Original Article

Differential expression of Cadherins switch and Caveolin-2 during stages of oral carcinogenesis

Rebeca B. Nascimento[1], Isadora A. R. Machado[1], Jamerson C. Silva[2], Lorena A. S. Faria[3], Fernanda C. Borba[3], Lia P. A. Porto[4], Jean N. Santos[5], Luciana M. P. Ramalho[5], Camila O. Rodini[6], Maria Fernanda S. D. Rodrigues[7], Katiúcia B. S. Paiva[8], Flávia C. A. Xavier[5]

[1]PhD in Dentistry and Health, Department of Propaedeutics and Integrated Clinical, School of Dentistry, Federal University of Bahia, Salvador, [3]DDS, Department of Propaedeutics and Integrated Clinical, School of Dentistry, Federal University of Bahia, Salvador, [5]PhD Professor, Surgical Pathology Laboratory, Department of Propaedeutics and Integrated Clinical, School of Dentistry, Federal University of Bahia, Salvador, BA, [2]PhD Student in Dentistry and Health Postgraduated Program, Department of Propaedeutics and Integrated Clinical, School of Dentistry, Federal University of Bahia, [4]PhD in Dentistry, Health Sciences Center, School of Dentistry, Federal University of Pernambuco, Recife, PE, [6]PhD Professor, Department of Biological Sciences, School of Dentistry, University of São Paulo, [7]PhD Professor, Biophotonics Applied to Health Sciences Postgraduated Program, University of the Ninth of July, [8]PhD Professor, Extracellular Matrix Biology and Cellular Interaction Laboratory, Department of Anatomy, Institute of Biomedical Sciences, University of São Paulo, São Paulo, SP, Brazil

Abstract

Background: Oral squamous cell carcinoma (OSCC) accounts for 90% of oral malignancies, which may be preceded by oral potentially malignant disorders (OPMDs). Cancer progression involves the downregulation of epithelial markers (E-cadherin) and the upregulation of mesenchymal markers (N-cadherin), which together characterise the epithelial–mesenchymal transition (EMT). Furthermore, caveolin can act on cell adhesion and migration events that regulate the expression of the E-cadherin/α-β-catenin complex, thus favouring aggressive biological behaviour. This study aimed to analyse the immunoexpression of E-cadherin, N-cadherin and caveolin-2 at different stages of oral carcinogenesis to identify reliable biomarkers to predict malignant potential.

Methods: Expressions of E-cadherin and N-cadherin in 14 normal oral mucosae (NOM), 14 OPMD and 33 OSCC specimens were evaluated using immunohistochemistry. Clinicopathological parameters were also assessed.

Results: E-cadherin immunoexpression was significantly reduced during the progression of oral carcinogenesis ($P = 0.0018$). N-cadherin immunoexpression did not show any statistical differences between these groups. However, a representative number of N-cadherin-positive OSCC cases did not express E-cadherin. The expression of caveolin-2 increased significantly with the progression of the disease, from NOM to OSCC (*P* value: 0.0028).

Conclusion: These findings indicate that cadherin switch and caveolin-2 immunoexpression may be regulatory events in oral carcinogenesis.

Keywords: Cadherins, caveolin-2, immunohistochemistry, oral squamous cell carcinoma

Address for correspondence: Dr. Flávia C. A. Xavier, Universidade Federal da Bahia, Faculdade de Odontologia - Avenida Araújo Pinho, 62/64, Canela – Salvador, BA – Brasil.
E-mail: f.calo@uol.com.br

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INTRODUCTION

The global estimate for lip and oral cavity cancer in 2020 is 377,713 new cases and about 177,757 deaths, according to Glocal Cancer Observatory (GLOBOCAN).[1] Despite advances in the treatment, the survival rate is 50% in five years.[2] Oral squamous cell carcinoma (OSCC) accounts for 90% of the histological types of oral malignant neoplasms and may be preceded by oral potentially malignant disorders (OPMDs).[3] The most commonly used method for predicting the malignant transformation of OPMD is still the histological grade of epithelial dysplasias, but this parameter is often inefficient in its predictive value.[4] In this context, the search for tumour biomarkers could help greatly in the early diagnosis and prognosis of the disease.

The progression of epithelial malignancies involves the epithelial–mesenchymal transition (EMT) event, characterised by the dissociation of tumour cells and the acquisition of an invasive phenotype.[5] In this process, the cells reduced the expression of epithelial markers including E-cadherin and overexpress mesenchymal markers such as N-cadherin.[6] The loss of E-cadherin expression and concomitant upregulation of N-cadherin (cadherin switching) are key EMT events.[7] The existence of a close association between this switching and oral cancer has been suggested, both as part of its development and its metastasis.[8–10] EMT has been observed in murine tumour models where it has been proposed to play a role in invasion, haematogenous dissemination and chemoresistance.[11]

Caveolins (CAV-1, CAV-2 and CAV-3) constitute a family of proteins that play a central role in the transport of intracellular components and signal transduction because they control the biogenesis of caveola, an invagination of the plasma membrane that is important to cellular processes.[12] These molecules act on cell adhesion and migration events that affect cell motility,[13] and regulate the expression of the E-cadherin/α-β-catenin complex, thus favouring aggressive biological behaviour.[14]

Alteration in CAV-2 expression has been detected in several types of cancer and is associated with decreased survival.[15] This expression varies in different types of tumours;[15,16] however, the role of this protein in tumours is not well defined.[16] To our knowledge, the presence of this protein in OSCC and its relation with the progression of this neoplasia have not yet been analysed.

In addition, though many studies have demonstrated the role of E-cadherin and N-cadherin in OSCC and its association with tumour progression and metastases, to our knowledge, few studies have attempted to evaluate the expression of these markers during tumour development. Additionally, the participation of CAV-2 in OSCC seems to be poorly understood. Therefore, this study aimed to investigate the immunoexpression of E-cadherin, N-cadherin and caveolin-2 in OSCC and OPMDs, to verify the association of these proteins with EMT events at different stages of oral carcinogenesis.

SUBJECTS AND METHODS

Tissue samples

We selected 33 OSCC and 14 OPMD cases. The samples were obtained from incisional biopsies from 2004 to 2013 and were retrospectively selected from the Anatomic Pathology Laboratory, School of Dentistry, Federal University of Bahia (UFBA). Fourteen NOM cases, obtained from tooth extraction, were used to represent morphologically healthy tissue. Clinical data from the OPMD and OSCC cases, such as age, sex, smoking habits, lesion size and site, were collected. This study was approved by UFBA's Research Ethics Committee (protocol number 102.359).

OSCCs were classified to the histological grade of malignancy as following World Health Organization (WHO) criteria.[3] The OPMD exhibiting oral epithelial dysplasia (OED) was graded according to a binary classification system (high and low risks).[4]

Immunohistochemistry

Initially, the paraffin-embedded blocks were sectioned (3 μm) and extended on 2% silanised glass slides. For the immunoperoxidase method, the sections were deparaffinised in xylene and rehydrated in ethanol, followed by incubation in 3% hydrogen peroxide for 45 minutes. Following antigenic exposure in 10 mM citrate (pH 6.0) buffer in a steamer at 95°C for 20 minutes, the sections were incubated for 10 minutes in Protein Block Serum-Free (K0909, Dako, Carpinteria, CA, USA), only for N-cadherin antibody, to block nonspecific sites, followed by incubation with primary antibody E-cadherin (M3612, monoclonal, 1:50, Dako), N-cadherin (M3613, monoclonal, 1:50, Dako) and caveolin-2 (AF5788, polyclonal, 1:100, R and D Systems, Minneapolis, Minnesota, United States) in a wet chamber at 4°C for 18 hours. Incubation using the EnVision™ Dual Link (K4061, Dako) detection system lasted 30 minutes. The reaction was revealed using 3,3'-diaminobenzidine (K3468, Dako Liquid DAB Plus, Dako) and counterstained with Mayer's haematoxylin. The sections were washed with PBS/0.1% Triton (pH 7.4) buffer between washings. The sections were clear and mounted in Permount resin (Fisher Scientific, Fair Lawn, NJ).

Samples of human (normal mucosa for E-cadherin) or animal (mouse embryo for N-cadherin and mouse brain for CAV-2) were used as control. The same tissues, wherein the primary antibody was replaced by non-immune serum, were used as a negative control.

Immunostaining analysis

Protein immunoexpression was analysed for the extent and intensity of immunostaining and cellular compartment (nucleus, cytoplasm and/or cell membrane) in NOM, OPMDs and OSCCs and the epithelium stratum (basal and suprabasal) in NOM and OPMDs. The membranous immunoexpression of E-cadherin was considered preserved, and when abnormal expression (cytoplasmic or nuclear staining) was present, it was thus determined.

The immunoreactivity of E-cadherin and N-cadherin was determined according to the intensity of the staining and the percentage of positive cells, as recommended by Zhao *et al*.[17] The immunostaining index was obtained by multiplying the intensity and percentage scores being characterised as negative (0), weakly positive (1 and 2), moderately positive (3 and 4) and strongly positive (6 and 9). For statistical purposes, the cases were then separated into negative (categories from 0 to 2) and positive (categories from 3 to 9).

CAV-2 immunoexpression was analysed by adapting Koo *et al*.'s[18] criteria. The extent of the staining was assessed via the following scores: score 0: staining in < 10% of cells; score 1: from 10 to 30% of cells; score 2: from 31 to 60% of the cells; and score 3: in ≥60% of the cells. The intensity of the staining was classified as weak, moderate or intense. CAV-2 expression was considered positive when the score was 2 and moderate intensity or more.

All data recorded were submitted to statistical analysis, using GraphPad Prism 5 (GraphPad Software, Inc., CA, USA). A *P* value < 0.05 was considered significant. The comparison of E-cadherin, N-cadherin and CAV-2 immunostaining, association with protein expression between groups and clinical data were assessed using the Chi-square or Fisher's exact test. Spearman's rank correlation coefficient was used for the correlation between the immunostaining of E-cadherin, N-cadherin and CAV-2 in OPMDs and OSCCs.

RESULTS

E-cadherin immunoexpression

In NOM, E-cadherin was positive in 13 (92,85%) cases [Table 1]. All cases presented preserved expression in the membrane and distribution in the suprabasal layer [Figure 1a]. Of the 14 cases of OPMDs, seven (50%) were negative for E-cadherin, one of them with no expression. No statistically significant difference was observed for E-cadherin among high- and low-risk OPMDs [Table 1] ($P = 1.00$, Chi-square test). The majority of these cases (10 cases) maintained a preserved E-cadherin immunoexpression pattern, and three cases presented an abnormal pattern [Figure 1b] but were not associated with the immunoexpression index ($P = 0.5594$). E-cadherin was distributed in the suprabasal layer in seven cases and in the basal/suprabasal layer in six cases.

In the 33 OSCC cases analysed, 12 (36,36%) presented E-cadherin positivity. No statistically significant difference was observed for E-cadherin and histological grade ($P = 0.22$, Table 1). Furthermore, in moderately differentiated tumours, loss of E-cadherin expression was observed in neoplastic islands adjacent to the surface epithelium and invasion fronts. An abnormal pattern was predominant in 31 cases of OSCC [Figure 1c], but it was not associated with the immunoexpression index ($P = 1.000$).

No association was observed between the clinical data and E-cadherin immunoexpression in OPMDs and OSCCs [Table 1].

N-cadherin immunoexpression

In NOM, nine (64,28%) cases presented N-cadherin positivity [Table 1]. Thirteen cases showed staining in the basal/suprabasal layer, seven cases presented a cytoplasmic/nuclear staining pattern [Figure 1d] and five cases were also expressed in the membrane.

Of the 14 OPMD cases, 10 (71.42%) were positive for N-cadherin. No statistically significant difference was observed for N-cadherin among high- and low-risk OPMDs ($P = 1.000$, Table 1). Regarding the compartment, the majority presented a membranous/cytoplasmic pattern (n = 9, Figure 1e), but without association with the immunoexpression index ($P = 1.00$). In all positive cases, the distribution was observed in the basal/suprabasal layer.

Of the 31 OSCC cases analysed (two cases were lost through processing), 17 presented positive expression (54,83%). No statistically significant difference was observed for N-cadherin and histological grade ($P = 0.587$, Table 1). As for the cell compartment, there was a large predominance of cytoplasmic/nuclear staining (n = 13, Figure 1f), this pattern is associated with a gain in N-cadherin immunoexpression ($P = 0.0083$).

No association was observed between the clinical data and N-cadherin immunoexpression in OPMDs and OSCC [Table 1].

Table 1: Clinicopathological association of the immunoexpression of E-cadherin, N-cadherin and caveolin-2

	No. of cases	E-cadherin expression		*P*	No. of cases	N-cadherin expression		*P*	No. of cases	Caveolin-2 expression		*P*
		-	+			-	+			-	+	
Diagnosis												
OSCC	33	21	12	0,0018**	31	14	17	0,549	32	9	23	0,0028*
OPMD	14	7	7		14	4	10		14	9	5	
NOM	14	1	13		14	5	9		14	11	3	
Oral squamous cell carcinoma												
Age#												
<60 years	15	8	7	0,24	14	3	11	0,12	15	2	13	0,1
≥60 years	15	12	3		14	8	6		14	6	8	
Gender#												
Female	6	3	3	0,37	7	5	2	0,076	6	2	4	1
Male	24	17	7		21	6	15		23	6	17	
Lesion site#												
Buccal floor	14	9	5	0,37	14	4	10	0,52	15	5	10	0,18
Tongue	4	2	2		3	2	1		4	0	4	
Buccal mucosa	4	4	0		4	1	3		4	0	4	
Other sites	6	3	3		6	3	3		6	3	3	
pT												
T1+T2	12	7	5	0,6	11	7	4	0,13	12	5	6	0,33
T3+T4	6	5	1		6	1	5		6	1	5	
Histological grading												
Well-differentiated	20	12	8	0,22	20	9	11	0,587	19	7	12	0,25
Moderately differentiated	8	5	3		7	4	3		8	2	6	
Poorly differentiated	5	5	0		4	1	3		5	0	5	
Oral potentially malignant disorders												
Age#												
<60 years	7	5	2	0,1	7	2	5	1	7	5	2	0,59
≥60 years	6	1	5		6	1	5		6	3	3	
Gender#												
Female	6	4	2	0,59	6	1	5	0,58	6	2	4	0,09
Male	8	3	5		8	3	5		8	7	1	
Lesion site#												
Tongue	3	3	0	0,09	3	2	1	0,255	3	3	0	0,09
Buccal mucosa	6	3	3		6	1	5		6	2	4	
Other sites	5	1	4		5	1	4		5	4	1	
Lesion size												
<2 cm	3	0	3	0,45	3	1	2	0,459	3	3	0	0,15
2-4 cm	3	1	2		3	0	3		3	1	2	
>4 cm	1	0	1		1	0	1		1	1	0	
Smoking												
Smoker	5	2	3	1	5	1	4	1	5	4	1	0,14
Non-smoker	2	0	2		2	0	2		2	0	2	
WHO histological grading												
Without dysplasia	11	4	7	0,14	11	3	8	0,65	11	6	5	0,34
OED mild	2	2	0		2	1	1		2	2	0	
OED moderate	1	1	0		1	0	1		1	1	0	
Binary grading												
Low risk	13	6	7	1	13	4	9	1	13	8	5	1
High risk	1	1	0		1	0	1		1	1	0	

*$P \leq 0,05$ #Missing data

Caveolin-2 immunoexpression

The majority of NOM (11 cases, 78,57%) was CAV-2-negative. Most NOM cases demonstrated a membranous pattern (n = 12), and only two cases had a membranous/cytoplasmic pattern [Figure 1g]. CAV-2 was predominantly distributed in the basal stratum (n = 11), with only three cases in the basal/suprabasal layer.

Of the 14 OPMD cases, nine (64,28%) were negative for CAV-2, two of them with no expression. No statistically significant difference was observed in CAV-2 between high- and low-risk dysplasia ($P = 1,000$). The membranous pattern was predominant, with nine cases [Figure 1f], which was associated with an immunoexpression index ($P = 0.018$).

In 23 (71,87%) of 32 OSCC cases, CAV-2 immunopositivity was noted. However, no statistically significant difference was observed for CAV-2 and histological grade of malignancy ($P = 0.25$). The majority of OSCC cases demonstrated a membranous/cytoplasmic immunoexpression pattern (n = 29, Figure 1i), but there was no association with the immunoexpression index ($P = 0.1669$).

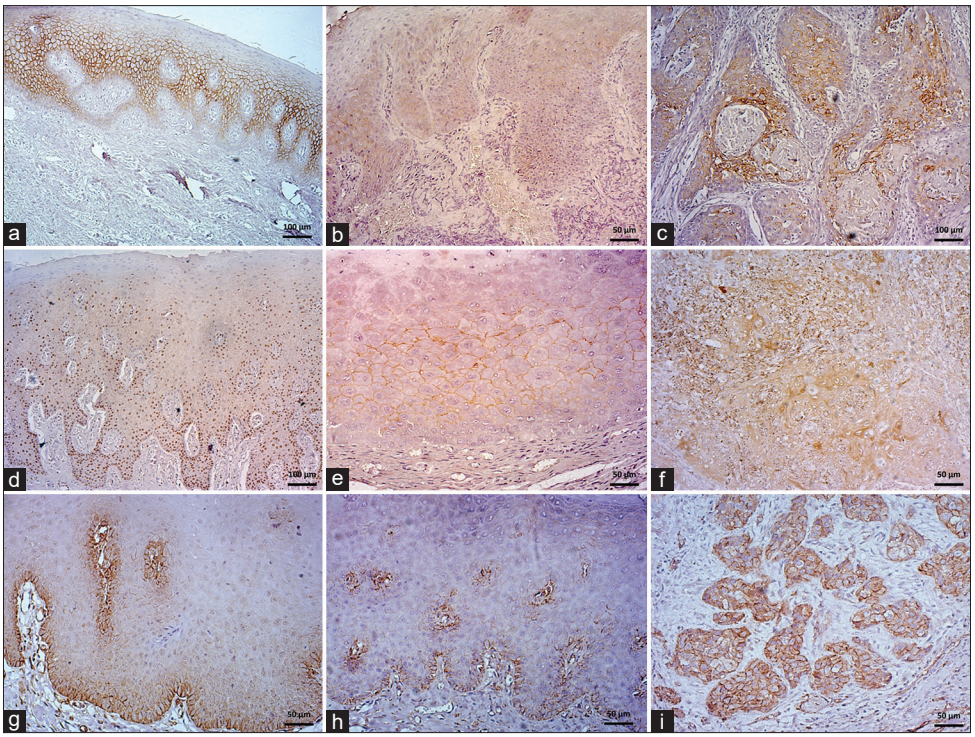

Figure 1: Immunoexpression of E-cadherin, N-cadherin and caveolin-2 during stages of oral carcinogenesis. E-cadherin shows intense membranous immunoexpression in normal oral mucosa (NOM) in almost all layers (a), weak cytoplasmic immunoexpression in oral potentially malignant disorder (OPMD) in basal/suprabasal layer (b) and moderate membranous/cytoplasmic immunoexpression distributed in an irregular pattern of oral squamous cell carcinoma (OSCC) tumoral islands (c). N-cadherin shows intense nuclear immunostaining in NOM (d), moderate membranous immunoexpression in OPMD (e) and moderate membranous/cytoplasmic immunoexpression in OSCC tumoral island (f). Caveolin-2 shows intense membranous/cytoplasmic immunoexpression in the basal/suprabasal layer of NOM (g), predominantly in the basal layer of OPMD (h) in an irregular pattern and intense membranous/cytoplasmic immunoexpression in the invasive island of OSCC (i). The scales indicate 100 μm (a, c and d) and 50 μm (b, e, f, g, h and i)

It was not possible to establish an association between the clinical data and CAV-2 immunoexpression in OPMDs and OSCC.

Association of E-cadherin, N-cadherin and Caveolin-2 immunoexpression with the stages of oral carcinogenesis

E-cadherin was significantly reduced during oral carcinogenesis progression (P = 0.0018, Table 1), comparing NOM, OPMD and OSCC. N-cadherin did not show any relationship within these groups (P = 0.549, Table 1), but a representative number of N-cadherin positive OSCC did not express E-cadherin (R = -0.1890, P = 0.3172, Spearman), mainly poorly differentiated cases.

As for CAV-2, a statistically significant difference was observed between the NOM, OPMD and OSCC stages (P = 0.0028, Chi-square test, Table 1), indicating that the tumour tissue exhibits a higher CAV-2 in relation to normal or dysplastic tissue. There was no correlation between CAV-2 and E-cadherin (R = 0.1645, P = 0.3938, Spearman) and N-cadherin (R = 0.2038 P = 0.2982, Spearman) in the OSCCs.

In regard to the cell compartments, E-cadherin expression was preserved in the 14 NOM cases (100%) and 10 (76.9%) of the OPMD cases, whereas in OSCC the abnormal pattern was predominant, with 31 cases (93.9%) (P < 0.0001). In N-cadherin staining, the predominant pattern in NOMs was cytoplasmic/nuclear (seven cases, 50%), in OPMDs it was membranous/cytoplasmic (nine cases, 64.3%) and in OSCC the cytoplasmic/nuclear pattern was predominant (13 cases, 43.3%) (P = 0.0022) [Figures 1a-f and 2]. With CAV-2, the majority of OSCCs (29 cases, 90,62%) exhibited a membranous/cytoplasmic pattern, especially poorly differentiated cases, while in both OPMDs (nine cases, 64.3%) and NOMs the membranous pattern was predominant (P < 0.0001) [Figures 1g-i and 2].

DISCUSSION

Adhesion molecules, such as E-cadherin, N-cadherin and caveolin-2, help maintain adhesion between epithelial cells, preventing their spread to other sites.[7,12] Many studies have demonstrated the role of E-cadherin and N-cadherin in OSCC, and recently, these proteins were used for early diagnosis of malignant transformation.[19]

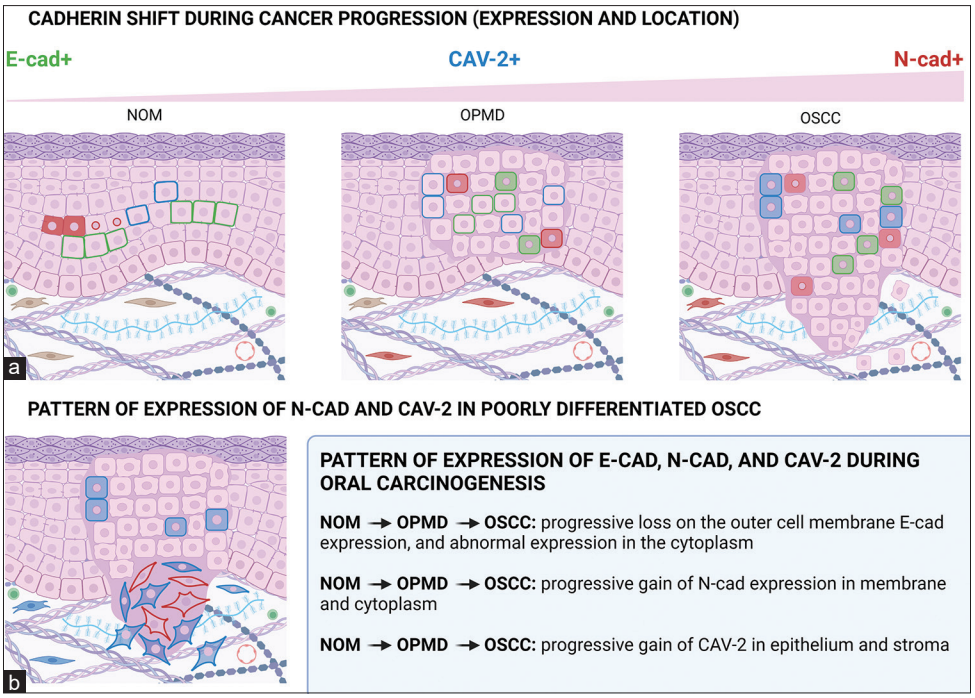

Figure 2: Cadherins and caveolin-2 differential immunoexpression in cell compartment during oral carcinogenesis. (a) During the cancer progression model, the epithelial–mesenchymal transition signalling promotes cancer cell invasion. Preserved E-cadherin expression (membrane) in the suprabasal layer in normal oral mucosa (NOM) is followed by abnormal (membranous/cytoplasmic) expression in oral potentially malignant disorder (OPMD) and oral squamous cell carcinoma (OSCC). Otherwise, the N-cadherin cytoplasmic/nuclear staining pattern in the basal/suprabasal layer of NOM is followed by membranous/cytoplasmic pattern in OPMD and cytoplasmic/nuclear in OSCC. Membranous CAV-2 immunoexpression pattern in NON and OPMD is followed by membranous/cytoplasmic pattern, through a gain of expression. (b) In poorly differentiated tumours, we observe membrane N-cadherin and CAV-2 and cytoplasmic CAV-2 expression

However, more studies need to evaluate the expression of these markers simultaneously in OPMD. In addition, there is a lack of knowledge as to the participation of CAV-2 in OSCC. In the present study, we observed that E-cadherin decreases with the progression of the disease, but N-cadherin expression did not increase significantly. However, the cytoplasmic/nuclear pattern was associated with an increased immunoexpression, especially in the OSCC. However, there was a progressive increase in the expression of CAV-2, especially with the membranous/cytoplasmic pattern, when progressing from the NOM to OSCC stages.

Many studies have shown that E-cadherin is decreased in many types of cancers.[8,20] The loss of E-cadherin critically influences an infiltrative phenotype and the occurrence of metastases in OSCC.[8,21] In the present study, the relationship between E-cadherin expression and the OSCC histological grade of malignancy was not found, nor with the risk of dysplasia in OPMD. Puneeta *et al*.[22] demonstrated that the E-cadherin expression in low-grade OED was similar to the control normal group of patients. In contrast, Al-Rawi *et al*.[23] showed that the expression of E-cadherin was significantly correlated with histological grades and metastasis status.

Costa *et al*.[10] evaluated the E-cadherin and N-cadherin immunoexpression in OSCC and observed even lower E-cadherin expression in the invasion fronts and highly invasive tumours. According to Nambiyar *et al*.,[24] E-cadherin expression was significantly reduced in cases with lymph node metastasis; however, this study did not analyse expression in the invasive front separately, because the tumour depth orientation was not feasible due to the small biopsies obtained. In our study, it was possible to detect the reduction in E-cadherin expression in the invasion front in some cases of moderately differentiated OSCC, but the association of E-cadherin expression with the clinical–pathological profile was not established.

We found the loss of membranous E-cadherin expression in OSCCs. A similar study by Kaur *et al*.[25] also noted this decrease during carcinogenesis. The results from Puneeta *et al*.[22] suggested a greater loss of E-cadherin expression with a shift in localisation to cytoplasmic with increasing grades of OED and OSCC. Another study in tongue OSCC observed E-cadherin immunoexpression in all cases, with a reduction in the membranous pattern accompanying a loss in the degree of differentiation and an increase in the invasion, whereas the opposite was observed with the cytoplasmic pattern.[9] Lopes *et al*.[26] showed the

cytoplasmic E-cadherin immunoexpression as a marker of OPMD transformation into OSCC. Thus, we attribute great importance to the determination of the cellular compartment of E-cadherin in the pathological processes because of its crucial role as a cell adhesion molecule. Evaluating a widely used mouse model of pancreatic ductal adenocarcinoma, Aiello *et al*.[11] suggested that the loss of membranous ECAD (M-ECAD) precedes a gain of mesenchymal markers in most tumour cells undergoing EMT and they concluded that the loss of membranous ECAD, rather than the gain of any single mesenchymal marker, would result in the identification of most cells exhibiting morphological features of EMT in this model.

N-cadherin helps the tumour cells to invade the adjacent stroma. Its high expression has been observed in several tumours, including advanced stages of OSCC.[10,20,27] In the present study, N-cadherin was positive in 71.4% of the OPMDs. In OSCCs, just over half of the cases presented positivity for N-cadherin. Therefore, no association with the progression of carcinogenesis was noted. Most of the OSCC cases analysed in our study were graded as well-differentiated, so it was not possible to establish a relationship between N-cadherin expression and the degree of malignancy. Afrem *et al*.[9] also reported positivity for N-cadherin in a few cases of OSCC; however, this expression was higher in cases diagnosed as poorly differentiated. Conversely, Costa *et al*.[10] reported immunonegativity for N-cadherin in all OSCCs, while Di Domenico *et al*.[21] demonstrated in OSCC a high N-cadherin expression associated with a worse clinical response and poor prognosis. Furthermore, the expression of N-cadherin, but not E-cadherin, at the invasive front was associated with the OSCC prognosis.[28]

Furthermore, herein representative N-cadherin-positive OSCC cases did not express E-cadherin, as also found by Ozaki-Honda *et al*.[28] N-cadherin positivity was useful for identifying small tumour satellites and individual OSCC cells with EMT in contrast to the negative E-cadherin immunoexpression.[28] In addition, the tumour profile may be related to E-cadherin and N-cadherin immunoexpression, as recently demonstrated by Abe *et al*.[29] who observed high E-cadherin and low N-cadherin immunoexpression in less aggressive OSCCs, while more aggressive OSCCs, a switch between the cadherins. It has been increasingly recognised that EMT also encompasses a range of hybrid states, a phenotype that has been referred to "partial EMT" (p-EMT). It is unknown whether this hybrid status signifies an intermediate phase during a mesenchymal transition or represents its end state. While EMT involving classical (transcription-dependent) mechanisms can give rise to single cells capable of crossing basement membranes and invading blood vessels, many tumours have been noted to exhibit "collective" migratory patterns whereby cells retain cell–cell contacts and activate mesenchymal programmes, resulting in dissemination of multicellular tumour cell clusters. Aiello *et al*.[11] showed that individual tumours utilise different plasticity programmes—a classical EMT programme involving transcriptional repression and an alternative programme in which the epithelial phenotype is lost post-transcriptionally.

In our study, although we observed N-cadherin in just over half of the OSCC cases, most demonstrated a cytoplasmic/nuclear pattern. In this context, Pyo *et al*.[8] observed NOM with negative expression for N-cadherin; however, they also noted membranous/cytoplasmic expression in the OSCC surgical margin and some lymph node metastases. In our study, this same membranous/cytoplasmic pattern was observed in most OPMD cases (64.3%), demonstrating the importance of analysing these disorders, thus helping to provide an earlier diagnosis of the malignancy.

The role of CAV-2 in tumour development is still controversial, and its function may differ by cancer type.[30,31] However, CAV-2 is generally coexpressed with CAV-1 and high CAV-1 expression has been detected in OSCC[32] and head and neck squamous cell carcinoma (HNSCC).[14] In our study, only 20% of NOMs were immunopositive for CAV-2; however, other studies have indicated the presence of this protein in small amounts in normal tissues, mainly adipose.[33] However, a greater percentage of OPMDs and the majority of OSCCs were CAV-2 immunopositive, pointing to an increase in disease progression. A similar result was found by Koo *et al*.[18] in breast cancer, where they observed an increase in CAV-2 immunoexpression in tumour progression.

Furthermore, most OSCCs expressed the CAV-2 membrane/cytoplasmic pattern; however, no association with the histological grade of malignancy was observed. Sugie *et al*.[15] also observed CAV-2 membranous/cytoplasmic pattern and did not establish a correlation with the histological grade and clinicopathological factors, but did detect high expression levels in the plasma of prostate cancer patients, matching a significant correlation with the disease progression.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, *et al*. Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 2015;136:E359–86.
2. Warnakulasuriya S. Global epidemiology of oral and oropharyngeal cancer. Oral Oncol 2009;45:309–16.
3. Sloan P, Gale N, Hunter K, Lingen MW, Nylander K, Reibel J, *et al*. Malignant surface epithelial tumours. In: El-Naggar AK, Chan JK, Grandis Jennifer R, Takata T, Slootweg PJ, editors. WHO Classification of Head and Neck Tumours. 4th ed. Lyon: WHO; 2017. p. 109–11.
4. Kujan O, Oliver RJ, Khattab A, Roberts SA, Thakker N, Sloan P. Evaluation of a new binary system of grading oral epithelial dysplasia for prediction of malignant transformation. Oral Oncol 2006;42:987–93.
5. Thiery JP. Epithelial-mesenchymal transitions in tumour progression. Nat Rev Cancer 2002;2:442–54.
6. Fuchs BC, Fujii T, Dorfman JD, Goodwin JM, Zhu AX, Lanuti M, *et al*. Epithelial-to-mesenchymal transition and integrin-linked kinase mediate sensitivity to epidermal growth factor receptor inhibition in human hepatoma cells. Cancer Res 2008;68:2391–9.
7. Yilmaz M, Christofori G. EMT, the cytoskeleton, and cancer cell invasion. Cancer Metastasis Rev 2009;28:15–33.
8. Pyo SW, Hashimoto M, Kim YS, Kim CH, Lee SH, Johnson KR, *et al*. Expression of E-cadherin, P-cadherin and N-cadherin in oral squamous cell carcinoma: Correlation with the clinicopathologic features and patient outcome. J Craniomaxillofac Surg 2007;35:1–9.
9. Afrem MC, Mărgăritescu C, Crăiţoiu MM, Ciucă M, Şarlă CG, Cotoi OS. The immunohistochemical investigations of cadherin "switch" during epithelial-mesenchymal transition of tongue squamous cell carcinoma. Rom J Morphol Embryol 2014;55:1049–56.
10. Costa LCMC, Leite CF, Cardoso SV, Loyola AM, Faria PR de, Souza PEA, *et al*. Expression of epithelial-mesenchymal transition markers at the invasive front of oral squamous cell carcinoma. J Appl Oral Sci 2015;23:169–78.
11. Aiello NM, Maddipati R, Norgard RJ, Balli D, Li J, Yuan S, *et al*. EMT subtype influences epithelial plasticity and mode of cell migration. Dev Cell 2018;45:681-95.e4. doi: 10.1016/j.devcel. 2018.05.027.
12. Kim D, Kim H, Koo JS. Expression of caveolin-1, caveolin-2 and caveolin-3 in thyroid cancer and stroma. Pathobiology 2012;79:1–10.
13. Bailey KM, Liu J. Caveolin-1 up-regulation during epithelial to mesenchymal transition is mediated by focal adhesion kinase. J Biol Chem 2008;283:13714–24.
14. Masuelli L, Budillon A, Marzocchella L, Mrozek MA, Vitolo D, Di Gennaro E, *et al*. Caveolin-1 overexpression is associated with simultaneous abnormal expression of the E-cadherin/α-β catenins complex and multiple erbb receptors and with lymph nodes metastasis in head and neck squamous cell carcinomas. J Cell Physiol 2012;227:3344–53.
15. Sugie S, Mukai S, Yamasaki K, Kamibeppu T, Tsukino H, Kamoto T. Significant association of caveolin-1 and caveolin-2 with prostate cancer progression. Cancer Genomics Proteomics 2015;12:391–6.
16. Lee S, Kwon H, Jeong K, Pak Y. Regulation of cancer cell proliferation by caveolin-2 down-regulation and re-expression. Int J Oncol 2011;38:1395–402.
17. Zhao D, Tang X-F, Yang K, Liu J-Y, Ma X-R. Over-expression of integrin-linked kinase correlates with aberrant expression of Snail, E-cadherin and N-cadherin in oral squamous cell carcinoma: implications in tumor progression and metastasis. Clin Exp Metastasis 2012;29:957–69.
18. Koo JS, Park S, Kim S Il, Lee S, Park BW. The impact of caveolin protein expression in tumor stroma on prognosis of breast cancer. Tumor Biol 2011;32:787–99.
19. Ivina AA, Syomkin VA, Babichenko II. [Morphology of epithelial-mesenchymal transformation in neoplasias of oral mucosa]. Stomatologiia (Mosk) 2017;96:11–3.
20. Islam S, Carey TE, Wolf GT, Wheelock MJ, Johnson KR. Expression of N-cadherin by human squamous carcinoma cells induces a scattered fibroblastic phenotype with disrupted cell-cell adhesion. J Cell Biol 1996;135:1643–54.
21. DI Domenico M, Pierantoni GM, Feola A, Esposito F, Laino L, DE Rosa A, *et al*. Prognostic significance of N-Cadherin expression in oral squamous cell carcinoma. Anticancer Res 2011;31:4211–8.
22. Puneeta N, Santosh T, Mishra I, Gaikwad P, Sahu A. Evaluation of e-cadherin and vimentin expression for different grades of oral epithelial dysplasia and oral squamous cell carcinoma - An immunohistochemical study. J Oral Maxillofac Pathol 2022;26:285–6.
23. Al-Rawi N, Al Ani M, Quadri A, Hamdoon Z, Awwad A, Al Kawas S, *et al*. Prognostic significance of E-Cadherin, β-Catenin and cyclin D1 in oral squamous cell carcinoma: A tissue microarray study. Histol Histopathol 2021;36:1073–83.
24. Nambiyar K, Ahuja A, Bhardwaj M. A study of epithelial-mesenchymal transition immunohistochemical markers in primary oral squamous cell carcinoma. Oral Surg Oral Med Oral Pathol Oral Radiol 2021;132:680–6.
25. Kaur J, Sawhney M, DattaGupta S, Shukla NK, Srivastava A, Walfish PG, *et al*. Clinical significance of altered expression of β-Catenin and E-Cadherin in oral dysplasia and cancer: Potential link with ALCAM expression. PLoS One 2013;8:e6736.
26. Lopes NM, Xavier FCA, Ortiz RC, Amôr NG, Garlet GP, Lara VS, *et al*. Subcellular localization and expression of E-cadherin and SNAIL are relevant since early stages of oral carcinogenesis. Pathol Res Pract 2018;214:1185–91.
27. Stemmler MP. Cadherins in development and cancer. Mol Biosyst 2008;4:835–50.
28. Ozaki-Honda Y, Seki S, Fujiwara M, Matsuura M, Fujita S, Ikeda H, *et al*. Prognostic prediction of oral squamous cell carcinoma by E-Cadherin and N-Cadherin expression in overall cells in tumor nests or tumor cells at the invasive front. Cancer Microenviron 2017;10:87–94.
29. Abe Y, Ogasawara S, Akiba J, Naito Y, Kondo R, Nakamura K, *et al*. Expression and role of regulator of G-protein signaling 5 in squamous cell carcinoma of the tongue. Clin Exp Dent Res 2019;5:160–9.
30. Ando T, Ishiguro H, Kimura M, Mitsui A, Mori Y, Sugito N, *et al*. The overexpression of caveolin-1 and caveolin-2 correlates with a poor prognosis and tumor progression in esophageal squamous cell carcinoma. Oncol Rep 2007;18:601–9.
31. Liu Y, Jang S, Xie L, Sowa G. Host deficiency in caveolin-2 inhibits lung carcinoma tumor growth by impairing tumor angiogenesis. Cancer Res 2014;74:6452–62.
32. Auzair LBM, Vincent-Chong VK, Ghani WMN, Kallarakkal TG, Ramanathan A, Lee CE, *et al*. Caveolin 1 (Cav-1) and actin-related protein 2/3 complex, subunit 1B (ARPC1B) expressions as prognostic indicators for oral squamous cell carcinoma (OSCC). Eur Arch Otorhinolaryngol 2016;273:1885–93.
33. Low J-Y, Nicholson HD. Epigenetic modifications of caveolae associated proteins in health and disease. BMC Genet 2015;16:71.