentiated (grade III). Tumor invasion depth was at least 4 mm in 72 (61.7%) of cases. Fifty (39.0%) of the TSCC patients had cervical LN metastasis. Of these, 35 (70.0%) had pathological signs of metastatic growth (e.g. pNb) in cervical LNs. The median (min–max) number of pN cervical LNs was 2 (1–8) and the median (min–max) number of cervical LNs with extranodal growth was 1 (1–6). Distant metastasis occurred in 7 (5.5%) of cases. Extranodal extension in cervical LN metastasis was found in 23 (18.0%) operated patients. One hundred and ten (86.1%) of the TSCC patients had surgical treatment and 87 (78.9%) of the operated patients had neck dissection. Seventy-nine (61.7%) of TSCC patients received chemoradiotherapy and 58 (45.3%) received cisplatin.

Cancer recurrence occurred in 59 (46.1%) patients, second primary in 5 (3.9%) and neck recurrence in 38 (30.0%) of cases. After the 5-year follow-up, 85 (66.4%) patients were alive or had died of other causes. Follow-up period of all patients at the end of the study was 61.5 months (median) and 57.4 months (mean) (range, 2–163).

CDH3 expression in tongue samples

CDH3 expression was detected in the basal part of epithelium both in the control and in the TSCC groups (Fig. 1). CDH3 immunohistochemical staining was performed on 128 TSCC samples and on 83 controls. In TSCC, it was negative in 1 (0.8%), weakly positive in 5 (3.9%), moderately positive in 46 (35.9%), and strongly positive in 76 (59.4%) samples. In the control group, CDH3 expression was negative in 0 (0.0%), weakly positive in 11 (13.3%), moderately positive in 51 (61.4%), and strongly positive in 21 (25.3%) samples. CDH3 expression in the invasive front of the primary tumor was negative in 1 (0.8%), weakly positive in 8 (6.3%), moderately positive in 70 (54.7%), and strongly positive in 49 (38.3%) samples. The median of CDH3 expression was 3.00 (mean 2.54, Q1 = 2.00, Q2 = 3.00, Q3 = 3.00) in the primary TSCC tumor and 2.0 (mean 2.12, Q1 = 2.00, Q2 = 2.00, Q3 = 3.00) in the control group. The difference was statistically significant ($p < 0.000$, by MWU test, Fig. 2A). The median of CDH3 expression in the tumors' invasive front was 2.00 (mean 2.30, Q1 = 2.00, Q2 = 2.00, Q3 = 3.00).

Age, smoking, heavy alcohol use, or previous lichen diagnosis had no effect on CDH3 expression in TSCC. Tumor grade-specific difference in CDH3 expression ($p = 0.000$, $p = 0.377$, Fig. 2B) was significant. CDH3 expression was stronger in the samples of patients without a recurrence compared with patients with recurrence ($p = 0.032$, $p = 0.716$, Fig. 2C). Tumor invasion depth had an effect on the staining intensity in the tumors' invasive front ($p = 0.116$, $p = 0.033$, Fig. 2D). CDH3 expression did not differ significantly between the subgroups regarding base of tongue group and the other tongue parts, tumor stage, cN classification, pN classification, cervical LN metastasis (pN+ or cN+), distant metastasis, perineural invasion, LN invasion, extranodal extension, occurrence of second primary, or neck recurrence ($p > 0.050$, by Fisher's exact test, data not shown).

CDH3 expression correlated significantly with CDH3 expression of the invasive front, differentiation grade, pN classification, and recurrence ($p < 0.034$, $r = -0.196$–0.340, by Spearman rank correlation test, data not shown). CDH3 expression in the tumors' invasive front correlated significantly with tumor invasion depth ($p = 0.015$, $r = -0.244$ by Spearman rank correlation test, data not shown). CDH3 expression and CDH3 expression in tumors' invasive front did not correlate with other parameters ($p > 0.05$, by Spearman rank correlation test).

CDH3 prognostic relevance in TSCC

CDH3 expression did not affect disease-specific survival or overall survival (p = .460, p = .965, respectively, data not shown). When analyzing the base of tongue group and the other tongue parts – group separately, the result remained similar. When observing TSCC primary tumor samples from patients who developed a recurrence during follow-up, strong CDH3 expression predicted poor disease-specific and overall survival (p = 0.004, p = 0.011, respectively, Fig. 3). When observing the TSCC subgroups, it was found that tumor grade, cN classification, pN classification, cervical LN metastasis, other metastasis, tumor invasion depth, extranodal extension, second primary, and neck recurrence did not associate with poor disease-specific or overall survival (p > 0.05, by Log-rank test, data not shown). CDH3 expression in the tumors' invasive front did not affect disease-specific survival or overall survival (p = 0.280, p = 0.155, respectively, data not shown).

When observing the TSCC subgroups regarding tumor size, tumor grade, cN classification, pN classification, cervical LN metastasis, other metastasis, tumor invasion depth, extranodal extension and second primary, CDH3 expression in tumors' invasive front, it was found that these did not associate with poor disease-specific or overall survival (p > 0.05, by Log-rank test, data not shown).

CDH3 nodal expression and prognostic relevance in LNs

CDH3 expression in LNs without metastasis was negative in all 15 (100.0%) cases. CDH3 expression in LN metastasis was negative in 11 (31.4%), weakly positive in 10 (28.6%), moderately positive in eight (22.9%), and strongly positive in six (17.1%) samples. CDH3 expression differed significantly between LNs without metastasis and those with metastasis (p = 0.000, Fig. 2E). The median of CDH3 expression was 1.0 (mean 1.26, Q1 = 0.00, Q2 = 1.00, Q3 = 2.00). CDH3 expression in LN metastasis did differ significantly in relation to extranodal extension (p = 0.033, Fig. 2F). When extranodal extension was absent, CDH3 expression in LN metastasis was negative in 11 (42.3%) cases and positive in 15 (57.7%) case s. When extranodal extension was present, CDH3 expression was positive in all (100%) cases. CDH3 expression did not differ significantly between other subgroups (p > 0.05, data not shown). The significant difference between nodal CDH3 expression was not associated with disease-specific survival or overall survival (p > 0.050, by Fisher's exact test, data not shown). Nodal CDH3 expression correlated significantly with the pN class and extranodal extension (p < 0.027, r = 0.373–0.398, by Spearman rank correlation test, data not shown). Nodal CDH3 did not correlate with other parameters (p > 0.05, by Spearman rank correlation test). CDH3 expression in LN metastasis did not affect survival (p = 0.138, p = 0.504, respectively, data not shown). When observing the TSCC subgroups regarding tumor size, tumor grade, tumor invasion depth, extranodal growth, recurrence,

second primary cancer, and CDH3 expression in LN metastasis, it was found that these did not associate with survival ($p > 0.05$, by Log-rank test, data not shown).

Cox regression analysis

Disease-specific survival was tested for the following 12 factors by Cox regression: age, gender, smoking, tumor size, tumor depth, resection margin, cervical LN metastasis, extran odal extension, recurrence, CDH3 expression, CDH3 expression in the tumors' invasive front and CDH3 expression in nodal metastasis. In the unadjusted model, the following six factors were associated with TSCC mortality: tumor invasion depth ($p = 0.032$, HR = 3.63, CI = 1.11-11.8, Table 3), resection margin ($p = 0.005$, HR = 2.53, CI = 1.33-4.81, Table 3), LN metastasis ($p = 0.046$, HR = 1.80, CI = 1.01–3.21, Table 3), extranodal extension ($p = 0.000$, HR = 3.65, CI = 1.82–7.33, Table 3), recurrence ($p = 0.000$, HR = 14.6, CI = 6.19–34.4, Table 3), and nodal CDH3 expression ($p = 0.048$, HR = 2.22, CI = 1.00–4.92, Table 3). When analyzing these six factors in the multivariable model, tumor recurrence correlated with TSCC mortality ($p = 0.000$, HR = 27.0, Cl = 4.94–148.2, Table 3).

Discussion

CDH3 is a classic cell-to-cell adhesion molecule with a homeostatic function in several normal tissues [23]. CDH3 expression in normal tissue is usually low [29]. In our study, CDH3 expression in the TSCC was significantly higher than in the benign tongue tissue. In early oral tumor, development higher CDH3 expression plays a critical role in neoplastic signaling networks [30]. Recently, it has been shown that absence of CDH3 expression constitutes a hallmark of aggressive biological behavior in OSCC [31]. CDH3 could upregulate the expression of b-catenin protein with an association with oncogenic transformation and cell proliferation [32]. CDH3 assists to the survival of aggressive cancer cells and it downregulates E-cadherin [23]. Low E-cadherin expression is linked to poorer survival compared with normal or higher E-cadherin expression in OSCC [32]. Increased tumor motility and invasiveness are related to low E-cadherin expression [32].

CDH3 expression had a negative correlation with tumor recurrence. According to our study, CDH3 expression in the primary tumor was lower in patients with recurrent disease compared with patients with no evidence of disease during follow-up. On the other hand, we demonstrated that among those patients with TSCC recurrence during follow-up, a high CDH3 expression in the primary tumor was associated with poor disease-specific and overall survival. Hence, high expression of CDH3 in primary tumor would predict poor survival in case of recurrent disease during follow-up. The CDH3 activity might be different in aggressive and non-aggressive cancers [30]. In cell models or early tumor development, CDH3 enables the ligand-dependent signaling of tumor growth factors IGF-1R

and EGFR [30]. CDH3 overexpression can enhance steady-state levels of the mesenchymal transcription factor Snail and presenilin-1/c-secretase expression, which might cause increased accumulation of CDH3 in the cytoplasm and hence reduced cell-cell adhesion and dysplastic cell motility [30, 33, 34]. Additional immunohistochemical studies are important to clarify CDH3 usefulness in TSCC.

A Finnish study group has recently reported on serological biomarkers that could reveal early-stage OSCC prior to clinical signs. N-glycopeptide serum levels of patients with stage I OSCC could be separated from those of healthy controls [35]. Interestingly, in a previous study regarding the diagnosis of recurrent colorectal cancer, CDH3 plasma levels and immunohistochemical expression in tumor tissue were assessed preoperatively and these showed a positive correlation [36]. The authors reported that the serum CDH3 levels seem to be a promising target in colorectal cancer for primary tumor diagnostics. In addition, they suggested that CDH3 could be paired with other blood-derived tumor markers in the diagnostics of primary and recurrent tumors. Thus, at the follow-up visits, it would be feasible to determine CDH3 plasma levels both pre- and postoperatively [36]. This might be useful also in TSCC diagnostics and forms an interesting area for future research.

One of the predominant causes of death in patients with TSCC is invasion to LNs [37]. The causal relationship between LN metastasis and poor prognosis of head and neck squamous cell carcinoma (HNSCC) patients is not well understood [38–41]. To the knowledge of the authors, CDH3 expression in LN metastasis in TSCC has not been studied previously. In our study, there was no significant association of CDH3 expression between the involvement of primary tumor and LNs. Instead, nodal CDH3 expression correlated significantly with extranodal extension. Extranodal extension refers to infiltration of metastatic cancer cells beyond the nodal capsule, which is frequently associated with high rate of locoregional and distant failures [38–41]. Our study shows that positive CDH3 expression in LN metastasis did differ significantly in cases with extranodal extension. Previous studies have shown that extranodal extension in patients with HNSCC can greatly influence the prognostic outcome [42]. Further, CDH3 overexpression is associated with cancer cell invasion and metastatic dissemination [23]. Shingaki et al. analyzed the risk factors in OSCC and showed that extranodal extension was a significant predictor for patient survival [43]. In cases with extranodal extension, CDH3 expression in LN metastasis was positive in all cases.

The limitations of the study include the lack of knowledge of HPV status. In addition, further limitations were low sample size, and lack of mRNA expression data. We acknowledge that despite we selected pAb anti-CDH3 that has been shown to be a specific antibody to detect P-cadherin [44],

CDH3 polyclonal antibody has a disadvantage compared to monoclonal antibody such as high chance of cross-reactivity due to a recognition of multiple epitopes.

Conclusions

Based on our cohort, CDH3 is not useful as a predicting marker for TSCC. The role of CDH3 in the development and progression of TSCC still remains open for further investigations.

Funding

The study was supported by the Competitive Research Funding of Tampere Medical Research Fund & Tampere University Hospital, research grants from the Finnish Medical Foundation, Finnish Association of Otorhinolaryngology and Head & Neck Surgery, Minerva Foundation, Emil Aaltonen Foundation, Väinö and Laina Kivi Foundation, Paulo Foundation, Jane & Aatos Erkko Foundation, The Foundation of Finnish Female Dentist and The Finnish-Norwegian foundation of Medicine. We thank Eini Eskola and Teemu Honkanen for excellent technical assistance

Conflict of interest

We declare that we have no conflict of interest.

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Figure legends

Fig. 1. The expression of P-cadherin (CDH3) in tongue specimens and lymph node specimens by immunohistochemical methods. (A) Strong CDH3 expression in tongue hyperplasia, original magnification ×40. (B) Moderate CDH3 expression in tongue hyperplasia, original magnification ×100. (C) Weak CDH3 expression in tongue squamous cell carcinoma (TSCC), original magnification ×40. (D) Strong CDH3 expression in TSCC original magnification ×100. (E) Strong CDH3 expression in TSCC, original magnification ×20. (F) Moderate CDH3 expression in tumors' invasive front, original magnification ×20. (G) Moderate CDH3 expression in tumors' invasive front original magnification ×100. (H) Strong CDH3 expression in cervical lymph node metastasis, original magnification ×40. (I) Weak CDH3 expression in cervical lymph node metastasis, original magnification ×100.

Fig. 2. Comparison of the epithelial P-cadherin (CDH3) expression in control group, tongue squamous cell carcinoma, and lymph nodes. *Y*-axis indicates the percentage of patients with different CDH3 expressions. In tongue specimens, CDH3 expression was scored as: low expression = no, weak and moderate staining and strong expression = strong staining. In lymph node specimens, CDH3 expression was scored as: negative expression = no staining, positive expression = weak, moderate, and strong staining. (A) CDH3 expression in control group and in tongue squamous cell carcinoma ($p = 0.000$). (B) CDH3 expression in different tumor grades ($p = 0.000$). (C) CDH3 expression in patient group with or without recurrence ($p = 0.032$). (D) CDH3 expression in tumors' invasive front in tumor invasion depth <4 mm or ≥4 mm ($p = 0.033$). (E) CDH3 expression in lymph node with or without metastasis ($p = 0.000$). (F) CDH3 expression in lymph node metastasis with or without extranodal extension ($p = 0.033$). p-Values by Mann–Whitney *U* test and Fischer's exact test.

Fig. 3. Survival rates of patients with tongue cancer associating with CDH3 expression according to the Kaplan–Meier method. (A) Disease-specific survival for CDH3 expression in the tongue cancer patients ($n = 51$, $p = 0.460$). (B) Overall survival for CDH3 expression in the tongue cancer patients ($n = 73$, $p = 0.965$). (C) Disease-specific survival for CDH3 expression in tumors' invasive front in the tongue cancer patients ($n = 51$, $p = 0.280$). (D) Overall survival for CDH3 expression in tumors' invasive front in the tongue cancer patients ($n = 73$, $p = 0.155$). (E) Disease-specific survival for patients with recurrence ($n = 45$, $p = 0.004$). (F) Overall survival for patients with recurrence ($n = 48$, $p = 0.011$). p-values by the log-rank test.

Table 1. Characteristics of the patients

	Control, $n = 83$		TSCC, $n = 128$		p-Value
	n	%	*n*	%	
Gender					
Male	41	49.4	72	56.3	0.397
Female	42	50.6	56	43.7	
Age					
<60 years	48	57.8	51	39.8	**0.011**
≥60 years	35	42.4	77	60.2	
Smoking					
No	37	44.6	36	28.1	**0.001**
Yes	27	32.5	75	58.6	
Unknown	19	22.9	17	13.3	
Heavy alcohol use					
No	39	47.0	58	45.3	0.082
Yes	10	12.0	32	25.0	
Unknown	34	41.0	38	29.7	
Previous lichen ruber planus					
No	24	28.9	30	23.4	**0.024**
Yes	4	4.8	19	14.8	
Unknown	55	66.3	79	61.7	

p-Values by Fisher's exact test.
Bold values indicate $p < 0.05$ and are considered significant.

Table 2. Characteristics of the tumors

	TSCC, $n = 128$	
	n	%
Tumor type		
Primary tumor	117	91.4
Recurrence	10	7.8
Second primary	1	0.8
Tumor location		
Oral tongue	91	71.1
Base of tongue	31	24.2
Unknown	6	4.7
Tumor class		
T1	30	23.4
T2	61	47.7
T3	22	17.2
T4	15	11.7
Tumor grade		
I	38	29.7
II	63	49.2
III	27	21.2
cN class		
cN0	30	23.4
cN+	20	15.6
Unknown	78	61.0
pN class		
pN0	15	11.7
pN+	35	27.3
Unknown	78	61.0
Cervical LN metastasis		
LN	15	11.7
LN+	35	27.3
Unknown	78	61.0
Distant metastasis		
No	120	93.8
Yes	7	5.5
Unknown[1]	1	0.8
Perineural invasion		
In primary tumor	7	5.5
In residive nodal metastasis	1	0.8
Unknown[1]	120	93.8
Lymphatic vessel invasion		
Yes	4	3.1
Unknown[1]	124	96.9
Tumor depth		
Low (<4 mm)	19	14.8
High (≥4 mm)	79	61.7
Unknown[1]	30	23.4
Extranodal extension		
No	24	74.3
Yes	9	25.7
Surgery		
No need to operate after biopsy	1	0.8
Inoperable	13	10.2
Resection	43	33.6
Resection with microvascular reconstruction	71	55.5
Resection margin		
Clear (≥3 mm)	55	43.0
Close (<3 mm) or involved	58	45.3
Unknown[1]	15	11.7
Neck dissection		
No	27	21.1

Table 2 *(continued)*

	TSCC, $n = 128$	
	n	%
Removal of sentinel lymph nodes	3	2.3
Modified or functional neck dissection	96	75.0
Radical neck dissection to the side of tumor	1	0.8
Radical neck dissection to the both sides	1	0.8
Adjuvant chemoradiotherapy		
No	49	38.3
Yes	79	61.7
Adjuvant cisplatin		
No	70	54.7
Yes	58	45.3
Recurrence[2]		
No	69	53.9
Yes	59	46.1
Second primary		
No	123	96.1
Yes	5	3.9
Neck recurrence		
No	20	57.1
Yes	15	42.9
Status (5 years of follow-up)		
Alive or dead of other causes	85	66.4
Dead of disease	43	33.6

[1]Including biopsies, inoperable patients, or no information in patient data.
[2]Recurrence during follow-up.

Table 3. Unadjusted and adjusted Cox's regression analysis models for the variables analyzed

	Events		Univariate			Multivariate		
	N	%	HR	95% CI	p-Value	HR	95% CI	p-Value
Gender								
Female	23	18.0	1					
Male	28	21.9	1.03	0.60–1.80	0.906		Not entered	
Age								
Under 60 years	17	13.3	1					
60 years or older	34	26.6	1.50	0.84–2.70	0.171		Not entered	
Smoking								
No	18	16.2	1					
Yes	27	24.3	1.42	0.78–2.58	0.250		Not entered	
Tumor size								
<40 mm	35	27.3	1					
>=40 mm	16	12.5	1.45	0.80–2.63	0.215		Not entered	
Tumor invasion depth								
Low (<4 mm)	3	3.1	1			1		
High (>=4 mm)	36	36.7	3.63	1.11–11.8	**0.032**	2.34	0.26–20.8	0.445
Resection margin								
Clear (>= 3mm)	14	12.4	1			1		
Close or involved	29	25.7	2.53	1.33–4.81	**0.005**	1.12	0.33–3.88	0.854
Lymph node metastasis								
N_0	18	14.1	1			1		
N_1	33	25.8	1.80	1.01–3.21	**0.046**	3.24	0.58–18.0	0.179
Extranodal extension								
No	25	25.8	1			1		
Yes	12	12.4	3.65	1.82–7.33	**0.000**	2.63	0.74–9.32	0.134
Recurrence								
No	6	4.7	1			1		
Yes	45	32.5	14.6	6.19–34.4	**0.000**	27.0	4.94–148.2	**0.000**
CDH3								
$CDH3^{low}$	19	14.8	1					
$CDH3^{high}$	32	25.0	1.24	0.70–2.19	0.464		Not entered	
Invasive front CDH3								
$CDH3^{low}$	29	22.7	1					
$CDH3^{high}$	22	17.2	1.35	0.78–2.36	0.285		Not entered	
Nodal CDH3								
$CDH3^{neg}$	10	20.0	1			1		
$CDH3^{pos}$	16	32.0	2.22	1.00–4.92	**0.048**	1.20	0.31–4.65	0.797

Bold values indicate $p < 0.05$ and are considered significant.

Fig. 1

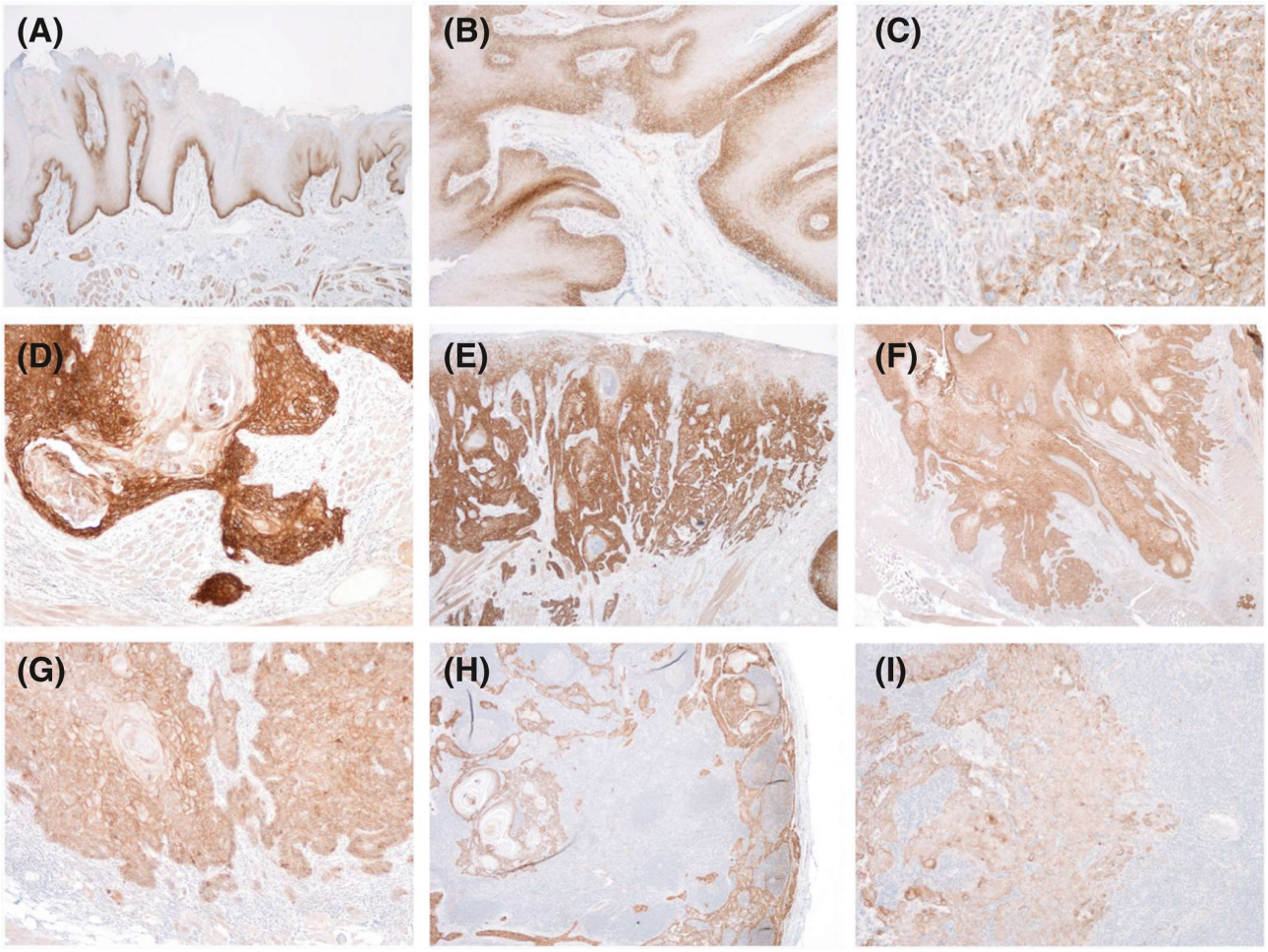


Fig. 2

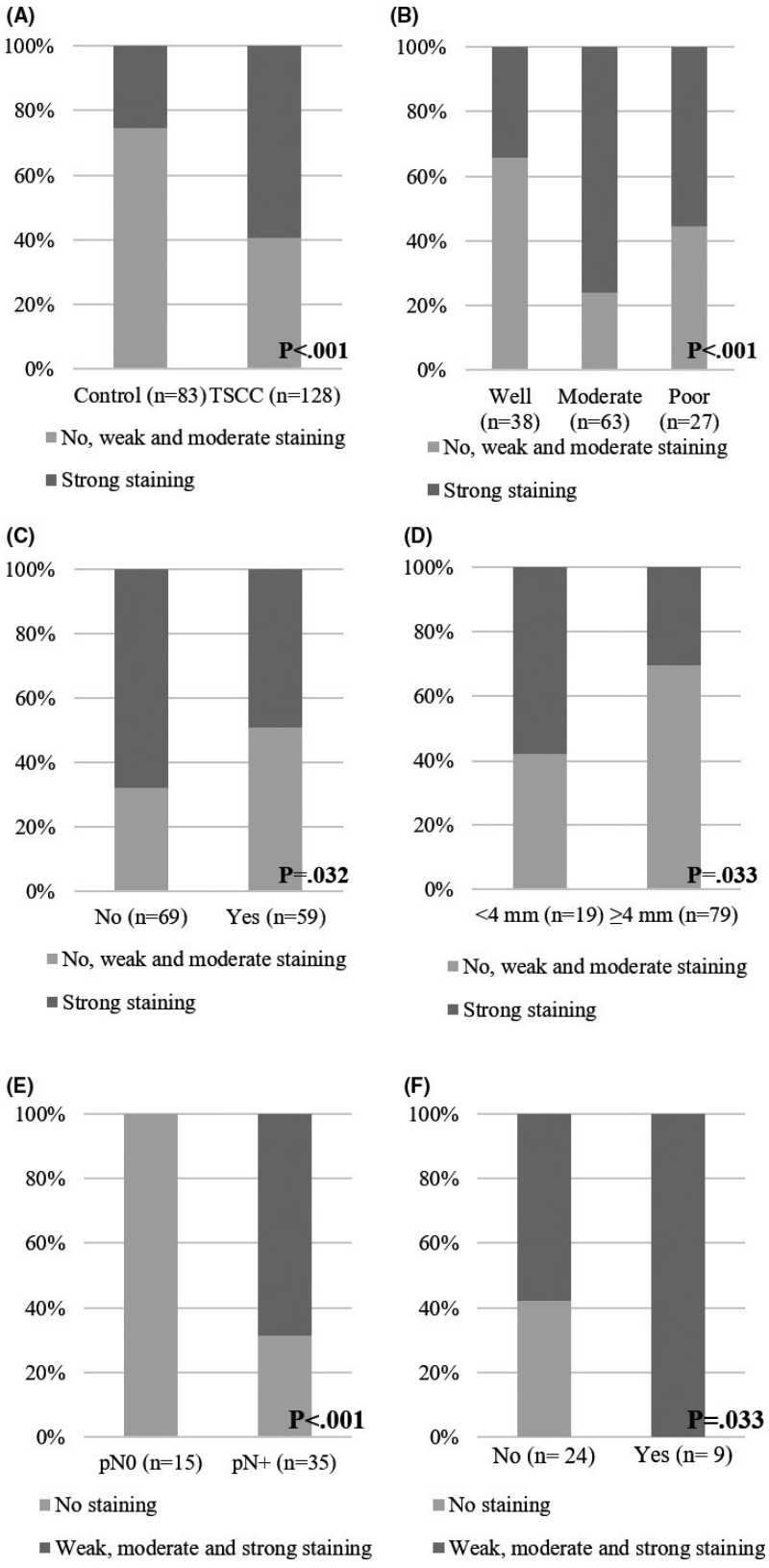
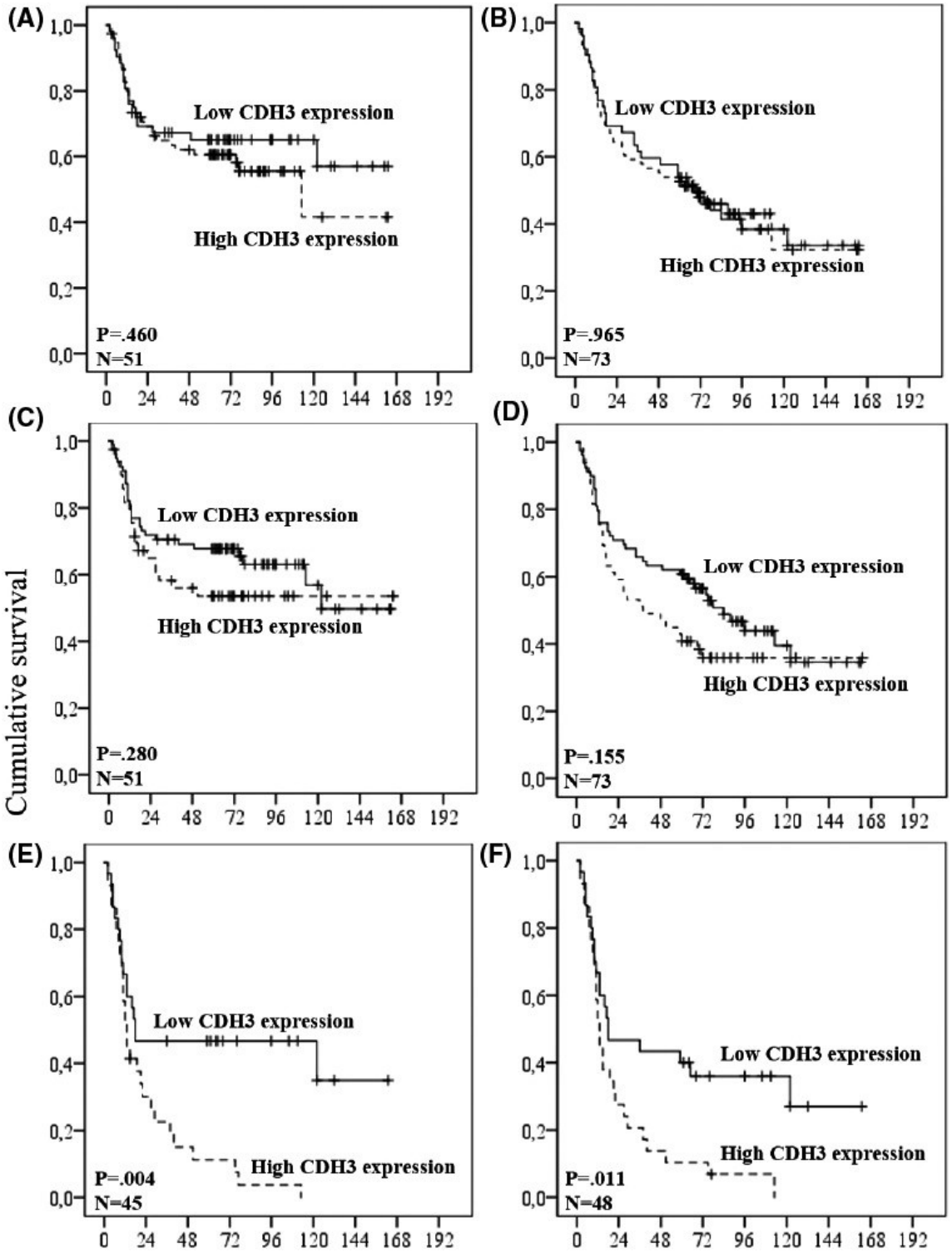

(A)
100%
80%
60%
40%
20%
0%
P<.001
Control (n=83)
TSCC (n=128)
No, weak and moderate staining
Strong staining
(B)
100%
80%
60%
40%
20%
0%
P<.001
Well (n=38)
Moderate (n=63)
Poor (n=27)
No, weak and moderate staining
Strong staining
(C)
100%
80%
60%
40%
20%
0%
P=.032
No (n=69)
Yes (n=59)
No, weak and moderate staining
Strong staining
(D)
100%
80%
60%
40%
20%
0%
P=.033
<4 mm (n=19)
≥4 mm (n=79)
No, weak and moderate staining
Strong staining
(E)
100%
80%
60%
40%
20%
0%
P<.001
pN0 (n=15)
pN+ (n=35)
No staining
Weak, moderate and strong staining
(F)
100%
80%
60%
40%
20%
0%
P=.033
No (n= 24)
Yes (n= 9)
No staining
Weak, moderate and strong staining

Fig. 3.


(A)
Low CDH3 expression
High CDH3 expression
P=.460
N=51
(B)
Low CDH3 expression
High CDH3 expression
P=.965
N=73
(C)
Low CDH3 expression
High CDH3 expression
P=.280
N=51
(D)
Low CDH3 expression
High CDH3 expression
P=.155
N=73
(E)
Low CDH3 expression
High CDH3 expression
P=.004
N=45
(F)
Low CDH3 expression
High CDH3 expression
P=.011
N=48
Cumulative survival
1,0
0,8
0,6
0,4
0,2
0,0
0 24 48 72 96 120 144 168 192
Follow up in months starting from curative treatment if it exists, otherwise from diagnosis