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Inverted ductal papilloma of minor salivary gland origin: morphological aspects and cytokeratin expression

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Abstract Ultrastructural features and cytokeratin expression of inverted ductal papillomas of minor salivary gland origin were studied. Under the electron microscope, an increased number of desmosomes and mucus-like granules in some cells were the most striking features. Immunohistochemical study revealed that tumor cells displayed strongly positive reactions with cytokeratins 13 and 14, and less strong reactions with cytokeratins 7, 8, 18 and 5D3. These results support the hypothesis that an inverted ductal papilloma can be derived from the proximal portion of a salivary gland excretory duct.

Key words Salivary gland tumors · Inverted ductal papilloma · Ultrastructure · Cytokeratin

Introduction

The inverted ductal papilloma (IDP) of minor salivary gland origin was first described by White et al. [11] in 1982, and has been included among the ductal papillomas in the latest classification of tumors by the World Health Organization [9]. Even though few cases of salivary gland IDP have been reported in the available literature [4, 5, 8, 11, 12], inverted papillomas arising in sites other than salivary glands are well-recognized entities, with many cases and extensive series now reported [3, 6]. In the present investigation we used cytokeratins (CKs) of different molecular weights to examine ultrastructural and immunohistochemical differences in two cases of IDP, intending to clarify their histogenesis.

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Materials and methods

Two examples of IDP of minor salivary gland origin (palate and buccal mucosa) were accessioned by the Department of Oral Pathology, University of São Paulo. The palatal tumor was removed from a 22-year-old woman, while the buccal tumor was taken from a 51-year-old man. Both tumors were studied by light microscopy and immunohistochemically. The palatal tumor was also studied ultrastructurally.

For the light microscopic and immunohistochemical study the gross material from each tumor was fixed in 10% formalin for 24 h and embedded in paraffin. For light microscopy, 5-μm sections were obtained and stained with hematoxylin and eosin. For immunohistochemical study, 3-μm-thick paraffin sections were subjected to deparaffinization and were treated twice during 5 min in 10 mM citric acid (pH 6.0) in a microwave oven at 700 W (modified method according to Shi et al. [10] and Gerdes et al. [5]). Immunohistochemistry was carried out by the streptavidin method employing a Biogenex complex (1:50, ZP000LM). Antibodies used (with their sources), concentrations and time of incubation are listed in Table 1. In brief, after incubation with primary antibody sections were thoroughly washed and exposed to a streptavidin complex. Diaminobenzidine was used as chromogen followed by 0.5% copper sulfate and counterstained with Mayer's hematoxylin. Positive and negative controls were included in all the reactions.

For transmission electron microscopic study, tissues from the palatal tumor were cut into small pieces and fixed for 2 h in 2% phosphate-buffered glutaraldehyde. They were then postfixed in 1% osmium tetroxide, washed in sucrose phosphate buffer, and immersed in 0.5% aqueous uranyl acetate overnight. Fragments were dehydrated in ethanol and embedded in Araldite. Thin sections were stained with lead citrate and examined using a Zeiss EM 9S2 electron microscope.

Table 1 Concentration and incubation times for monoclonal antibodies

Cytokeratin[a]	Concentration	Incubation time (min)
CK 7	1:10	60
CK 8	1:100	30
CK 10	1:10	30
CK 13	1:80	120
CK 14	1:40	30
CK 18	1:700	30
5D3 (CK 8, 18, 19)	1:80	30

[a] Biogenex Lab., San Ramon, Calif.

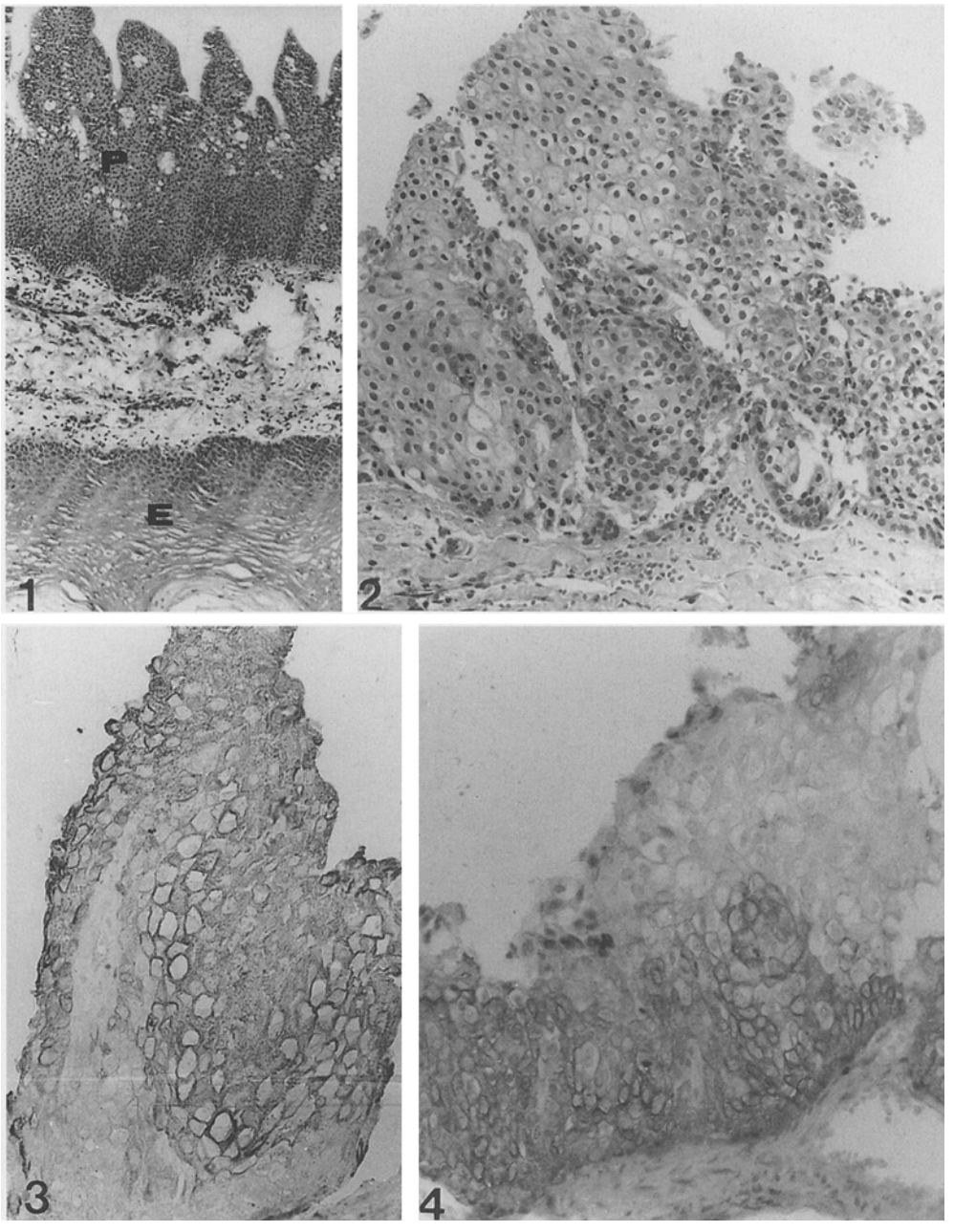


Fig. 1 Low-magnification view of inverted ductal papilloma in buccal mucosa. Mucous cells are seen among epidermal cells (*E* surface epithelium, *P* inverted papilloma). HE, × 80

Fig. 2 Squamous aspect of cells in a papillary projection. HE, × 250

Fig. 3 Expression of cytokeratin 13 in tumoral cells above the basal layer. × 400

Fig. 4 Expression of cytokeratin 14 in tumoral cells. × 400

Results

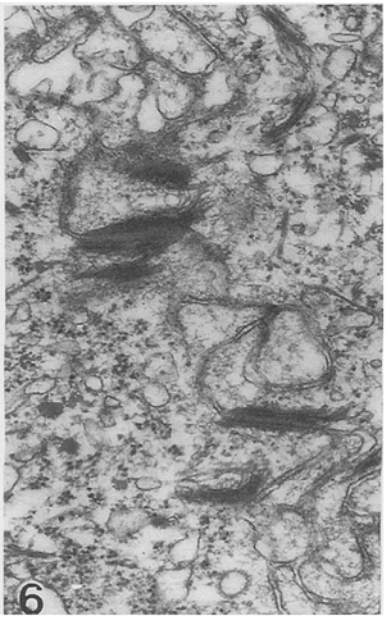
Light microscopy

Both cases presented similar histological aspects: a dilated cystic space present in the lamina propria lined by an epithelium that formed large, broad-based papilliferous projections which extended into the lumen and subjacent connective tissue. Projections were formed in a squamous pattern by one or two layers of cuboid cells and numerous polyhedral cells of various sizes. Mucous cells were present among the cells as well as small microcystic spaces. There was no evidence of invasion. In the surrounding fibrous connective tissue, lobules of minor salivary gland tissue were present and showed mild chronic inflammation (Figs. 1, 2).

Immunohistochemistry

In terms of a current numbering system for CKs [7], 5D3 reacted with CKs 8, 18 and 19, and AE8 reacted with CK 13. Except for CK 10, tumoral cells were positive for all the CKs studied. The most pronounced reactions occurred with CKs 13 and 14. CK 13 was positive in squamous cells but not in basal and mucous cells, whereas CK 14 was positive in basal cells and also in some layers above the basal layer. The remaining keratins 7, 8, 18 and 5D3

Fig. 5 Ultrastructural aspect of epidermal-like cells exhibiting large interdigitations. × 5,700



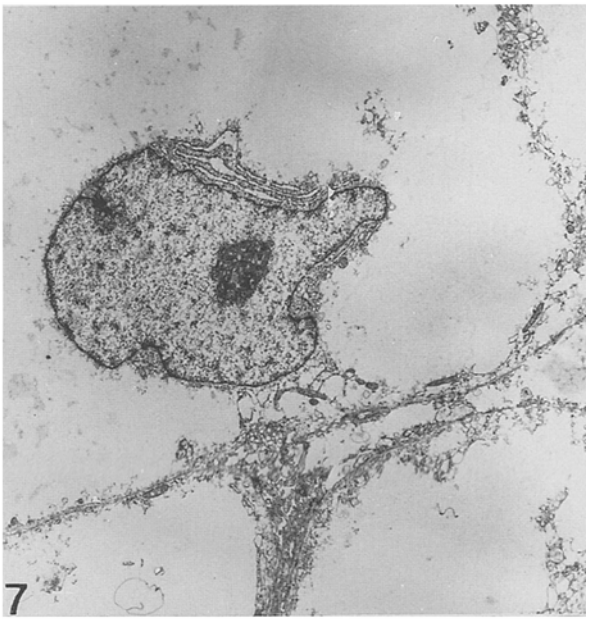
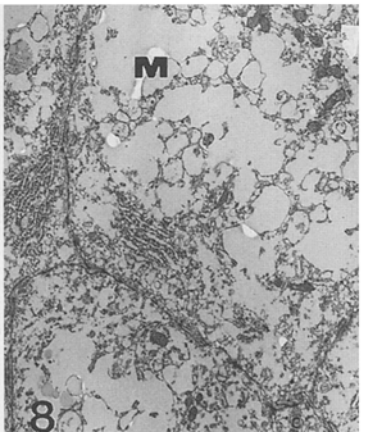
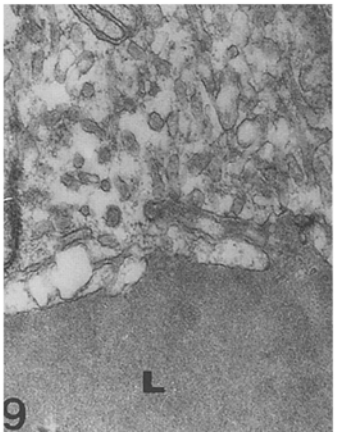


Fig. 6 Enlarged view of Fig. 5 showing typical desmosomes anchored in bundles of tonofilaments. × 30,000

Fig. 7 Stacked rough endoplasmic reticulum cisternae are seen near the nucleus. × 3,400





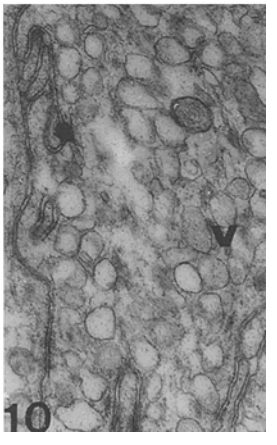


Fig. 8 Mucous-type secretory granules (*M*) in mucous cells. × 59,000

Fig. 9 Tubular and round profiles with material of the same electron density as that seen in the lumen (*L*). × 22,800

Fig. 10 Vacuoles of 150–300 nm diameter (*V*). × 26,000

(CKs 8, 18 and 19) were present focally but not significantly in all cell layers (Figs. 3, 4).

Electron microscopy

Under the electron microscope papillary projections showed epidermal-like cells of different profile sizes that varied in subcellular structures. The plasmalemma of adjoining smaller cells possessed many desmosomes and interdigitated in a marked and complex fashion. Bundles of tonofilaments anchored in these desmosomes were seen in the nearby cytoplasm. These cells showed rough endoplasmic reticulum (RER) cisternae and variable amounts of free polysomes. The progressively larger cells had wide, clear and empty cytoplasmic spaces, occasionally with stacked RER cisternae. Adjoining plasmalemma did not interdigitate in the largest cells. In areas of mucus-like cells no plasma membrane interdigitations were seen. Stacked RER cisternae and mucus-like secretory granules were observed consistently. Occasional profiles filled with clear vesicles of 150–300 nm diameter were seen. Cystic spaces encountered close to these mucus-like cells had a villus-free, almost smooth surface (Figs. 5–10).

Discussion

The IDP is a well-established entity that is now believed to arise from the excretory ducts of minor salivary glands. These neogrowths become lined with squamous epithelial cells and extend into surrounding connective tissue.

Our present immunohistochemical results show a CK immunoprofile in agreement with the immunoprofile of the excretory duct seen in the normal salivary gland: a strong presence of CK 14 and CK 13. In the normal salivary gland CK 14 is seen in basal cells of excretory ducts, while CK 13 is present in luminal cells[2]. Although expression of 5D3 (CKs 8, 18 and 19) and CK 7 in the tumors studied was very light, it supports the hypothesis that the IDP is derived from the salivary gland duct system.

Our ultrastructural study of our tumor specimens has shown features resembling the proximal portion of the excretory duct near the glandular oral orifice, where squamous differentiation occurs. These features were characterized by adjoining cells exhibiting a plasmalemma interdigitating in a complex fashion and possessing many desmosomes with bundles of tonofilaments anchored in them. These cells were interspersed by cells filled with RER and mucus-like secretory granules.

Based on salivary gland origin from surface epithelium, any of its cells have the theoretical potential of becoming squamous. However, excretory duct cells are most likely to suffer this change due to a loss of specialization and their proximity to the glandular buccal opening.

The large number of desmosomes in IDP may reflect a benign course or the degree and direction of cell differentiation of these lesions, as has been observed in inverted papilloma in the urinary bladder [1]. This contrasts significantly with nasal and paranasal papillomas, which have a tendency to recur and may undergo malignant transformation [3].

Despite the presence of epidermoid and mucous cells and cystic spaces, lack of multicystic and multinodular infiltrative growth allows differentiation of the IDP from cells of mucoepidermoid carcinoma.

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