POLYMORPHOUS LOW-GRADE ADENOCARCINOMA. AN ULTRASTRUCTURAL AND IMMUNOHISTOCHEMICAL STUDY

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■ ABSTRACT: Six cases of polymorphous low-grade adenocarcinomas were studied by light and electron microscopy and immunohistochemistry. The following patterns of histological appearance have been found: papillary, solid, tubular, pseudocystic, trabecular and rarely cribriform. Utilizing the peroxidase-antiperoxidase (PAP) method, the intermediate filament vimentin, keratin and S100 protein are observed in tumoural cells. Under the electron microscope, tumour cells have relatively clear cytoplasms and limited amounts of rough endoplasmic cisternae. Intermediate size filament bundles are observed close to the nuclei and in more distant cytoplasmic areas. Cells with microvilli lining lumen like spaces are also seen. Morphological and immunohistochemical analysis reveal two types of neoplastic cells: myoepithelial and luminal. Cells with intermediate features between these two types are also seen, indicating a possible conversion of luminal cells into myoepithelial ones.

■ KEYWORDS: Salivary gland neoplasms; adenocarcinoma.

Introduction

Under the generic designation of "adenocarcinoma" of salivary gland origin there is a group, previously described, of clinically and microscopically low-grade polymorphous tumours. The importance of separating this clinicopathologic entity from other adenocarcinomas lies chiefly in its distinctly favorable prognosis.

These neoplasms have been variously designated in different studies as "Lobular Carcinomas of Minor Salivary Glands",[1, 7] "Terminal Duct Carcinomas",[4, 8] "Papillary

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Low-Grade Adenocarcinomas",[2, 12] and "Polymorphous Low-Grade Adenocarcinomas".[6]

They are composed essentially of cuboid, low columnar or occasionally spindle-shaped cells with uniform round to ovoid nuclei, and cytoplasm modest in quantity. As the name indicates, the Polymorphous Low-Grade Adenocarcinomas (PLGA) may have a variety of histomorphologic features. The spectrum of histologic appearance includes solid nests, trabeculae, tubules, papillary areas, pseudocystic formations and strands. Combinations and transitions among these patterns are frequent. Perineural infiltration may be found.

The purpose of this study is to describe the histological, immunohistochemical and ultrastructural features of six oral PLGA and to discuss the histogenesis of this minor salivary gland tumour.

Material and methods

Cases reported as adenocarcinomas at the Oral Pathology Service, University of São Paulo, since 1968, were reviewed.

Within this heterogeneous group of cases, one clinicopathologic tumour entity was identified and was designated, according to the Evans & Batsakis[6] histopathological criteria, as "Polymorphous Low-Grade Adenocarcinomas".

There were three male and three female patients. The ages at diagnosis ranged from 28 to 57 years, with the average of 47 years. All six tumours arose from minor salivary glands (five in the palate and one in the tongue).

Histologic sections were available for all of these cases.

Immunostaining was caried out with the peroxidase-antiperoxidase (PAP) method.[19]

The dilutions of antiserum were as follows: anti-vimentin 1:200 (Biogenex), anti-keratin (45-55Kd) 1:800 (Dakopatts), and anti-S100 protein 1:1500 (Dakopatts). All the cases were incubated overnight at room temperature.

Aminoethylcarbazole was used as the chromagen. Positive and negative controls were included in all procedures.

For electron microscope examination formalin-fixed tissue fragments from two cases, washed in sucrose, were post fixed in 2% phosphate buffered glutaraldehyde and secondarily post fixed in 1% osmium tetroxide, washed in sucrose phosphate buffer, and immersed in 0.5% aqueous acetate overnight. These tissues were dehydrated in ethanol and embedded in Araldite. Ultrathin sections were stained with lead citrate and examined with a Zeiss (D-7082 Oberkochen, West Germany) EM 952 electron microscope.

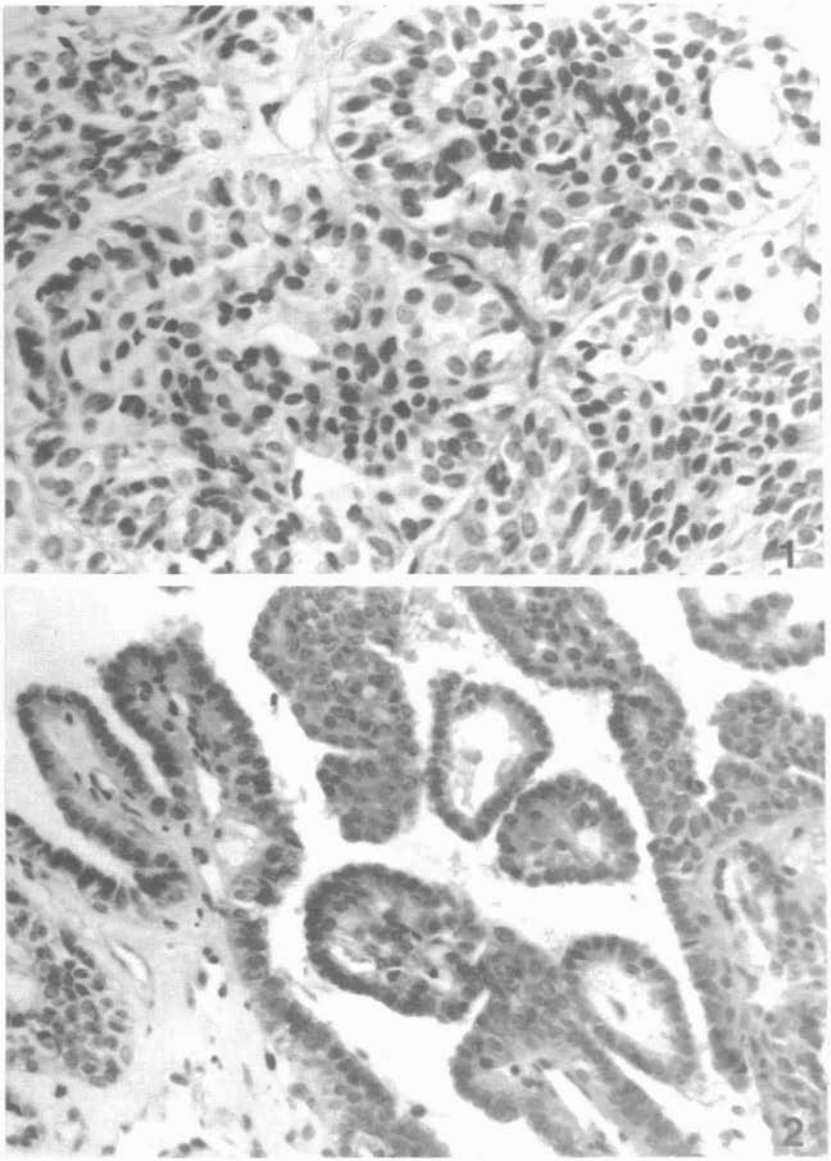

FIGURE 1 – Lobules of tumour cells showing some luminal spaces. HE, X350
FIGURE 2 – Neoplastic cells in a papillary pattern. HE, X250.

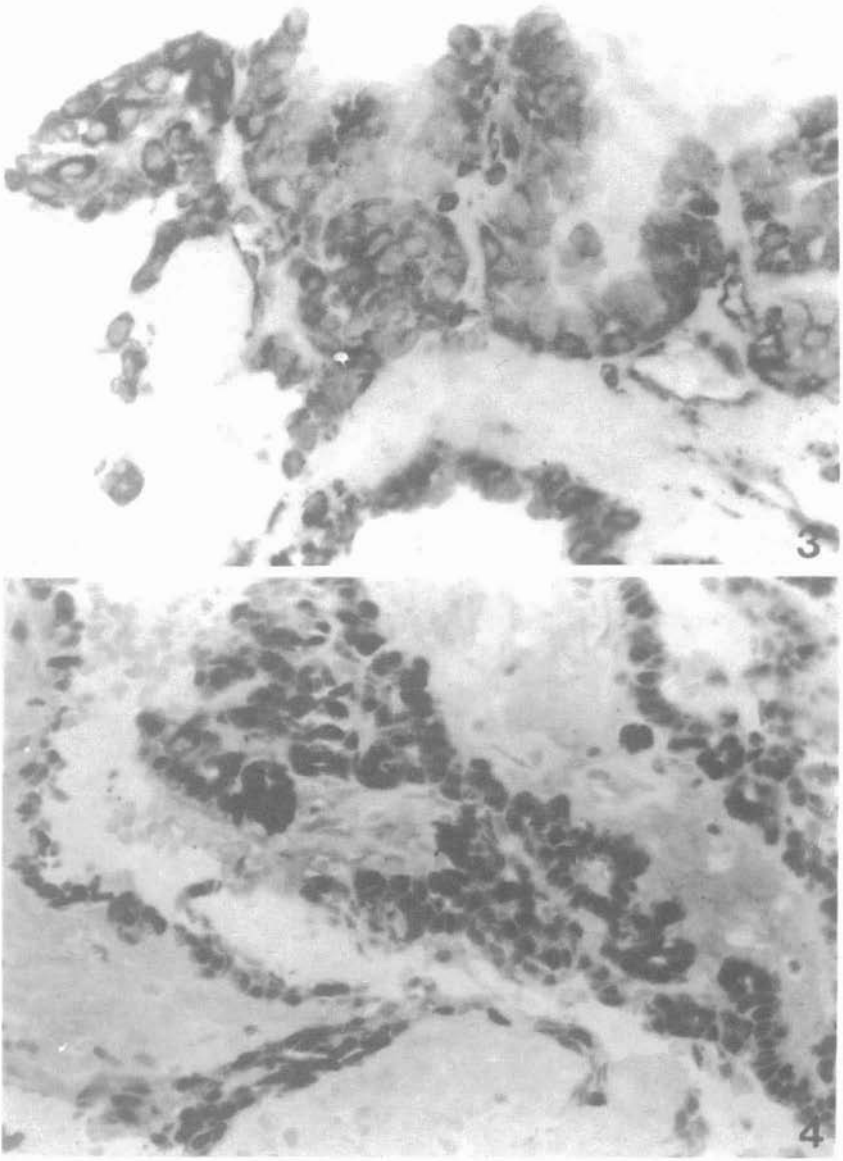

FIGURE 3 – Antiserum to vimentin. Reaction of cells lining the papillae. X380.
FIGURE 4 – Antiserum to S100 protein. Reaction of some cells in a papillary area. X310.

Results

This group of tumours displayed a variety of growth patterns, many of which could be found within the same lesion. In five cases, the neoplastic cells were arrayed in solid masses and lobules (Figure 1). Four cases exhibited a papillary pattern, showing papillae with fibro-vascular cores and lined by a single layer of cells (Figure 2). One of these cases was characteristically papillary whereas the others showed different morphologic features. In four tumours, tubules of a single cell layer or more and pseudocysts, some of which with a mucinous content, could be seen. In three cases there were anastomosing strands of cells in a trabecular pattern, and two tumours presented rare areas of cribriform pattern.

The neoplastic cells, in general, were characterized by their regularity and lack of nuclear atypia. Most cells were cuboid or low columnar with round to ovoid nuclei which had slightly stippled chromatin and a small inconspicuous nucleolus. The cytoplasm was usually scant, eosinophilic and showed indistinct cell borders. Some tumour cells exhibited clear cytoplasm.

The stromal component of these lesions was sometimes scant, being strongly eosinophilic and hyalinized, or muco-hyalinized showing a bluish tint, or highly cellular. Foci of calcification were present in one case.

All tumours were non-capsulated and exhibited infiltration into surrounding tissues. One palatal tumour showed bone invasion and spreaded into the vicinity of the maxillary sinus, whereas another palatal tumour showed invasion of the mucosal epithelium. Perineural invasion was seen around small nerves in some specimens.

In solid or lobular areas most cells were positive to vimentin and some scattered non luminal cells were positive for keratin and S100 protein. Vimentin was especially found in outer edges of tumour masses and S100 protein sometimes showed the same pattern. Small groups of cells within these areas were positive for keratin, characterizing luminal cells of small ducts.

Cells lining pseudocystic spaces were negative for keratin, positive for vimentin and some were also positive for S100 protein.

In areas of papillary pattern, cells lining the papillae were vimentin positive (Figure 3). Keratin and S100 protein were found in groups of cells situated among lining cells of the papillae, without exhibiting uniform staining pattern (Figure 4).

In tubules with more than one cell layer, the inner layer of luminal cells was positive for keratin and negative for vimentin and S100 protein, whereas the outer nonluminal cells were negative for keratin and some were positive for S100 protein and vimentin (Figure 5).

In areas of trabecular pattern, some cells were positive for keratin, vimentin and S100 protein, without exhibiting uniform pattern of staining.

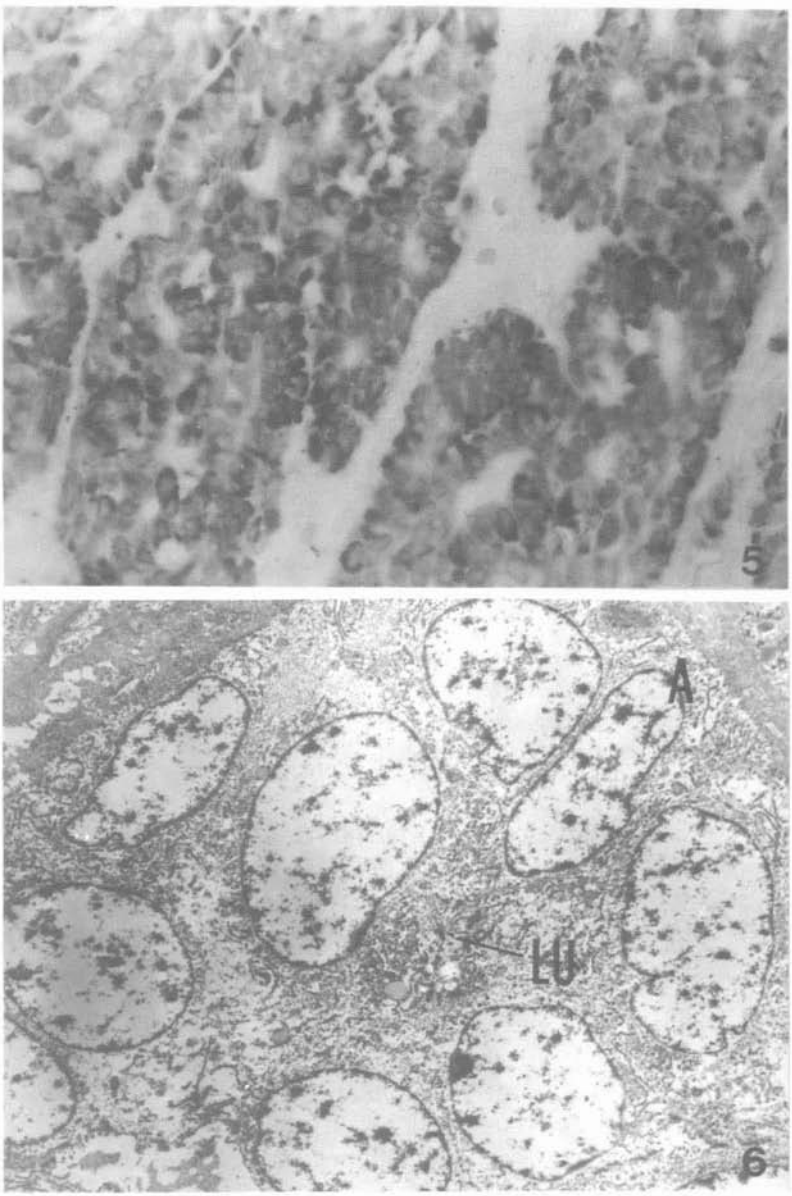

FIGURE 5 – Antiserum to vimentin. Reaction of cells in a tubular area. X375.

FIGURE 6 – Cell cluster with a lumen like space (Lu). A shows a cell lining a lumen and also in contact with a basal lamina. X3,800.

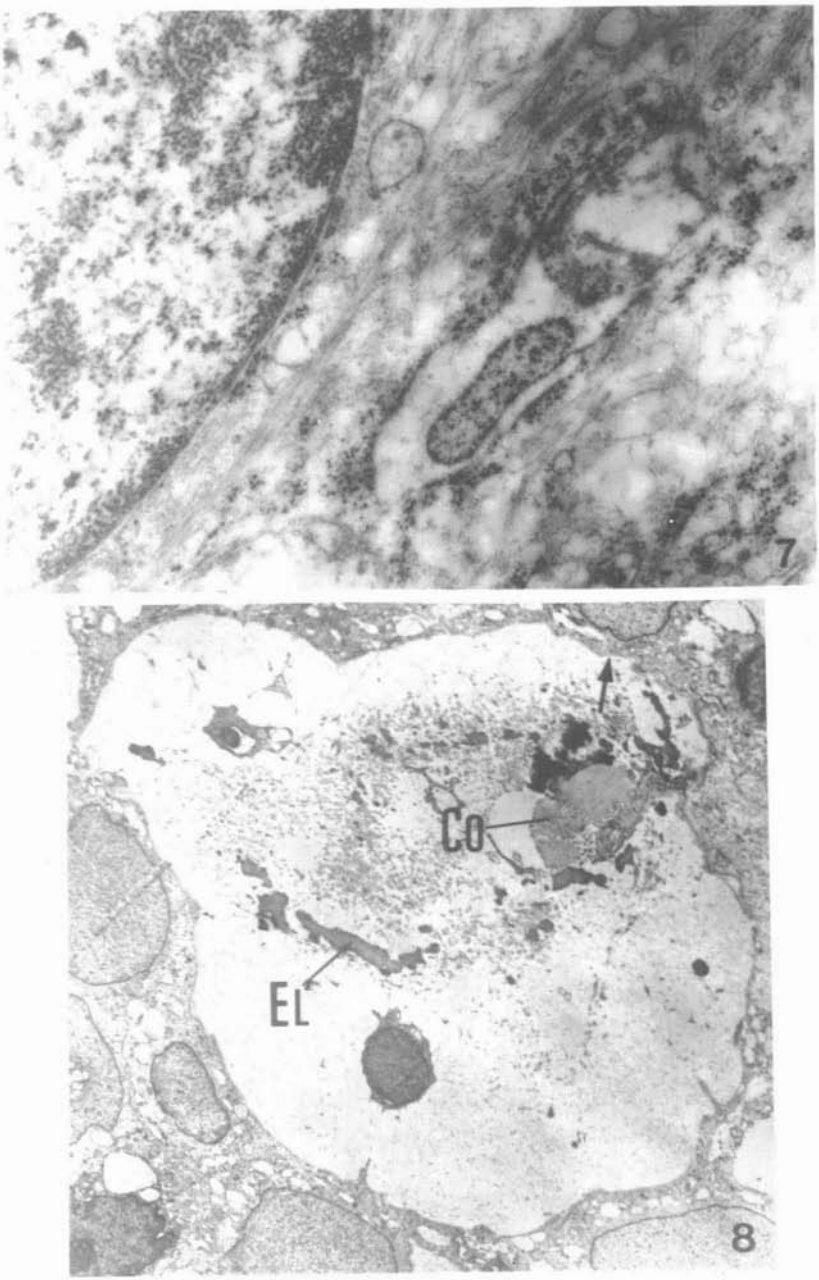

FIGURE 7 – Cell containing clear nuclear profile, rough ER cisternae and intermediate filaments. X39,000.
FIGURE 8 – At the figure borders one notices the cells or cell parts limiting a pseudocystic space containing profiles from connective tissue cells processes, collagenic fibrils (Co) and elastic fibers (El) and basal lamina (arrow). X3,825.

Regardless the morphologic pattern of the tumour, the S100 protein stained both the cytoplasm and the nucleous of the cells, whereas keratin stained only the cell cytoplasm. Vimentin usually stained cells with a scant cytoplasm, appearing as a narrow ring around the nucleus.

Electron Microscopy

The most central cells from the groups seen under the electron microscope limited one or more lumen-like spaces of variable sizes (Figure 6). Microvilli were seen at the space borders. Sometimes the cell lining a lumen was also in contact, at the opposite end, with a basal lamina (e.g., cell A in Figure 6). On other occasions, a second cell with no contact with this lumen intervenes and is in contact with the basal lamina. In the extracellular space close to the basal lamina, collagenic fibrils with apparently irregular cross sections and variable amounts of elastic fibers were seen. In general, the cytoplasm of epithelial cells appeared relatively clear with limited and variable amounts of rough endoplasmic reticulum cisternae.

Commonly, intermediate sized filament bundles were observed either close to the nuclei (Figure 7) or in more distant cytoplasmic areas. Pseudocysts were observed in one case. Such spaces were completly encircled by the epithelial cells and contained collagenic fibrils and elastic fibers (Figure 8).

Discussion

Parameters from Polymorphous Low-Grade Adenocarcinomas (PLGA) described in this series, such as site, sex distribution, and average age, compared very well with those reported by others.[1, 4, 8, 12, 13, 16, 17]

Histologically, our six cases of PLGA presented features similar to those previously described in the literature.[15, 16, 17, 18] These neoplasms exhibited a spectrum of microscopic appearances that included papillary, solid, tubular, pseudocystic, trabecular and rare cribriform patterns. These tumours were non-capsulated and locally infiltrative. Accordingly to Slootweg & Muller[18] they are prone to reappear and should be treated by wide local excision to avoid recurrences.

Immunohistochemical study using anti-keratin and anti-vimentin antibodies demonstrated two types of neoplastic cells in these PLGA: luminal cells and myoepithelial cells. Vimentin has been detected in all tumours in which the participation of myoepithelial cell as a tumoural component has been postulated. This fact supports the view that the vimentin filament is one of the earliest indicators of neoplastic myoepithelial cell differentiation.[3] In the PLGA, we could find myoepithelial cells in the lining of the cystlike spaces, in the solid or lobular areas, in the outer layer

of tubules with more than one cell layer, and in the lining of the papillae. Luminal cells, evidenced by anti-keratin antibody, were seen in the inner layer of tubular areas and in solid growth areas, often arranged in a ductlike pattern.

The staining by anti-S100 antibody showed variable results. Occasionally, myoepithelial cells expressed S100. Similar findings with anti-S100 and anti-cytokeratin antibodies are described in a PLGA from the nasal cavity.[5]

Submicroscopic examination of the cell groups demonstrated lumens lined by cells with microvilli. These cells were characterized immunohistochemically as true luminal cells, by their reactivity to keratin antibody. Other cells without microvilli presented filaments around the nucleus. It was demonstrated immunohistochemically that they were myoepithelial cells.

Dardick & Van Nostrand[5] showed well differentiated myoepithelial cells and luminal cells in a PLGA from the nose whereas Nicolatou et al.[13] observed myoepithelial-like cells, besides stem cells, intercalated duct cells and secretory cells in a PLGA of the palate. In accordance with Frierson et al.[8] our electron microscopic studies failed to definitely identify myoepithelial cells in the tumours examined. However, utilizing the two methods – electron microscopy and immunohistochemistry – we have characterized some cells as immature myoepithelial ones.

An interesting finding in our material was that some luminal spaces were lined by cells that extended from the lumen to a basal lamina. This observation might be revealing cells with intermediate features between luminal and myoepithelial cells. If this suspicion corresponds to facts, then the more structurally complex myoepithelial cells would derive from the luminal ones, suggesting a myoepithelial cell lineage for the polymorphous low-grade adenocarcinoma.

A transitional cell between luminal cells and myoepithelial cells had already been suggested by Kierszenbaum[11] in pleomorphic adenoma and by Hayashi et al.[9] in tumours produced in nude mice.

Pseudocysts were observed under the electron microscope, showing a direct continuity with the interstitial connective tissue, as reported by Hoshino & Yamamoto.[10] Elastic fibers were seen in these pseudocystic spaces and in the extracellular space, close to the basal lamina. Elastic fibers have been demonstrated in some salivary tumours like pleomorphic adenomas, myoepitheliomas and adenoid cystic carcinomas.[14] It is possible that salivary neoplastic cells elaborate elastic matrix under certain conditions, and this might be an additional criterion for the recognition of their myoepithelial nature.[14]

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■ *RESUMO: Seis casos de adenocarcinomas de baixo grau de malignidade foram estudados por microscopia de luz e eletrônica e imuno-histoquímica. Os seguintes padrões histológicos foram identificados: papilar, sólido, tubular, pseudocístico, trabecular e raramente cribriforme. Utilizando o método da peroxidase-antiperoxidase (PAP), o filamento intermediário vimentina, queratina e proteína S100 foram observados nas células tumorais. Ao microscópio eletrônico, as células tumorais exibiam citoplasma relativamente claro e quantidade limitada de cisternas de retículo endoplasmático rugoso. Feixes de filamentos intermediários foram verificados próximo ao núcleo e em áreas citoplasmáticas mais distantes. Células com microvilos, revestindo espaços semelhantes a lumes, foram também observadas. A análise morfológica e imuno-histoquímica revelou dois tipos de células neoplásicas: mioepitelial e luminal. Células com aspectos intermediários entre estes dois tipos estavam presentes, indicando uma possível conversão de células luminais em mioepiteliais.*

■ *UNITERMOS: Neoplasias das glândulas salivares; adenocarcinoma.*

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