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Distribution of Epithelial Membrane Antigen in Benign and Malignant Lesions of the Salivary Glands

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Summary. An antiserum against epithelial membrane antigen has been used to stain a variety of lesions arising in the salivary glands. In normal major and minor glands staining was localised to the ductal systems. There was no evidence of myoepithelial cell staining. The mucous elements of the submandibular and sublingual glands were negative, but in the mucous elements of the minor glands there was focal cytoplasmic positivity. There was no cytoplasmic staining of serous elements in major or minor salivary glands. In pleomorphic adenomas the luminal membrane of ductal elements was strongly positive, with focal cytoplasmic positivity in some myxoid areas. In mucoepidermoid tumours both adjacent cell membranes and cytoplasm were strongly positive. The ductal structure of adenoid cystic carcinomas were clearly delineated while the pseudoducts produced by enclosed areas of stroma were negative. All mesenchymally derived tumours were negative and a tumour previously considered as a chondroma was strongly positive. The results are discussed in relation to phenotypic heterogeneity and the histogenesis of salivary gland tumours.

Key words: Epithelial membrane antigen – Salivary glands

Antisera have been raised to milk fat globule membranes prepared by defatting human cream. After suitable absorptions, these antisera show a specific reaction with a component on the surface membranes of many glandular epithelia (Heyderman et al. 1979). More recently, an extensive study in normal and neoplastic human tissues has demonstrated that this epithelial

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membrane antigen (EMA) is confined to the luminal surface membranes (and to a lesser extent to the cytoplasm) of normal epithelial tissues and is present in tumours of surface epithelial or mesothelial derivation. Normal and malignant tumours of mesenchymal origin are consistently negative (Sloane and Ormerod 1981). In view of the controversy concerning the degree of epithelial and myoepithelial differentiation in some salivary gland tumours (Hamperl 1970; Lucas 1976; Dardick et al. 1982; Batsakis 1980; Caselitz et al. 1981b) it was decided to examine the distribution of EMA in normal salivary glands and in some benign and malignant conditions arising in these glands. Apart from potential histogenetic interest it was also intended to examine the diagnostic use of this reagent in the field of salivary gland pathology.

Materials and Methods

Tissue Specimens

In this study both normal major and minor glands were examined in addition to a number of benign and malignant disorders.

Epithelial Tumours		*Connective Tissue Tumours*	
Adenoma		Sarcoma	3
Pleomorphic adenoma	14	Fibroma	1
Monomorphic adenoma	4	Chondroma	1
Mucoepidermoid tumour	3		
Acinic cell tumour	1	*Non-Neoplastic Disorders*	
Carcinoma			
Adenoid cystic carcinoma	7	Lymphoepithelial lesions	5
Adenocarcinoma	3		
Epidermoid carcinoma	2		

Immunocytochemical Staining

The antisera used in this study were obtained by injecting into a rabbit defatted human cream suspended in complete Freund's adjuvant (Heyderman et al. 1979; Sloane and Ormerod 1981). The antisera were absorbed with human plasma, 3 M KCl extracts of liver and kidney, non-specific cross-reacting antigen (Von Kleist et al. 1972), lactoferrin and fractions from human milk, which were eluted from a Sepharose 4B column in the molecular weight range 50,000–100,000 Daltons. All immunoabsorents were immobilised by conjugation to Sepharose 6B.

Affinity purified sheep anti-rabbit-γ-globulin antibodies were conjugated to horseradish peroxidase by Nakane's method (Nakane and Kavaroi 1974). Sections were cut from formalin-fixed, paraffin embedded blocks. After dewaxing, slides were bleached with 7.5% hydrogen peroxide in water and treated with 0.1 M periodic acid followed by 0.05 M potassium borohydride to block endogenous peroxidase (Heyderman and Neville 1976). Sections were incubated with the rabbit antiserum at a dilution of 1:50 for 1 h, washed, and then incubated with the peroxidase conjugate for 1 h. The stain was developed in a solution of diaminobenzidine and hydrogen peroxide and the sections counterstained in Meyer's haemalum.

Specificity was demonstrated by the total abolition of staining when the antiserum was prior absorbed with a preparation of EMA from milk (Sloane and Ormerod 1981).

Results

1. Normal Salivary Glands

Sections from the parotid, sublingual, submandibular and the minor glands of the lip and the palate were examined. In all tissues the cytoplasm of the serous elements was consistently negative and there was no staining of any stromal elements or of myoepithelial cells. The mucous elements of the submandibular and sublingual glands were also negative, but there was focal acinar staining of some of the mucous elements in the minor salivary glands. The staining in these mucin-containing cells was weak and cytoplasmic. The luminal membranes and the luminal contents throughout the ductal system of all glands were heavily stained (Fig. 1) and in the intercalated, striated and interlobular ducts the cytoplasm on the luminal aspect of the cell was also strongly stained with weaker staining of the basal portion of the cell.

2. Salivary Gland Tumours

The pleomorphic adenomas showed a variety of histological appearances. In the cellular areas in which the tumours were growing in sheets or clumps the cells were negative, but wherever tubular or ductal structures were formed there was strong staining of the luminal membrane and contents. In areas where there was stratification of the cells lining the lumina, the myoepithelial-like cells at the epithelial/stromal junction were consistently negative, whereas the lining cuboidal cells often had strong cytoplasmic staining. The myxoid and chondroid areas were variably stained, but in three tumours the cytoplasm of the stellate cells in the myxoid areas was positive. In one tumour it was not possible on the basis of standard histological stains to distinguish the lesion from a chondroma, but the 'chondrocytes' were strongly stained (Fig. 2). The myxoid stroma was also weakly positive in some of these tumours, suggesting that the tumour cells may secrete the antigen in a manner analogous to the ductal cells of the normal gland. Areas of squamous metaplasia showed weak staining, both membranous and cytoplasmic, of the more differentiated elements.

The seven adenoid cystic carcinomas were very variable in their growth patterns, with examples of both cribriform and solid types. In the cribriform areas of the tumours only the luminal membranes of the true ductal structures stained (Fig. 3), while the characteristic pseudoducts and the cytoplasm were uniformly negative. In the more solid regions small lumina were clearly depicted with strong staining of both the luminal membrane and the secreted contents. It is evident that this is not an artefactual edge effect, since the 'stromal' lumina do not express the antigen.

Four monomorphic adenomas were examined. One was an adenolymphoma in which the glandular elements were strongly positive, both cytoplasmically and on the luminal surface. Two tubular adenomas were strongly positive in areas of ductal differentiation and both the cytoplasm and the luminal surface were positive. In one alveolar adenoma the cells were ar-

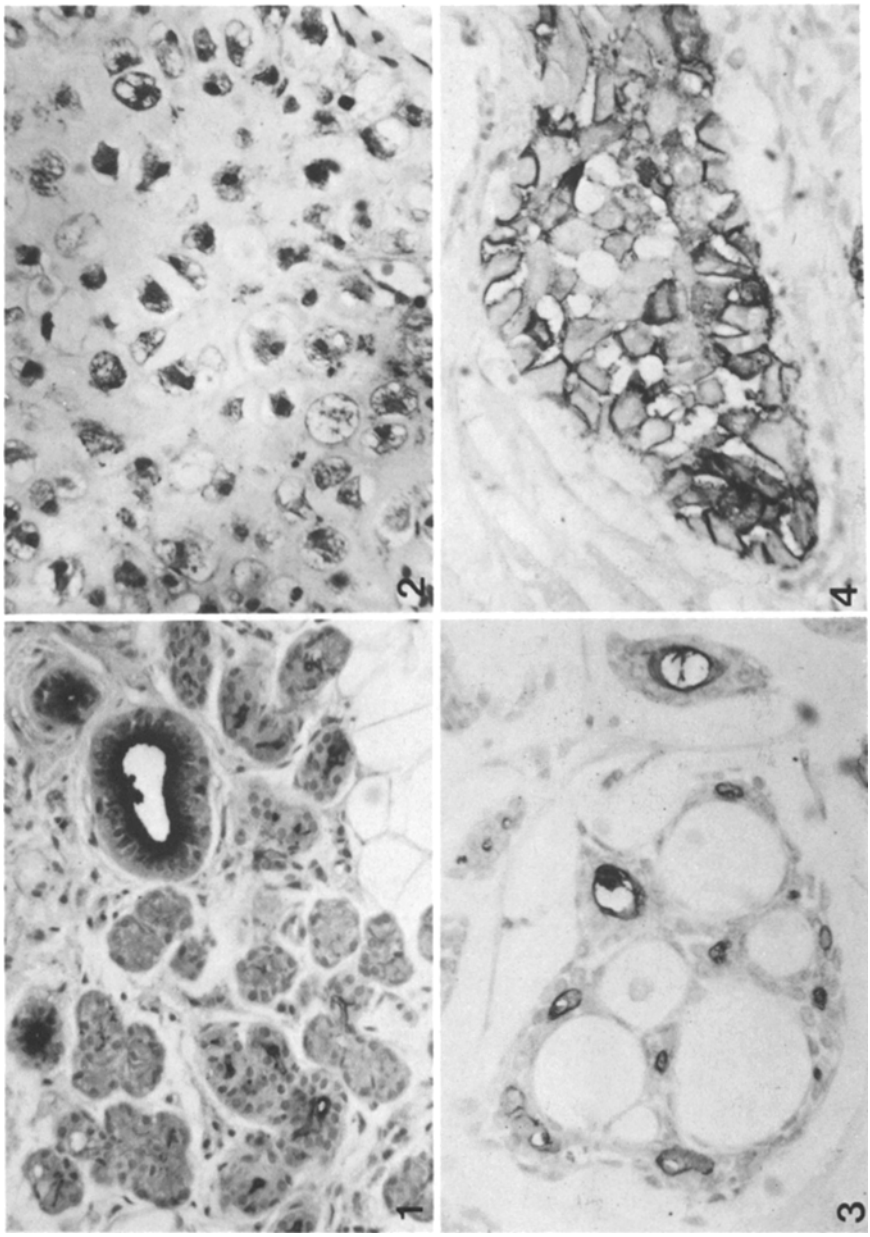

Fig. 1. Photomicrograph of a portion of normal parotid gland demonstrating luminal staining of the ducts. Magnification ×250

Fig. 2. Photomicrograph of a chondromatous lesion with strong cytoplasmic staining indicating a non-mesenchymal histogenesis. Magnification ×400

Fig. 3. Photomicrograph of an adenoid cystic carcinoma with clear delineation of lumina. Magnification ×310

Fig. 4. Photomicrograph of a mucoepidermoid tumour with both cytoplasmic and adjacent membrane staining. Magnification ×400

ranged in groups resembling mucous acini and in these areas the cells were cytoplasmically positive.

The three mucoepidermoid tumours showed strong positive cytoplasmic reactivity of all the cells with marked membrane staining (Fig. 4). In the squamous cell carcinomas there was strong cytoplasmic and membrane staining of the keratinising elements. The luminal surfaces of the adenocarcinomas were strongly stained as were the luminal contents. The acinic cell tumour and the sarcomas were all negative.

In the benign lymphoepithelial lesions the islands of salivary tissue were clearly demonstrated with both cytoplasmic staining of sheets of cells and staining of residual ducts as described in the normal glands. Lymphoid cells were not stained.

Discussion

This study shows that the staining pattern described previously for adenocarcinomas and squamous carcinomas in other primary sites (Sloane and Ormerod 1981; Sloane et al. 1982) is also seen in similar tumours arising from salivary glands. The distribution of the antigen in the normal glands and in the salivary gland tumours supports the view that salivary gland tumours represent a spectrum of myoepithelial and epithelial differentiation which varies both throughout a single tumour and between tumour types. In salivary gland tumours Epithelial Membrane Antigen (EMA) provides a reagent for the further investigation of tumour histogenesis and heterogeneity and has potential diagnostic value in identifying the predominantly spindle celled pleomorphic adenomas, chondroid pleomorphic adenomas as opposed to chondromas and in establishing the true identity of the solid variety of adenoid cystic carcinomas.

In the field of diagnostic histopathology EMA has proved useful in the differential diagnosis of anaplastic lesions where epithelial differentiation can be detected by expression of the antigen (Sloane and Ormerod 1981). In the normal salivary glands, with the exception of occasional staining of mucous acini, the staining was localised and concentrated on the luminal surface of the ductal cells. Thus it might be expected that ductal differentiation would lead to expression of the antigen. This was indeed the case in a variety of tumours, ranging from areas of ductal differentiation in pleomorphic adenomas and tubular monomorphic adenomas to the ductal structures in adenoid cystic and adenocarcinomas. This may be taken as suggestive of epithelial as opposed to myoepithelial differentiation.

In some myxoid areas of the pleomorphic adenomas the cells were stained cytoplasmically suggesting epithelial derivation, with staining of the myxoid material demonstrating secretory ability. This is seen to its extreme in the "chondromatous" tumour in which there was cytoplasmic staining of the cells in the chondroid-like matrix. Ultrastructural studies have suggested that these cells in myxoid areas show both epithelial and myoepithelial characteristics (Dardick et al. 1982). The staining in the chondroid tumour supports the view that there is epithelial differentiation in these

cells which by morphological criteria are demonstrating cartilaginous metaplasia. In contrast, the presence of vimentin in some of the cells in pleomorphic adenomas suggests a potential conversion to a mesenchymal phenotype (Caselitz et al. 1981 b). Caselitz has also reported the presence of keratin and actin immunoreactive materials in the spindle-shaped cells (corresponding to myoepithelial cells) in pleomorphic adenomas further supporting the potential interconversion of the secretory epithelial and the myoepithelial-like cells in salivary tumours (Caselitz et al. 1981 a, b). Thus it seems likely that in pleomorphic adenomas there is phenotypic lability in which both mesenchymal and epithelial elements may be expressed. Within the epithelial expression there are transitions from myoepithelial to secretory epithelial characteristics with many cells expressing both phenotypes of varying degrees.

In adenoid cystic carcinoma there is also evidence for dual myoepithelial and epithelial differentiation. The strong cytoplasmic staining of the mucoepidermoid tumours supports their derivation from ductal epithelium.

Current views on the histogenesis of salivary tumours regard the reserved intercalated ductal cells as the precursor population for the majority of these tumours, and thus the variable phenotypes seen are due to variability in cellular differentiation pathways (Eversole 1971). Recent autoradiographic evidence, however, indicates that both acinar and ductal cells are self-renewing populations (Dardick et al. 1982). Thus cells can potentially arise from any of these compartments and give rise to a transformed clone which can then undergo phenotypic change with variable gene expression and environmental modulation. If the possibility of variable gene expression is accepted, then the presence of myoepithelial or secretory epithelial characteristics within a single tumour is predicted, and when taken to the extreme the ability of epithelial or myoepithelial-like cells phenotypically of express mesenchymal characteristics – that is to say, chondroid metaplasia, is easily comprehended.

The present study supports the view that variable degrees of epithelial and myoepithelial differentiation are seen in pleomorphic adenomas which probably arise from a stem cell population capable to expressing more than one pathway under the action of local environmental factors. Adenoid cystic carcinoma would be the malignant counterpart of this lesion. The mucoepidermoid tumour however is restricted in its expression as shown by the uniformity of staining. Monomorphic adenomas appear to represent a midway point, although they are not always easy to distinguish from pleomorphic adenomas.

The heterogeneity of gene expression seen in salivary gland tumours is also seen in the rodent breast where stem cells that have been established in vitro from rat mammary tumours can be made to convert to myoepithelial or secretory-like epithelial cells (Bennett et al. 1978). Provisional tissue-culture studies have also demonstrated the potential stem cell nature of pleomorphic adenomas (Shirasuna et al. 1980) and it might be expected that future in vitro and xenograft studies will throw more light on salivary gland tumour histogenesis.

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