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Galectin-9 as an important marker in the differential diagnosis between oral squamous cell carcinoma, oral leukoplakia and oral lichen planus

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ABSTRACT

Objectives: To evaluate the expression of Galectins (Gal) 1, 3 and 9, Metalloproteinase 3 (MMP-3) and mast cell density in oral lesions of patients with potentially malignant disorders (PMD) and oral squamous cell carcinomas (OSCC) by comparison with the controls.
Study design: We selected 40 cases of PMD, 40 OSCC and 13 with normal histopathological profile. Immunohistochemistry was performed for Gal-1, Gal-3, Gal-9 and MMP-3.
Results: Gal-9 was significantly higher in patients with OSCC than in others groups ($p < 0.001$). Gal-1 expression was significantly lower in patients with leukoplakia than those with OSCC and controls ($p = 0.0001$). Gal-3 was significantly lower in patients with OSCC than those with leukoplakia ($p = 0.03$). MMP-3 was lower in patients with leukoplakia in comparison with the lichen planus group ($p = 0.013$).
Conclusion: The increased expression of Gal-9 may be helpful to differentiate of OSCC from other oral cavity lesions.

Introduction

Oral cavity squamous cells carcinoma (OSCC) is a malignant neoplasia arising from the oral mucosal epithelium, and which is classified as the most frequent type of cancer around the world (Konkimalla et al., 2007). Although the comprehension of cancer etiology is still unclear, several determining factors have been noticed and investigate (Parkin et al., 2005).

Biomarkers of pre-malignant lesions of oral epithelium have been sought for clinical application (Lippman and Hong, 2001). Although the comprehension of cancer etiology is still unclear, several determining factors have been noticed and investigated (Bundgaard et al., 1994). Biomarkers of pre-malignant lesions of oral epithelium have been sought for clinical application (Mishra, 2012), including some oncogenes and molecules whose expression is deregulated in oral cancer (Murugan et al., 2012).

Pre-malignant lesions or cancer precursor lesions are tissue changes that may turn into malignant tumor at any time, and they may also remain stable for a considerable length of time (Neville et al., 2009). These lesions are also called "potentially malignant disorders" (PMD), and leukoplakia is the most common disorder in the oral cavity (Gabriel et al., 2004). Oral lichen planus is a chronic inflammatory dermatosis and its etiology and pathogenesis remain obscure; also, some proposed etiologies are as follows: genetic predisposition, and psychogenic, neurological and immunological changes (Sugerman and Savage, 2002).

Over the past decades, numerous studies have indicated an increased risk of patients with PMD to develop cancer, and some

Abbreviations: Gal-1, galectin 1; Gal-3, galectin 3; Gal-9, galectin 9; MMP-3, metalloproteinase 3; PMD, potentially malignant disorders; OSCC, oral squamous cell carcinomas; BSA, bovine serum albumin.

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proteins such as galectins and metalloproteinases have been considered as possible risk markers (Ding et al., 2009).

Galectins (Gal) are animal lectins that have one or two carbohydrate recognition domains for ligands with affinity for β-galactose-containing oligosaccharides. In tumor biology, galectins have been associated with properties related to tumor progression or to the control of tumor development (Braeuer et al., 2012). They can bind to extracellular matrix proteins such as laminin, fibronectin, vitronectin, as well as integrin, through carbohydrate-dependent ligands (Elola et al., 2007). Galectins are also involved in cell modulation, acting as a potentiator or inhibitor of cell-extracellular matrix interactions as well as cell-cell interaction. Moreover, they can inhibit cell proliferation and, thus, interrupt G1-phase cell cycle (Baba et al., 2005).

Galectin-1 and galectin-3 are the most widely studied galectins, and some studies indicate that galectins may have profound effects on the growth and on the functional biological role of cells, such as cell–cell interaction and cell–extracelluar matrix interaction, cell differentiation, angiogenesis, apoptosis and inflammation (Dumic et al., 2006; Smetana et al., 2006). Galectin-9 has been connected with antineoplastic properties, regulating several cellular functions, such as cell adhesion, cell proliferation, and apoptosis (Elola et al., 2007; Okudaira et al., 2007).

Nonetheless, there are controversies over the exact role of each galectin. On the one hand, they can activate several cell types; on the other hand, they can have opposite effects, such as deactivation and inducement of apoptosis (Lv et al., 2012).

Similarly, it is believed that metalloproteinases (MMPs) play an important role in cell proliferation and in angiogenesis, hence they may play an important role in tumorigenesis as well (Egeblad and Werb, 2002).

In this study we evaluated the expression of Gal-1, Gal-3, Gal-9 and MMP-3, as well as mast cell density in biopsies diagnosed with oral squamous cell carcinoma, leukoplakia and lichen planus.

Material and methods

The samples comprised of 93 cases of oral lesions diagnosed at the Pathological Anatomy Service of the Odontology course of University of Uberaba (UNIUBE), and at the Laboratory of Pathological Anatomy and Cytopathology (PATMED). This study was approved by the Research Ethics Committee of University of Uberaba (UNIUBE), Brazil, under protocol CAAE 0005.0.227.000-10.

Tissue sample selection

We selected 40 paraffin blocks with diagnosis of squamous cell carcinoma in patients with lesions in different areas of the oral cavity. Those lesions presented dysplastic surface epithelium and were characterized histopathologically by invasive island and cords of malignant epithelial cell. Other 40 cases of PMD lesions were selected, with clinical and histopathological diagnosis of leukoplakia (20 cases) and lichen planus (20 cases), where all 40 cases of presented free of atypical cells. Lichen planus were characterized by hyperkeratosis, destruction of the basal cell layer, saw-toothed shape and lymphocytes infiltrate. Leukoplakia was an exclusion diagnosis from other lesions with oral white plates, with histological findings of hyperkeratosis and acanthosis. Control groups were composed by 13 cases with normal histopathological tissue from retro molar mucosa obtained from patients submitted to third molars extraction. The histologic diagnosis is illustrated in Fig. 1.

Histochemistry and immunohistochemistry

For mast cell quantification, the slides were stained with toluidine blue and were examined under a standard light microscope with a magnification of 1600×. Mast cell counts were performed in all areas of the sections. Counting was performed with the help of an ocular micrometer so as to calculate the area of each field.

For immunohistochemistry, desparaffinized sections were treated with 3% hydrogen peroxide in methanol for 10 min and incubated at 90°C for 30 min for antigen detection. The sections were incubated in 2% bovine serum albumin (BSA) at room temperature for 30 min to reduce nonspecific binding. Then, the specimens were individually incubated with anti-cytokine monoclonal antibodies specific for human anti-galectin-1 (1:50) (AF 1152; R&D Systems, Minneapolis, MN), anti-galectin-3 (1:75) (AF 1154; R&D, Systems, Minneapolis, MN), anti-galectin-9 (1:75) (AF 2045; R&D Systems, Minneapolis, MN) and anti-MMP-3 (1:10) (MAB 905; R&D, Systems, Minneapolis, MN), diluted in 2% BSA before use at 37°C for 2 h. The specimens were then incubated with secondary biotinylated anti-mouse immunoglobulin (Ig), anti-rabbit Ig, and anti-goat Ig antibodies using Link System 002488 (Dako, Carpinteria, CA) at 37°C for 30 min. The sections were washed and then incubated with the streptavidin–peroxidase conjugate (Dako) for 30 min. The reaction was developed by incubation with diaminobenzidine (Sigma, St. Louis, MO). The sections were counterstained with hematoxylin.

Morphometric analysis

For morphometric analysis, immunopositive cells were quantified using images of the histological sections captured with a digital system, and analyzed using ImageJ software (National Institutes of Health, Bethesda, MD). For this purpose, each field to be quantified was captured with a camera coupled to the standard light microscope and to a computer to digitize the image. The number of cells in each field was determined, as well as the area of each field (0.14 mm^2). The density of positive cells was expressed as the number of cells per square millimeter.

Statistical analysis

The data were analyzed using Statview software (Abacus Concepts, Berkeley, CA). After analysis for normality and variance of the data, Mann–Whitney and Kruskal–Wallis tests were performed. p Values < 0.5 were considered to be statistically significant.

Results

Our results demonstrated that immunostaining for all antibodies tested. The staining was evaluated in the cytoplasm of cells studied.

Staining for Gal-9 was significantly higher in the patients with OSCC than in the other groups (Kruskal–Wallis; $p < 0.001$) (Figs. 2A and 3A).

Immunostaining of Gal-1 was significantly lower in the patients with oral leukoplakia than in patients with OSCC and patients in the control group (Kruskal–Wallis; $p = 0.0001$) (Figs. 2B and 3B).

Gal-3 was significantly higher in patients with oral leukoplakia than in patients with OSCC (Mann–Whitney; $p = 0.032$). However, Gal-3 did not have a significant difference when compared to the other groups (Kruskal–Wallis; $p = 0.06$) (Figs. 2C and 3C).

When comparing the number of Gal-3 positive cells in patients with OSCC according to the degree of malignancy, it was observed that Gal-3 was significantly lower in patients with OSCC type 1 than in the control group (Kruskal–Wallis; $p = 0.001$) (Figs. 2D and 3D). MMP-3 immunostaining was significantly higher in patients with oral lichen planus than in patients with oral leukoplakia (Kruskal–Wallis; $p = 0.0130$) (Figs. 2E and 3E). Histochemistry analysis with toluidine blue revealed that mast cell density was significantly lower in patients with leukoplakia and OSCC