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Expression of adhesion proteins (E-cadherin and β-catenin) and cell proliferation (Ki-67) at the invasive tumor front in conventional oral squamous cell and basaloid squamous cell carcinomas

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ABSTRACT

Objective: Investigate, on a comparative basis, the expression of the adhesion molecules E-cadherin (E-cad), β-catenin (β-cat) and the proliferation index (Ki-67) at the invasive tumor front (ITF) in squamous cell carcinoma (SCC) and basaloid squamous cell carcinoma (BSCC).
Material and methods: Thirty-five SCC and 16 BSCC cases were evaluated by immunohistochemistry. Clinicopathological and survival data were also evaluated and compared.
Results: There was a low expression of E-cad in the cytoplasmic membrane ($p = 0.50$) as well as in the nucleus ($p = 0.31$) for both SCC and BSCC. A high expression of E-cad was seen in the cytoplasm for the SCC group (80%) when compared to the BSCC group (25%) ($p < 0.01$). The expression of β-cat was low in the cytoplasmic membrane and high in the cytoplasm in both SCC and BSCC groups. Both types of carcinoma presented low expressions of β-cat in the nucleus ($p = 0.03$). The Ki-67 expression was low irrespective of tumor variant. The high expression of E-cad in the cytoplasm was associated with T3/T4 tumors ($p = 0.04$) in the SCC group and there was no significant association of E-cad, β-cat, Ki-67 with the other clinical variables. In terms of disease-free survival and overall survival, there were no significant differences between SCC and BSCC.
Conclusion: The E-cad–β-cat system was found to be dysregulated in both oral SCC and oral BSCC. The Ki-67 cell proliferation index was extremely low in the cases investigated and consequently had no prognostic value.

1. Introduction

The squamous cell carcinoma (SCC) presents a classic histomorphological pattern. However, microscopic variants can be found, such as verrucous, basaloid squamous cell, papillary squamous cell, spindle cell, acantholytic squamous cell, and adenosquamous cell carcinomas (Fronie et al., 2013; Lindenblatt Rde et al., 2012). These variants or microscopic subtypes can present different biological behaviors. Basaloid squamous cell carcinoma (BSCC), for example, is a malignant tumor which is considered rare, aggressive and a variant of SCC (Coletta et al., 2002; Hanemann et al., 2014; Rodriguez Tojo, Garcia Cano, Infante Sanchez, Velazquez Fernandez, & Aguirre Urizar, 2005; Winters et al., 2008; Yu et al., 2008).

In general, BSCC has been reported as having a worse prognosis than conventional SCC, and existing studies have not reached any definitive conclusion about its more aggressive biological behavior, hence the need for further studies to investigate this variant (de

Abbreviations: SCC, squamous cell carcinoma; BSCC, basaloid squamous cell carcinoma; WHO, World Health Organization; E-cad, E-cadherin; β-cat, β-catenin; ITF, invasive tumor front; EMT, epithelial mesenchymal transition; HE, hematoxylin and eosin; PBS, phosphate buffer solution; CI, confidence interval; HPV, human papillomavirus.

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Sampaio Goes et al., 2004; Fritsch & Lentsch, 2014; Jayasooriya, Tilakaratne, Mendis, & Lombardi, 2013).

In recent years, with the identification of numerous biomarkers, it has been possible to investigate the different stages of carcinogenesis, from modulation to tumor progression, and thus glean useful information for understanding tumor initiation, proliferation, invasion, progression, migration and recurrence. In addition, these markers could contribute to establishing the prognosis for oral carcinomas (Mohtasham et al., 2013; Nilkaeo & Bhuvanath, 2006; Zhao et al., 2014). Included in these markers are the adhesion molecules, such as the cadherins, catenins, and the cell proliferation marker Ki-67 (Dragomir et al., 2012; Thomas & Speight, 2001).

Normal oral epithelial cells are strongly connected to each other through the cell–cell junction mediated by cadherins. Cadherins are regulated transmembrane, calcium-dependent proteins present in the cell which form a superfamily. One of the main cadherins is the E-cadherin (E-cad), which is expressed in all epithelial tissues. During proliferation, the neoplastic cells at the invasive tumor front (ITF) frequently lose their epithelial cell phenotype and acquire "mesenchymal-like" phenotype referred to as Epithelial Mesenchymal Transition (EMT) cells. Such cells facilitate the migration, invasion and metastasis of neoplastic cells. Therefore the reduction in E-cad and increase in N-cadherin are the strong points of the EMT cells. So it is crucial to investigate the role of reduced expression of E-cad in tumor progression and metastasis (Berx & van Roy, 2009; Chaw et al., 2012; Zhang, Filho, & Nor, 2012).

E-cad is comprised of extracellular, transmembrane and intercellular domains which bind with the catenins. These domains are involved in the transduction of the signal mediated by these catenins. The cytoplasmic domain of E-cad is directly bound to β-catenin (β-cat) or γ-catenin. The α-catenin links E-cad–β-cat and γ-catenin to the cytoskeleton. The β-cat and γ-catenin work on cell adhesion in the Wnt pathway and on the stability of the same when regulated by adenomatous polyposis of the colon. In malignancies this pathway becomes dysregulated (Howard, Deroo, Fujita, & Itasaki, 2011; Jeanes, Gottardi, & Yap, 2008). Changes in E-cad and β-cat expression are associated with loss of differentiation, acquisition of an invasive epithelial phenotype and poor prognosis (Freitas Rde, Silveira, Silveira, Silva, & Amorim, 2010; Lyons & Jones, 2007; Oliveira & Ribeiro-Silva, 2011).

The Ki-67 protein has been extensively studied as an indicator of proliferative activity (Oliveira & Ribeiro-Silva, 2011). The Ki-67 protein is present in all phases of the cell cycle, with the exception of G0 and as noted it has been used to estimate the proliferative potential of a lesion (Tumuluri, Thomas, & Fraser, 2004; Watanabe et al., 2010).

Against this background, this study aimed to investigate, on a comparative basis, the immunoexpression of E-cad, β-cat and Ki-67 in oral SCC and BSCC. In addition, it also analyzed the association of the expression of these proteins with the clinicopathologic variables of the BSCC variant in relation to conventional SCC.

2. Material and methods

For this study, formalin-fixed paraffin-embedded blocks from 35 cases of SCC and 16 cases of BSCC, diagnosed between 1998 and 2007, were retrieved from the archives of the Pathology Laboratory of Dental School at the Federal University of Goiás and the Anatomy Pathology Laboratory of Araújo Jorge Hospital, Goiânia, Brazil. Patients with incomplete information or with previous adjuvant therapy (radiotherapy and/or chemotherapy) or paraffin tissue blocks with insufficient sample were excluded. Seven samples of normal oral mucosa were used as control group. For the microscopic characterization of the samples, the paraffin blocks were sectioned by microtome (Leica RM 2165), 5 μm slices were then placed on histological slides and stained using Hematoxylin and Eosin (HE) techniques. The slides prepared were analyzed by 2 experienced pathologists (RCGA and EFM) to review and confirm the microscopic diagnosis of SCC or BSCC.

Clinical and demographic data (age, gender, tobacco and alcohol consumption and clinical stage), histological grading, presence or absence of metastasis in lymph nodes, established treatment modality, recurrence, date of diagnosis, date of recurrence, date of last consultation and date of death for the calculation of disease-free survival and overall survival were collected from medical records.

This study was approved by the Federal University of Goiás Research Ethics Committee (Protocol 022/13).

2.1. Sample analysis

The immunohistochemical technique was used for the qualitative and quantitative analysis. Serials of 3 μm sections were placed on silanized slides (A3648-EasyPath) and subjected to immunohistochemistry. The antibodies used in the study were the monoclonal mouse anti-human E-cadherin (dilution 1:200; SPM471Clone; Biotechnology Santa Cruz, CA, USA), monoclonal mouse anti-human β-catenin (dilution 1:100; E-5Clone; Biotechnology Santa Cruz, CA, USA), and monoclonal mouse anti-human Ki-67 (dilution 1:100; MM1Clone; Biotechnology Santa Cruz, CA, USA).

First the sections were deparaffinized using xylene and hydrated using ethanol. The sections were washed in a phosphate buffer solution (PBS) (pH 7.2) and incubated for 30 min in citrate buffer, pH 6.0 (for E-cad and Ki-67), and EDTA-tris base, pH 9.0 (for β-cat), in a digital water bath preheated to a temperature of 95 °C. The sections were removed for progressive cooling for 10 min. After washing with PBS, the slides were dipped in a 3% hydrogen peroxide solution for 30 min to block endogenous peroxidase. Sections were then incubated for 20 min in a bovine serum albumin (BSA) solution to block endogenous proteins. After that the sections were incubated for 18 h and kept at 4 °C (overnight) with the primary antibodies monoclonal mouse anti-human E-cadherin, monoclonal mouse anti-human β-catenin antibody and Ki-67 monoclonal mouse anti-human antibody. After an overnight the slices were incubated in the biotin–streptavidin–peroxidase system (LSAB System, Dako) for 30 min at a temperature of 22–25 °C (for β-cat and Ki-67) and in the polymer system (MACH 4-Universal HRP-Polymer, Biocare, USA) for E-cad for 30 min at 22–25 °C. The sections were incubated in 3.3′-diaminobenzidine (DAB) (Novolink system, Novocastra, RE7280-K) for 5 min for β-cat and Ki-67 and for 2 min for E-cad at room temperature. The sections were counter-stained with hematoxylin dehydrated with alcohol and sealed with coverslips in a non-aqueous resin solution (Entellan–Mikroskopie–Merck).

2.2. Qualitative analysis

For the qualitative analysis of the samples, the ITF region was evaluated. The ITF corresponds to the cells in the most advanced tumoral region at the tumor-host interface (Bryne, 1998; Piffko et al., 1997). In that region, the molecular characteristics of these cells are different from those of surface areas and this is the most important area of the tumor to assess prognosis in both SCC and BSCC (Bryne, 1998; Piffko et al., 1997). The samples were evaluated in terms of E-cad and β-cat cells with continuous membrane staining, cytoplasm and nucleus staining, and the anti-Ki-67 antibody with exclusively nuclear staining.