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Pleomorphic adenoma of oral minor salivary glands: An investigation of its neoplastic potential based on apoptosis, mucosecretory activity and cellular proliferation

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ABSTRACT

Objective: The aim of this study was to investigate the neoplastic potential of the PA of minor oral salivary glands measured by apoptosis (Bcl-2, Bax and p53), mucosecretory activity (MUC1), and cellular proliferation (Ki-67).

Design: Thirty-one cases of PA of the oral cavity and four controls (C) taken from normal oral minor salivary glands were analyzed using the immunohistochemistry technique. The proteins were detected utilizing a semi-quantitative method (scores) as follows: (−) negative ≤5%, (+) low 6–25%, (++) moderate 26–50% and (+++) high >50% of positive tumour cells. The apoptotic indices were evaluated by the ratio Bcl-2/Bax. Non-parametric comparison and correlation tests were used for analysis.

Results: The data showed high staining of anti-apoptotic protein Bcl-2 in both groups (PA = 57.9%; C = 67.7%) and a significantly lower expression of pro-apoptotic protein Bax (PA = 22.7%; C = 97.7%) and MUC1 (PA = 14%; C = 82.3%) in PA than in C ($p < 0.001$). On the other hand, a similar expression of Ki-67 and p53 proteins (≤5%) was seen in both PA and C. In PA, only 2/31 cases showed the ratio Bcl-2/Bax < 1.There was no difference in cellular expression with regard to clinical variables or clinical outcome ($p > 0.05$).

Conclusion: The neoplastic potential of PA could be associated with an imbalance in apoptotic processes and a lower index of cellular proliferation. Mucosecretory activity does not play a significant role in primary PA.

1. Introduction

The most common salivary gland tumour is the pleomorphic adenoma (PA), which accounts for 40–70% of such diseases.[1] Fifty-five percent of tumours of the minor salivary glands are PA, and the most common intraoral sites are the palate, followed by the lip and buccal mucosa.[2] High rates of malignant transformation have been reported in long-standing and recurrent PA.[3]

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Its biological behaviour and clinical and microscopic features are widely known. However, its etiopathogenesis remains unclear[4] and as a result certain proteins have been investigated in order to analyze the development mechanisms of PA in minor salivary gland. Among them are Bcl-2 (anti-apoptosis protein), p53 (tumour suppressor protein) and the Ki-67 (proliferation cell marker).[5–11] But for the Bax, a pro-apoptotic protein, only two studies have investigated its association in the pathogenesis of the PA.[5,6] According to these studies, Bax and Bcl-2 proteins have often been associated with the process of apoptosis in salivary gland tumours. In addition, it has been suggested that the apoptosis markers Bcl-2 and p53 could predict the behaviour of tumours,[12] and that the loss of the p53 function associated with upregulation of bcl-2 potentiate tumour growth.[13]

Data from the literature suggest that the positive staining of these markers may be related to the development, aggressiveness, differentiation, malignancy and prognosis of salivary gland tumours, but these studies are still controversial mainly in terms of PA.[7,9,11]

Few studies have explored the mucosecretory activity of mucins in PA, which have been associated with the development of several kinds of cancer.[14–19] In general, these mucins are glycoproteins which protect the mucosal surfaces from adverse environmental influences and may facilitate glandular secretory processes.[14] The minor salivary glands are small mucosal glands in the oral cavity with short ducts which produce a mucoprotein-rich secretion with a high IgA concentration. The glands with prominent production of mucins come into close contact with the teeth and mucosal surfaces and are key contributors to the protective mechanism of saliva.[20] Of these mucins, the MUC1, a membrane bound mucin detected in most epithelial tissues, is one of the best known, and it has been suggested that its overexpression could influence various physiological or biochemical events, including carcinogenesis and tumour invasion.[14–19]

Investigation of PA in parotid, submandibular and minor salivary gland tumours by immunohistochemical technique has shown that the expression of MUC1 is associated with the invasive growth of these tumours and a poor outcome in patients such as lymphatic invasion, lymph node metastasis and deep invasion in several tumors.[15] In our review, few samples of PA in minor salivary glands investigating MUC1 were found because of a lack of exploratory studies.[14,15,19]

Against this background, we investigated the neoplastic potential of the PA in the oral minor salivary glands because it is the most frequently found salivary gland tumour in the oral cavity. To date no study has investigated the association between apoptosis (Bcl2, Bax and p53), mucosecretory activity (MUC1) and cellular proliferation (Ki-67) specifically in the minor salivary gland.

2. Materials and methods

2.1. Patients and clinical specimens

Thirty-one cases of PA of the minor salivary glands located in the oral cavity and which had been submitted to excisional biopsy were selected for this research. As a control group, the study included four specimens of normal minor salivary glands acquired from specimens of non-neoplastic lesions without any inflammatory signs, such as, adjacent glands to mucous retention phenomenon (mucocele) located in the oral mucosa. All specimens were retrieved from the archives of the Oral Pathology Laboratory of the Dental School at the Federal University of Goiás, Brazil, between 1996 and 2013. Clinical data, such as age, gender, anatomical location, treatment, clinical outcome and recurrence were recorded. All the procedures for sample acquisition and preparation were approved by the Research Ethics Committee of the Federal University of Goiás (Protocol: 043/11).

2.2. Light microscopy

All specimens were fixed in 10% buffered formalin (pH 7.4) and paraffin embedded. The microscopical findings were evaluated from an analysis of a 5-μm section of each sample, routinely stained with haematoxylin and eosin. The microscopic features were analyzed and confirmed by two independent examiners (E.F.M and M.O.M) in accordance with the World Health Organization (WHO) classification of tumors.[4]

2.2.1. Histomorphological analyses

For histological analysis of the sample, the categorization used was that of Foote and Frazel et al.[2]: predominantly cellular, equally myxoid and cellular (mixed), extremely cellular and predominantly myxoid.

2.3. Immunohistochemistry

The immunohistochemical study of the expression of Bcl-2, Bax, p53, MUC1 and Ki-67 was carried out. Then, paraffin-embedded tissues were sectioned (3 μm) and collected in serial sections on glass slides coated with 2% 3-aminopropyl-triethylsilane (Sigma Chemicals, St. Louis, MO, USA). The sections were deparaffinized by immersion in xylene, and this was followed by immersion in alcohol and incubation with 3% hydrogen peroxide diluted in Tris-buffered saline (TBS) (pH 7.4) for 40 min. The sections were then immersed in a citrate (PH = 6.0) or EDTA buffer (pH 6.0; SIGMA, P4809, St. Louis, USA) for 30 min at 95 °C for antigen retrieval (Table 1), and then blocked by incubation with 3% normal goat serum diluted in distilled water, at room temperature, for 20 min. The slides were then incubated overnight (18 h) with the primary antibodies at 4 °C in a humidified chamber.

After washing in TBS, the sections were treated with labelled streptavidin–biotin–peroxidase (LSAB) kits (K0492, DAKO, Denmark). They were then incubated in 3.3′ diaminobenzidine in a chromogen solution (DAKO, K3468) for 2–5 min at room temperature. Finally, the sections were stained with Mayer's haematoxylin and covered. Negative controls were obtained by omitting the primary antibodies, and substituting them with 1% PBS-BSA and non-immune mouse (DAKO, X0910) serum.

2.3.1. Cell counting and statistical analysis

The immunoreactivity was evaluated in at least 1000 cells examined at 40× magnification (integration graticule - 4740680000000- Netzmikrometer × 12.5, Carl Zeiss, Göttingen,