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ORIGINAL ARTICLE

Myoepithelioma of minor salivary gland - An immunohistochemical analysis of four cases

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Summary

Introduction and Methods: We performed an immunohistochemical study in four cases of myopitheliomas with objective to realize a profile in respect of differentiation grade by the monoclonal antibodies CK14, vimentin and alph-SMA, besides to investigate the cell proliferation by anti-PCNA, besides, we compare the immunoreactive with glandular normal tissue. **Results:** In the glandular normal tissue the myoepithelials cells had shown expression for alpha-SMA and CK 14, while that in the ductals cells, only the presence of CK 14 was verified. All the cases was verified positivy for CK 14 and vimentin, however, CK 14 had been present only in epithelioid and fusiform cells, while that the vimentin revealed positive also in the cytoplasm of the plasmocytoid cells. alpha-SMA was not detected in the neoplasic cells. Immunopositivity for the PCNA was observed in more than 75% of the cellular component of the analyzed tumors, independent of the cellular type. **Conclusions:** We concluded that it did not have difference in the proliferative activity among the cellular types presents in the myoepitheliomas and, still, the results of this study suggest that the constituent cells of this neoplasia one really represent cells of the mioepitelial ancestry, but in different stages of differentiation.

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INTRODUCTION

Myoepitheliomas are rare benign neoplasias of the salivary gland, more commonly found in the parotid[1,2], being responsible for less than 7% of salivary gland tumors. It was first described in 1943[3,4]. Such lesion shows a varied pattern of morphological growth, it may be solid, myxoid or reticular. It differs from the pleomorphic adenoma because it does not bear any ductal component[5].

This tumor has varied cell morphology, being fusiform, plasmocytoid, epidermoid or clear cells[6]. Some studies have shown that fusiform cells have some muscular differentiation because they react to a-SMA and vimentin[4], while other investigations did not show any muscular differentiation in the plasmocytoid cells[4,7]. According to Jaeger et al. (1997), these cells could have origins other than myoepithelial, and they lost or had changed their capacity to express muscular evidence markers.

Besides the many phenotypes myoepithelioma cells may have, some authors report that fusiform and clear cells have a higher prolipherative capacity, when compared to the plasmocytoid cells, and they also stated that the production of myxoid material would be related to the low prolipherative activity of these tumors[9].

Medical literature mentions the use of different markers for histogenetic-related prolipherative activity both in the normal salivary gland and also in the glandular benign and malignant tumors. Thus, we should use immunohistochemistry to analyze differentiation patterns and the prolipherative activity of the different cell types present in salivary gland myoepitheliomas.

MATERIALS AND METHODS

We selected 4 cases of small salivary gland myoepitheliomas from the files of the Pathology lab of the Oral Pathology Department of the Federal University of Rio Grande do Norte. Paraffin-bounded specimens were cut in 5mm thickness slices and hematoxylin-eosin was used for the cellular morphology analysis.

An immunohistochemical study by the streptavidine-biotin technique was carried out using antibodies against vimentin, a-SMA, CK 14 and PCNA (Cell Proliferation Nuclear Antigen). Chart 1 lists the clones, antigenic recovery, dilution, incubation time and manufacturers of the antibodies used.

All the material selected was fixed in formaldehyde and embedded in paraffin, histological cross-sections of 3µm were made and placed on slides adhered by 3-aminopropyltriethoxy-silane (Sigma Chemical CO., St. Louis, MO, USA). The histology cross-sections were deparaffined in xylol, rehydrated in an alcohol sequence up to water and washed in two distilled water vials for 5 minutes each. Endogenous peroxidase was blocked by hydrogen peroxide 20vol, flushed with water and incubated in TRIS-HCL (Tris-hydroximethil-aminomethane), pH 7.4 for 10 minutes. The cross-sections were incubated with anti-mouse monoclonal antibody, diluted in a TRIS-HCL buffer solution (Chart 1), for incubation with the streptoavidine-Biotin complex, in a 1:100 dilution for 30 minutes. For development purposes, we used a 0.03% diaminebenzidine cromogen solution, diluted in TRIS-HCL added to 0.6ml of 20vol hydrogen peroxide in a dark chamber for 3 minutes. For counter-coloring we used Mayer Hematoxylin for 10 min, flushing in water after each step. To finish the process we used alcohol for dehydration and diaphanization in xylol for slide preparation with Permount.

Chart 1. Antibodies used.

Clone	Specificity	Dilution	Antigenic recovery	Incubation time
LL002	CK 14*	1:20	Citrate, pH6.0, Steamer	60'
∝-SM-1	∝-SMA*	1:50	Tripsine 0.1%, 37º	120'
V9	Vimentin**	1:50	No recovery	120'
PC10	PCNA**	1:50	Citrate, pH6.0, Steamer	Overnight

Fragments of normal salivary gland were used as internal positive control and for comparative purposes.

The immunopositiviness analysis was carried out by two examiners at different times, in a double-blinded study through light microscopy, and all the Brown colored cells in their cytoplasm or nucleus were considered positive (PCNA). Thus we investigated the presence or absence of markers, assigning the following scores: - (no marker); + (focal marker, less than 10% of cells marked) and ++ (diffuse marking).

RESULTS

Table 1 lists the patients' clinical data.

Morphological Results

Tumors were well circumscribed, and we frequently found a fibrous connective tissue capsule surrounding the specimens. The four tumors presented a predominantly solid growth and organizational patterns. The specimens were made up of nests of cohesive and non-cohesive cells in a matrix that varied between hyaline and myxoid. Tumor cells showed different morphologies, frequently fusiform, polygonal of eosinophilic cytoplasm (epithelioid) and, sometimes, with hyper chromatic nucleus. No tumor had necrotic areas; although in one case we did find squamous metaplasia and calcifications.

Immunohistochemical results

The immunohistochemical marking for the analyzed antibodies may be seen in Figures 1 and 2. All the cases