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Expression of Different Keratins in Salivary Gland Tumours

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Twenty-four salivary gland tumours (six pleomorphic adenomas, two myoepitheliomas, five basal cell adenomas, six adenoid cystic carcinomas and five polymorphous low grade adenocarcinomas) were investigated by an immunocytochemical technique using monoclonal antibodies against cytokeratins (CKs) 7, 8, 10, 13, 14, 18 and 19. The luminal cells of ductal structures of the tumours reacted with all the CKs studied except for CK 13 and CK 10 and sometimes CK 14, showing an immunoprofile comparable to that of the intercalated segment of a normal salivary gland. The outer cells of the ducts rarely stained with CK 14, confirming that full differentiation of the myoepithelial cells is seldom achieved in tumours. Considerations were made regarding the intriguing expression of CK 14, the heterogeneous expression of CKs in the modified myoepithelial cells and the immunoprofile of the polymorphous low-grade adenocarcinoma.

Keywords: salivary gland, tumours, immunohistochemistry, cytokeratins

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INTRODUCTION

Since the advent of immunohistochemical techniques, several markers have been studied in normal salivary gland tissue and in salivary gland tumours.

The most contributory studies have been on S-100 protein, glial fibrillary acidic protein (GFAP), myosin, desmin and vimentin [1–12]. The detection of the muscle-specific actin in both normal and tumoral myoepithelial cells [13–17] and the co-expression of intermediate filament proteins in modified tumoral myoepithelial cells [5, 18–20] have been particularly useful.

More recently, a series of monoclonal antibodies (mAbs) have become available against the subclasses of cytokeratins (CKs). Studies have shown the presence of CK 14 in normal salivary myoepithelial cells and in basal cells of excretory ducts [21–24]; the presence of CKs 13 and 16 in the basal excretory duct cells [21, 23, 25, 26], the presence of CK 19 in luminal ductal cells and in myoepithelial cells [21, 25]; the presence of CK 18 in normal acinic cells and in acinic cell carcinoma [25, 27, 28]; and a variability of CK subtype expression in the modified myoepithelial cells of pleomorphic adenoma [21].

Although such studies have demonstrated that the immunostaining expression in salivary gland neoplasms is complex [6, 25, 26] these studies seem to indicate that different neoplasms can be distinguished on the basis of their immunoprofile [29].

We have, therefore, compared the CK's immunoprofile in a group of salivary tumours presumed to arise from the intercalated duct reserve cells [30, 31].

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MATERIALS AND METHODS

Twenty-four salivary gland tumours were studied. The material consisted of six pleomorphic adenomas, two myoepitheliomas, five basal cell adenomas, five polymorphous low grade adenocarcinomas and six adenoid cystic carcinomas (two tubular type and four cribriform type). All histological material from the tumours was reviewed to confirm the accuracy of diagnosis.

Formalin-fixed tissue sections (3 μm) were used for immunohistochemical staining. Paraffin sections were treated after deparaffinisation three times for 5 min at 700 W in citric acid 10 mM, pH 6.0, in a microwave oven (modified method according to Shi *et al.* [32] and Gerdes *et al.* [33]).

Two of the tumours—one pleomorphic adenoma and one myoepithelioma—were also fixed in a modified methacarn fixative (60% methanol, 30% chloroform, 10% glacial acetic acid) to serve as a control for the material submitted to the microwave oven.

The antibodies used in this study were numbered according to Moll *et al.* [34], and their source, concentration and time of incubation are listed in Table 1.

After incubation with the primary antibody the sections were thoroughly washed and exposed to secondary antibodies and, after further washing, exposed to strepto-avidin complex. Diaminobenzidine was used as the chromogen followed by 0.5% copper sulphate and finally counterstained with Mayer's haematoxylin. Positive controls for each CK, as indicated by the supplier, were used. Negative controls were incubated in buffer without primary antibodies.

Normal major and minor salivary glands included in the specimens were also studied.

Table 1. Monoclonal cytokeratins used

Cytokeratin	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
CK 19†	1:75	60

*Supplier: Biogenex Lab., San Ramon, California, U.S.A.
†Supplier: Dako A/S, Glostrup, Denmark.

RESULTS

Using the microwave, the results of the formalin-fixed and paraffin-embedded histological material were comparable to the results obtained with the alcohol-fixed tissue, although a little improvement of the staining was achieved with the latter.

The reactivity pattern of the normal glands included in the specimens are shown in Table 2. The results for the tumour material were as follows.

Pleomorphic adenoma (six cases)

All tumours included in this study presented ductal structures and were predominantly cellular with hyaline or myxoid components. Luminal cells of the ductal structures reacted with all the CKs studied except for CK 13 and CK 10 and sometimes CK 14. The outer cells of the ducts stained with CK 14. Plasmacytoid or hyaline cells stained with all the CKs tested except for CK 10. The staining was not uniform; some groups of cells reacted while others did not. Polygonal cells were reactive only with CK 14. In the epidermoid islets, the central areas reacted with CK 10 and the peripheral areas with CK 14 (Figs 1–3).

Myoepithelioma (two cases)

The tumours were composed of blocks of plasmacytoid or hyaline cells. The staining observed was not uniform. The cells reacted with CK 7, CK 8, CK 18 and CK 19, and rarely with CK 13 and CK 14.

Basal cell adenoma (five cases)

Two tumours were predominantly solid (monomorphic type), with a few tubular and trabecular structures, one tumour was tubulo-trabecular, and two were tubular (one with well-differentiated ducts and a great amount of secretion in the ducts). In the non-tubular areas, some cells stained with CK 8, CK 18, CK 14 and CK 19. This staining was also seen in ductiform structures with one cell layer. In ductal structures with two cell layers, the luminal cells were positive for all the CKs except CK 13 and CK 10. The outer cells of the tubular structures did not stain for CKs. The structures with two cell layers in the tubular and trabeculo-tubular types, exhibited the same pattern as mentioned for the ductal structures, except in the case of the mucous-producing tumour in which the outer cells reacted with CK 14 whereas the luminal cells did not react with this CK (Figs 4 and 5).

Polymorphous low-grade adenocarcinomas (five cases)

Almost all cells stained for CK 8 and CK 18. The staining appeared as a thin ring around the nucleus (Fig. 6).

Adenoid-cystic carcinoma

Tubular type (two cases). The luminal cells stained for all the CKs except for CK 10 and CK 13. The staining for CKs 7, 8 and 18 was slight and the outer cells did not stain.

Cribriform type (four cases). The luminal cells stained for CK 7, CK 8, CK 14, CK 18, and CK 19. The cells lining the pseudocyst and the outer cells of the cylinders did not stain for CKs (Figs 7 and 8).

DISCUSSION

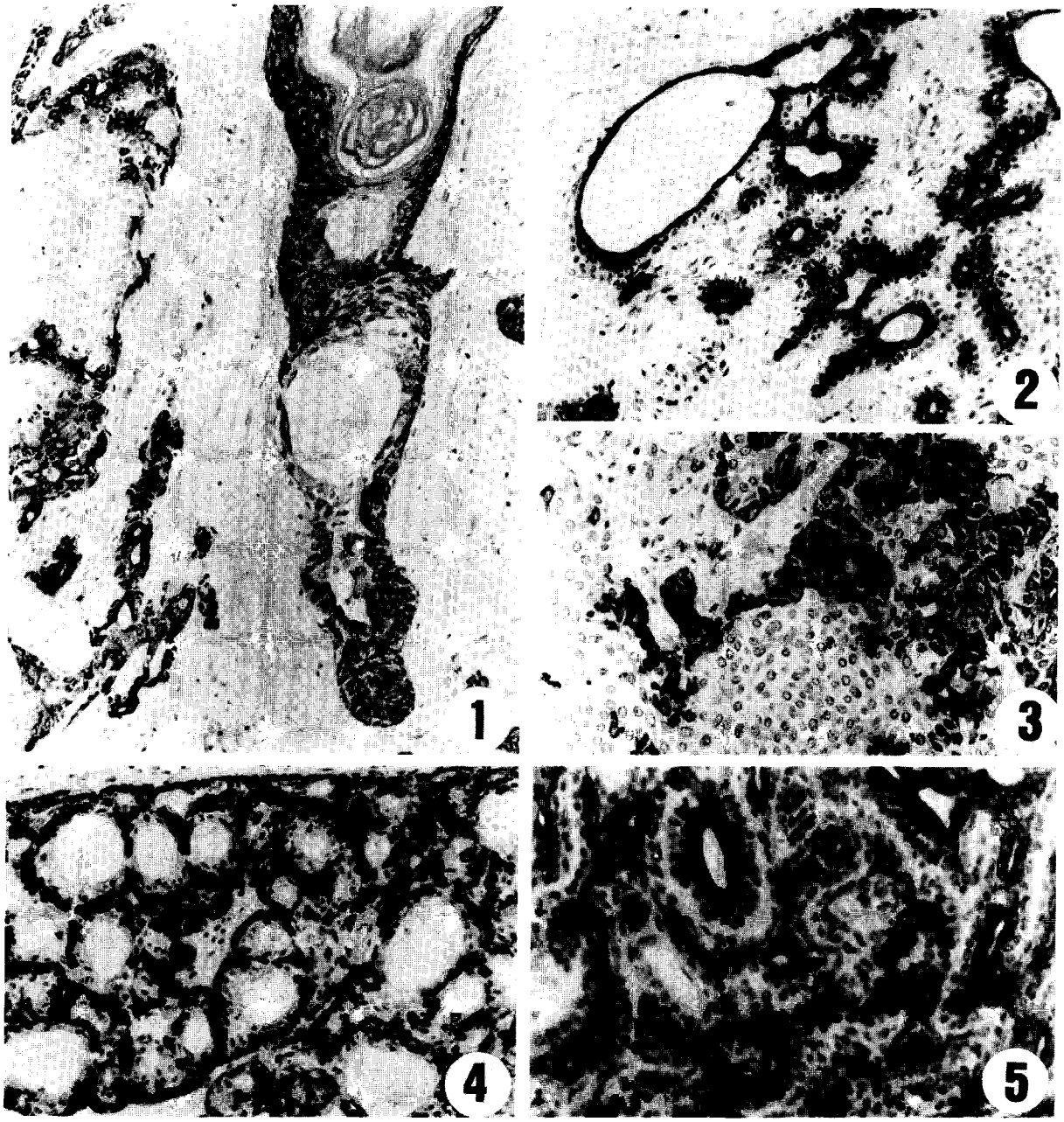
The results obtained in normal salivary glands included in the specimens showed staining patterns as described by others. CK 14 has been identified in myoepithelial cells and in the basal cells of excretory ducts [21–24]. Luminal cells reacted variously with anti-keratin mAbs. In our study CK 13 was detected only in excretory ducts and CK 10 was not found in normal gland structures.

In tumours, our results have shown that luminal cells of the ductal structures with two cell layers react with all the CKs studied except CK 13 and CK 10. These results are comparable to those found in the intercalated segment of the normal salivary gland. The outer cells of the ductal structures usually did not stain with CKs except in very well-formed ducts with

Table 2. Staining patterns of cytokeratins in normal salivary glands

	Cell type					
	Luminal				Acinar	
Cytokeratin	Excretory duct	Intercalated duct	Basal	Myoepithelial	Serous	Mucous
CK 7	++	+++	−	−	−	−
CK 8	+	++	−	−	++	++
CK 10	−	−	−	−	−	−
CK 13	+	−	−	−	−	−
CK 14	+◆	+/−	++	++	−	−
CK 18	+◆◆	+	−	−	++	+
CK 19	++	++	++	+◆◆◆	−	−

−, no staining; +, weak; ++, moderate, +++, strong staining.
◆ one cell layer duct; ◆◆ negative in the distal segment; ◆◆◆ only in one minor salivary gland.



Figs 1–3. Pleomorphic adenoma. Fig. 1. Expression of CK 14 in the outer cells of ducts and in peripheral cells of the epidermoid islets. The luminal cells show variable staining, × 125. Fig. 2. Expression of CK 18 in luminal cells, × 400. Fig. 3. Expression of CK 14 in some plasmacytoid cells. Observe groups of non-stained modified myoepithelial cells, × 250. Figs 4 and 5. Basal cell adenoma. Fig. 4. Tubular type. Expression of CK 14 in the outer cells of ducts, × 250. Fig. 5. Tubulo-trabecular type. Expression of CK 8, × 400.

mucous production, as seen in certain areas of the pleomorphic adenomas and in one basal cell adenoma of the tubular type. In these cases, the outer cells stain exclusively with CK 14, as seen in normal myoepithelial cells. This fact suggests that complete myoepithelial differentiation may be correlated with secretory and excretory functions. Actually, as shown by our results, the presence of the intermediate filament CK 14 in salivary gland tumours was intriguing: it was found in luminal cells only, sometimes in both luminal and outer cells and rarely exclusively in the outer cells as mentioned above.

In view of our results, some considerations must be made concerning the expression of CKs in modified myoepithelial cells of the pleomorphic adenoma and the myoepithelioma, as well as in the cells of the polymorphous low-grade adenocarcinoma and in some cells of the basal cell adenoma, monomorphic type.

Modified myoepithelial cells (plasmacytoid or hyaline cells) stained, although not uniformly, with all the CKs studied, including CK 13 which in normal glands is found only in the luminal cells of the excretory duct. Other studies have demonstrated considerable heterogeneity of keratin, vimentin, S-100 protein, desmin, and GFAP expression, as well as co-expression of these in the modified myoepithelial cells of pleomorphic adenomas. These facts are not surprising since

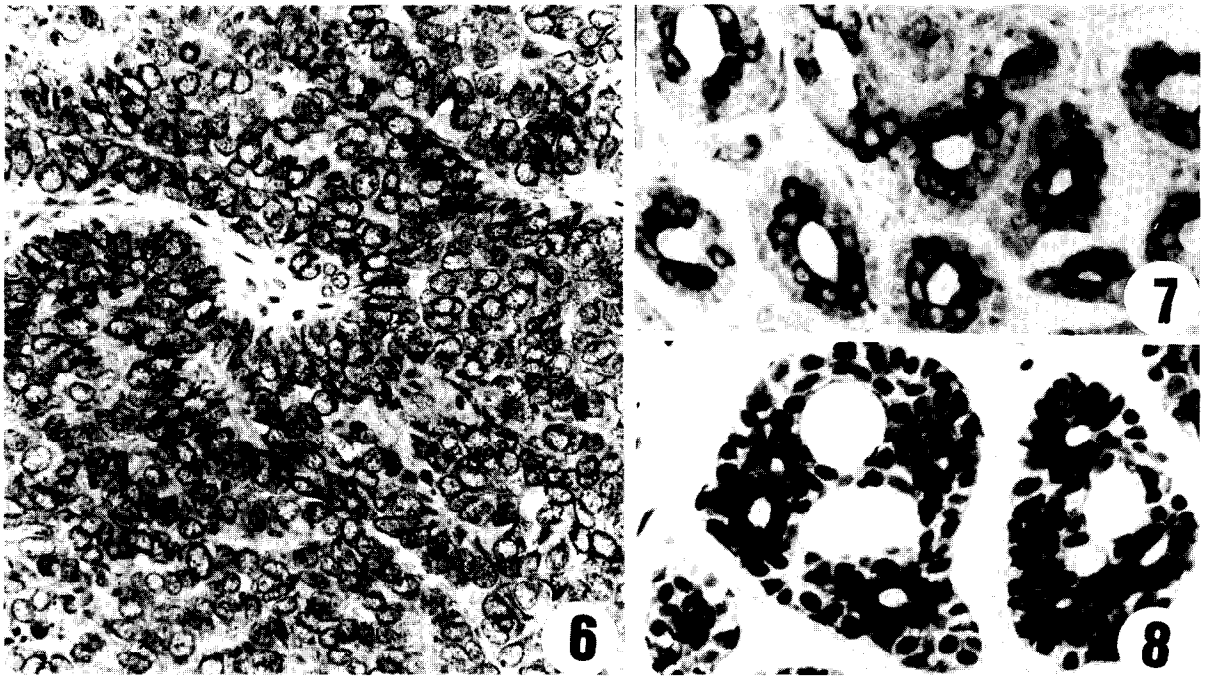


Fig. 6. Polymorphous low-grade adenocarcinoma. Expression of CK 8 in a great number of cells, × 250. Figs 7 and 8. Adenoid cystic carcinoma. Fig. 7. Tubular type. Expression of CK 14 in luminal cells, × 800. Fig. 8. Cribriform type. Expression of CK 7 in luminal cells, × 800.

there is evidence from ultrastructural studies that myoepithelial cells eventually become separated, loosing cell–cell contact and the basal lamina enveloping, which disrupts causing the dispersal of cells into the extracellular space [35, 36]. It is well established that cell–cell and cell–matrix interactions play an important role in biological events, such as tissue morphogenesis and cell differentiation. These facts may explain the diversity of phenotypes acquired by the modified myoepithelial cells and reinforce the concept that the morphology of pleomorphic adenomas is the result of diversity of tumour cell differentiation rather than the process implicit in a reserve cell histogenetic model [21]. For this reason pleomorphic adenomas and myoepitheliomas seem not to be the ideal models for the study of salivary gland tumour histogenesis, although the ductal structures show a CK immunoprofile comparable to the intercalated segment of the salivary duct system.

The cells of polymorphous low-grade adenocarcinomas and basal cell adenomas (monomorphic type) in areas without two cell layer duct formation stained with CK 8 and CK 18. Gustafsson *et al.* [29] found all cells reacted with PKK1 (CK 8, 18 and 19) in a monomorphic variant of basal cell adenomas. In basal cell adenomas of the monomorphic type and in polymorphous low-grade adenocarcinoma, ultra structural studies have demonstrated a predominance of luminal cells resting on the basal lamina—a hybrid of myoepithelial and epithelial cells—called transitional cells [37–40]. Therefore, we can conclude that transitional or hybrid cells between the luminal and the myoepithelial cells stain with CK 8 and CK 18.

Studies have suggested that the polymorphous low-grade adenocarcinoma and the basal cell adenoma are derived from pluripotential cells that can differentiate along two cell lines—myoepithelial and epithelial. The cell of origin would logically give rise to both myoepithelial and epithelial cells [41]. The present study has shown that differentiation towards the luminal cells is achieved only when there is a formation of two cell layer ductal structures and this may suggest that these tumours would not be composed exclusively of luminal or epithelial cells.

Based on these results, we may conclude that the so-called basal cell adenoma in the WHO classification [42] corresponds to several tumours with different grades of differentiation—from those where transitional cells predominate to those where duct formation is advanced.

Keeping these concerns in mind and the fact that the myoepithelial tumoral cell rarely achieves full differentiation, it seems to be possible to relate the CK immunoprofile of one tumour to a specific compartment of the salivary gland, contributing to the knowledge of the histogenesis of salivary gland tumours.

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