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Immunohistochemical expression of MAM-3 and MAM-6 antigens in salivary gland tumours

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Summary. MAM-3 and MAM-6 antigens of human milk fat globule membrane were detected immunohistochemically in 93 cases of salivary gland tumours as well as in normal glands. The antigens were visualized in 10% formalin-fixed paraffin sections. MAM-3 (MoAbs 115G3, 67D11) antigen was distributed in intercalated and striated duct cells of the normal salivary glands, and in luminal tumour cells and squamous metaplastic cells of pleomorphic adenomas. In pleomorphic adenomas the frequency of positive staining with MoAb 67D11 (54/67; 80.6%) was higher than that with MoAb 115G3 (36/67; 53.7%). MAM-6 (MoAbs 115D8, 115F5) antigen was expressed in luminal and lateral borders of serous acinar cells and ductal of the normal glands, and also in luminal borders of tubulo-ductal and glandular structures of salivary gland tumours. Ductal basal cells were characterized by existence of positive staining for MAM-6 antigen, in adenolymphomas MAM-6 antigen was restricted to the basal tumour cells. Some mucous cells of mucoepidermoid tumours were stained specifically with MoAb 115G3, and epidermoid cells of mucoepidermoid carcinomas manifested MAM-6 antigen staining. Immunohistochemical localization of MAM-6 antigen resembled that of epithelial membrane antigen (EMA) detected with MoAb.

Key words: MAM-3 and MAM-6 Antigens – Human salivary gland tumour – Immunohistochemistry

Introduction

Human milk fat globule membrane participates in the secretion of milk from mammary gland cells, and contains a glycoprotein known as epithelial membrane antigen (EMA). Polyclonal antiserum has been raised against EMA, and different types of monoclonal antibody (MoAb) have been employed and evaluated as MAM-3 and MAM-6 antigens, and HMFG-1, -2 antigen. Eight kinds of MoAb's against MAM-3 and MAM-6 antigens, each specific for a different epitope determinant, were tested in mammary glands and their tumours (Hilkens et al. 1984a, b), endometrial and endocervical carcinomas (Tsubura et al. 1985), and skin adnexia and their tumours (Tsubura et al. 1987). Another series of EMA immunohistochemistry has identified EMA in salivary gland lesions and tumours (Gusterson et al. 1982; Tatemoto et al. 1987a, b). Immunohistochemical identification of MAM antigen has been made in formalin-fixed paraffin-embedded sections, and these antigens are useful markers for cell differentiation (Hilkens et al. 1984a, b).

The present study was undertaken to determine immunohistochemically the distribution of MAM-3 and MAM-6 antigens in normal salivary glands and salivary gland tumours. Two kinds of MoAb's (67D11 and 115G3) against MAM-3 and also two MoAb's (115D8 and 115F5) against MAM-6 were used and the distribution patterns of these antigens were compared.

Materials and methods

A total of 93 cases of human salivary gland tumours and accompanying normal salivary glands were used. The neoplastic tissues included pleomorphic adenomas (67), adenolymphomas (5), mucoepidermoid carcinomas (3), and sialoadenocarcinomas (6). All the tumour materials obtained from surgery or biopsy were fixed in 10% formalin, embedded in paraffin, and cut into 4 μm sections.

Normal submandibular glands (10) obtained from surgery in the case of benign mandibular tumours, were fixed in one of 3 fixatives: 10% formalin, for 12 h: Bouin's for 18 h; or

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Table 1. Immunohistochemical method

(1) Deparaffinization

(2) Inactivation of endogenous peroxidase:
methanol with 0.3% H_2O_2, 30 min

(3) Background blocking:
normal rabbit serum, 1/20, 30 min

(4) 1st layer
Antibody (1/500, 1 h)
MAM-3: MoAbs 115G3 and 67D11
MAM-6: MoAbs 115F5 and 115D8

(5) 2nd layer
Biotinylated affinity-isolated rabbit immunoglobulin to mouse immunoglobulin (1/500, 30 min)

(6) ABC complex (1/200, 30 min)

(7) Visualization of peroxidase activity by incubation for 5 min in 0.02% 3-3′diaminobenzidine hydrochroride (DAB)/0.05 M Tris buffer solution (pH 7.6) containing 0.005% H_2O_2

Carnoy's solution, for 4 h. Fixed tissue specimens were embedded in paraffin and 4 μm sections were made for immunohistochemical staining and for H & E staining.

Paraffin sections were used to detect MoAb's to MAM-3 (67D11, 115G3) and MAM-6 (115D8, 115F5) antigens immunohistochemically by the ABC method. Details of the method are shown in Table 1. It is reported that MAM-3 antigen was recognized Lewis blood group a and b antigens, and MAM-6 antigen is an epitope of a 400KD glycoprotein which contains the Ca-1, HMFG-1, and 2 epitopes (Taylor-Papadimitriou et al. 1981; Tsubura et al. 1985).

Table 2. Effect of fixation on antigenicity of normal salivary gland

	Total cases	MAM-3		MAM-6	
		67D11	115G3	115D8	115F5
Carnoy's solution	3	3	2	3	3
10% Formalin solution	3	3	1	3	3
Bouin's solution	4	3	1	2	3

Table 3. Distribution pattern of MAM antigens in normal salivary glands

	MAM-3		MAM-6	
	67D11	115G3	115D8	115F5
Acinar cells				
Serous cells	0	0	1 (LLB)	0
Mucous cells	0–1	0	± (LLB)	0
Duct cells				
intercalated duct cells	2–4	0–±	0	0
striated duct cells	0–4	0–±	1 (LB)	1 (LB)
Excretory duct cells				
basal cells	0	0	2	2
luminal cells	3–4	1	0	0

LLB: luminal and lateral borders, LB: luminal border, 0: negative, ±: trace, 1: slight, 2: moderate, 3: strong, 4: most strong

Results

The effect of the fixative solution on immunostaining of MAM-3 and MAM-6 antigens in normal salivary glands, was that Carnoy's-fixed sections manifested immunohistochemical staining of MoAb's to MAM-3 and MAM-6 antigens (Table 2), and the staining in 10% formalin-fixed sections resembled that in the Carnoy's-fixed ones. In contrast, Bouin's-fixed specimens gave a much lower frequency of positive results. Thesefore, in the present study the expression and distribution of MAM-3 and MAM-6 antigens were evaluated in 10% formalin fixed sections of normal glands as well as salivary gland tumours.

In normal salivary glands different expressions of MAM-3 and MAM-6 antigens were found in the normal salivary glands. In the acinar compartment, MoAb 67D11 (MAM-3) stained some of the acinar mucous cells in the sublingual glands slightly (Fig. 1B), MoAb's 115G3 and 115F5 were unreactive, and MoAb 115D8 (MAM-6) staining was confined to luminal and lateral borders of serous acinar cells (Fig. 1C) and to luminal borders of mucous cells. In ductal segments, MoAb 67D11 reacted with intercalated, striated, and excretory duct cells. Intercalated duct cells were stained by MoAb 67D11 moderately to strongly (Fig. 1A), and by MoAb 115G3 slightly, but they were not stained with MoAb's 115D8 and 115F5. Striated duct cells showed irregular staining with 67D11, and positive luminal borders with MoAb's 115D8 and 115F5. For the large excretory duct epithelium which is composed of 3 to 4 cell layers; basal, intermediate, and luminal (Fig. 1D), MoAb's 115D8 and 115F5 were limited to the basal cells (Fig. 1F, G), whereas they were unreactive in intermediate and luminal cells. In contrast, MoAb's 67D11 and 115G3 bound to the luminal side of the duct cells (Fig. 1E). Immunohistochemical staining patterns for MAM-3 and MAM-6 antigens are summarized in Table 3.

Pleomorphic adenomas of salivary gland origin showed great variation in their histopathological features; the typical histology consisted of tubuloductal structures accompanied with modified myoepithelial cells, and occasional tumour tissues showed changes to hyalinous or myxomatous structures containing chondroid cells. Areas of

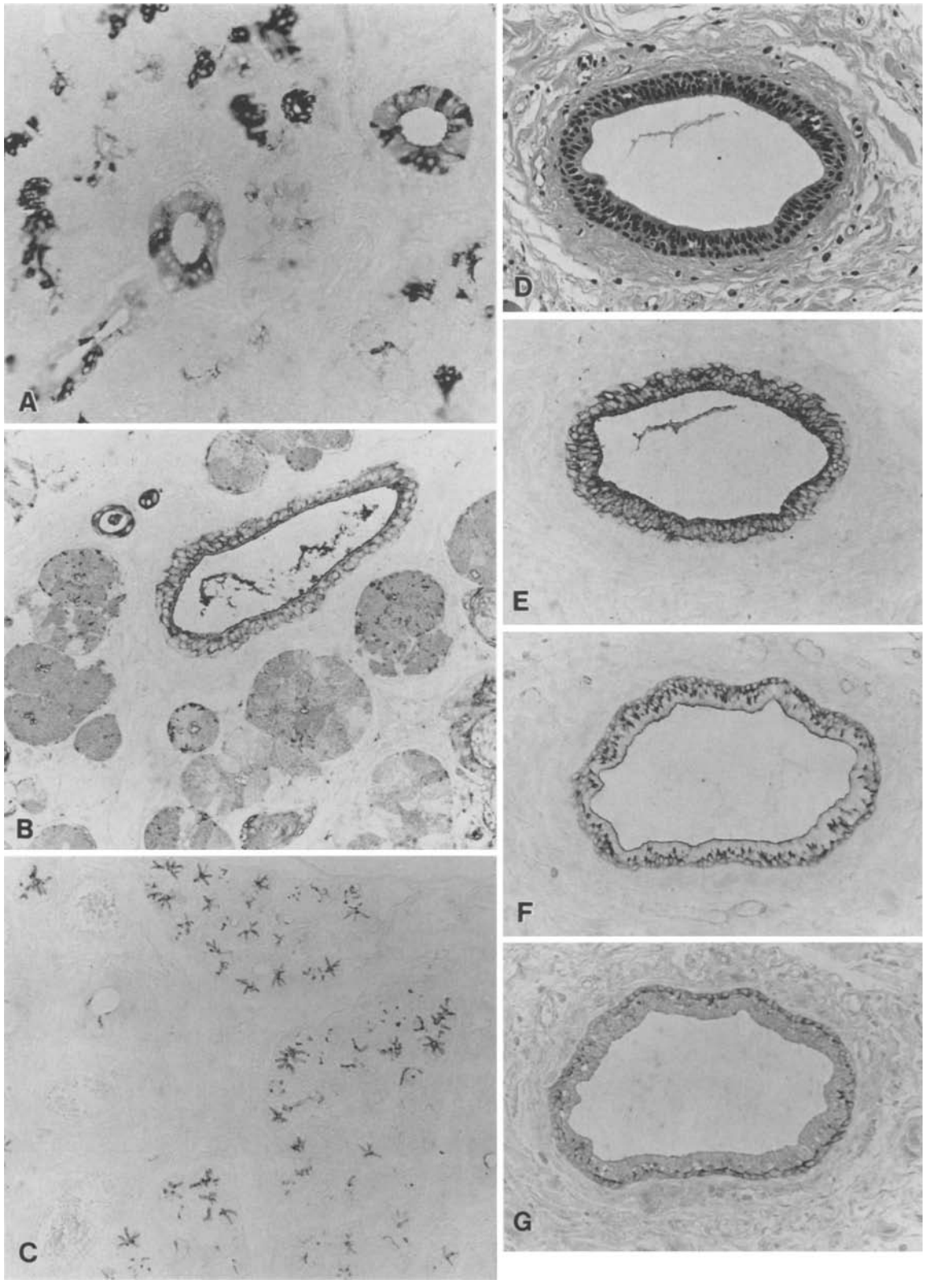


Fig. 1. Normal salivary glands ×100. (**A**) MoAb 67D11 staining in the submandibular gland. Intercalated duct shows strongly positive staining in some limited cells. (**B**) MoAb 67D11 staining in the sublingual gland. Mucous acinar cells show someone slightly positive, and excretory duct cells also positive to 67D11. (**C**) MoAb 115D8 staining in the submandibular gland. MoAb 115D8 staining is limited to luminal and lateral borders of acinar cells. (**D–G**) Excretory ducts of human salivary glands. (**D**) H & E stain. Large excretory duct epithelium consists of stratified squamous cells; basal, intermediate and luminal cells. (**E**) MoAb 67D11 staining. Excretory duct epithelium stains 67D11 except for basal cells. (**F**) MoAb 115D8 staining. Immunostaining of 115D8 is restricted in basal cells as well as some intermediate or parabasal cells. (**G**) MoAb 115F5 staining. Reaction product for 115F5 is confined to basal cells of excretory duct epithelium

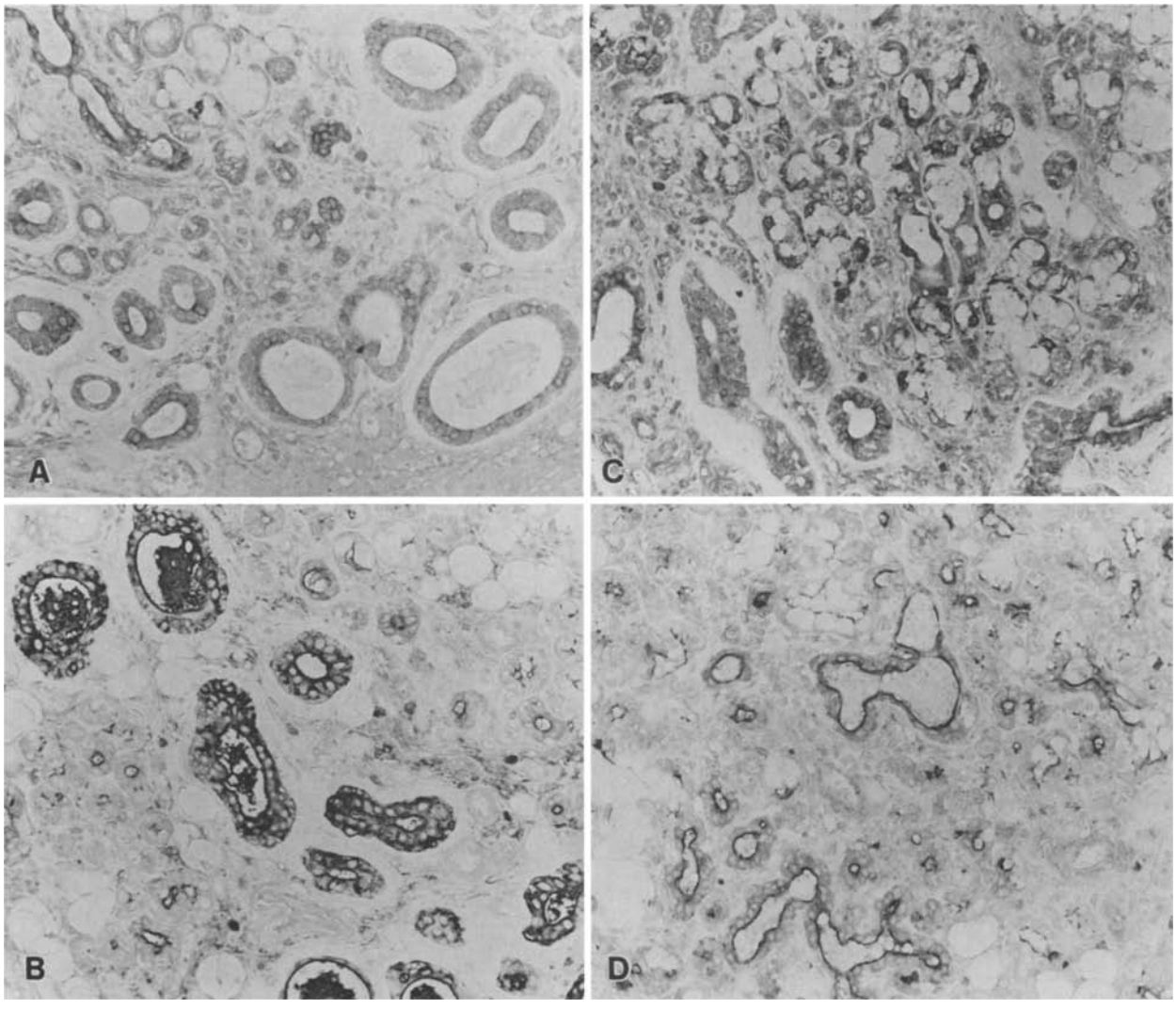


Fig. 2. Transforming area from the normal gland. ×100. **(A)** MoAb 115G3. All the duct-like structures are stained weakly, and some limited cells located duct-like epithelium are also stained. **(B)** MoAb 115D8. 115D8 staining is limited to duct-like structures and luminal or lateral borders of atrophic acinar cells. **(C)** MoAb 115G3. Most acinar cells, showing dilated lumens, stain moderately. **(D)** MoAb 115D8. Immunostaining of 115D8 is confined to luminal borders of dilated duct-like structures and atrophic acinar cells

transformation from the normal salivary gland to pleomorphic adenoma were associated with proliferation of duct-like structures and a diminished number of acinar cells or atrophic and dilated acinar-ductal components.

Duct-like epithelial cells showed no staining with MoAb 67D11, irregular positive staining with MoAb 115G3, and a strong reaction with MoAb's 115D8 and 115F5 (Fig. 2A, B). Acinar compartments were usually reduced in size and showed cystic change, and immunohistochemical expressions of MAM-3 and MAM-6 antigens in them were different in normal acinar cells. MoAb 115G3 gave cytoplasmic staining, and MoAb's 115D8 and 115F5 were reactive with the luminal surfaces of cystic and dilated acinar cells (Fig. 2C, D).

Tubulo-ductal and duct-like structures consisted of two layers, each having one or more rows cells of layers; luminal tumour cells and outer spindle tumour cells. MAM-3 antigen was confined to the cytoplasm of luminal tumour cells and/or their luminal surface, whereas MAM-6 antigen was limited to the luminal aspect only. MoAb 67D11 stained strongly, or in a rare specimen, slightly in the luminal tumour cells (Figs. 3B, E, 4B). The frequency of positive 115G3 staining was not so high, and the MoAb bounds to the luminal surface or to the cytoplasm of some luminal tumour cells

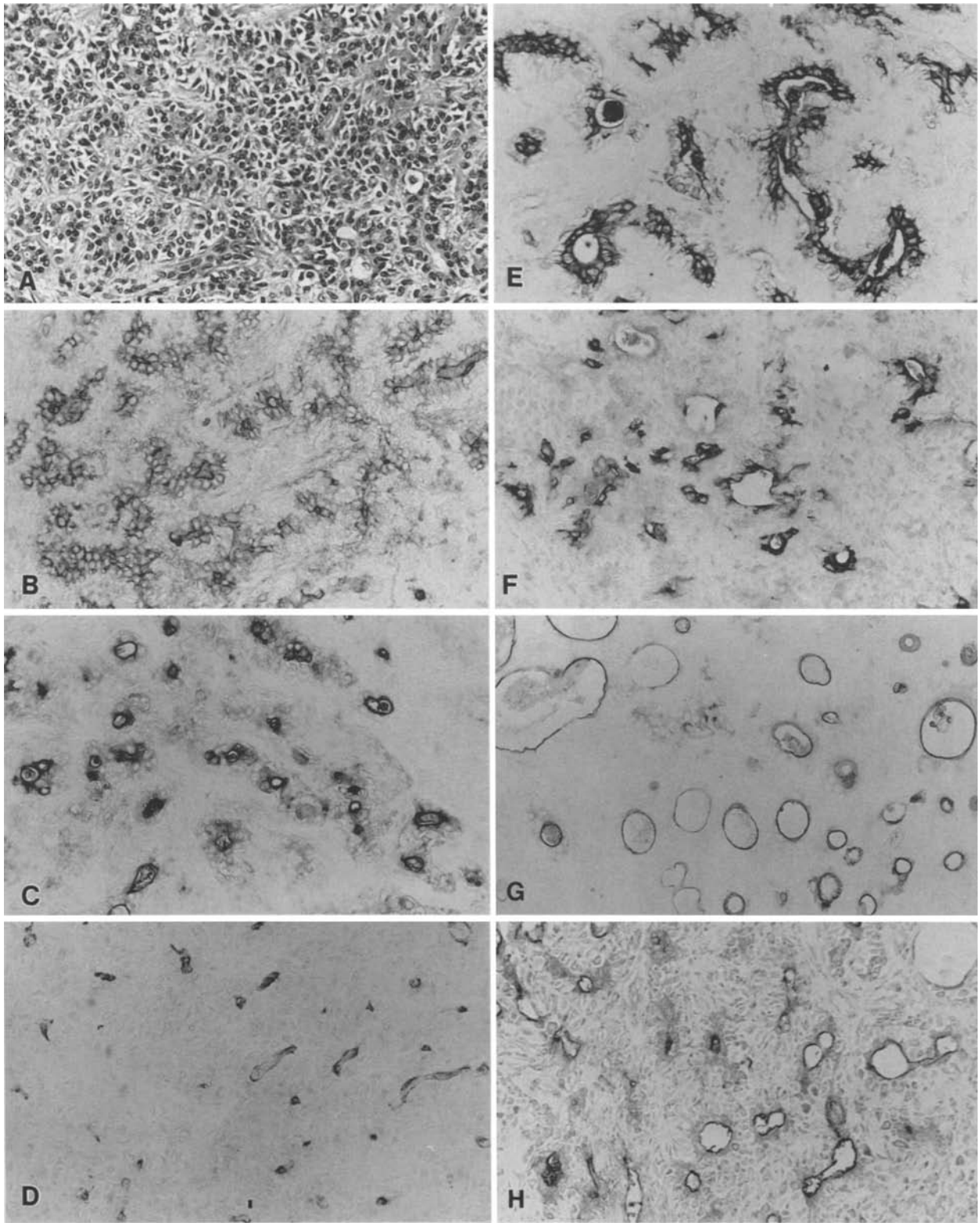


Fig. 3. Pleomorphic adenomas ×100. (**A–D**) Same tumour specimens. (**A**) Histology of pleomorphic adenoma. Typical tubular structures consist of two cell layers, luminal tumour cells and outer spindle tumour cells. (**B**) MoAb 67D11. Luminal tumour cells of tubular structures stain moderately with 67D11 whereas outer tumour cells not. (**C**) MoAb 115D8. Strong staining of 115D8 antibody is confined to luminal borders and slight staining to luminal tumour cells. (**D**) MoAb 115F5. Immunohistochemical staining of 115F5 antibody is limited to luminal surface. (**E–H**) Same tumour. (**E**) MoAb 67D11 antibody is distributed in luminal tumour cells of duct-like structures. (**F**) MoAb 115G3. Reaction product of 115G5 is manifested either some luminal cells or luminal borders of duct-like structures. (**G**) MoAb 115D8. Luminal borders of duct-like structures stain to 115D8 antibody. (**H**) MoAb 115F5. Luminal surfaces of the structures show positive staining to 115F5 antibody

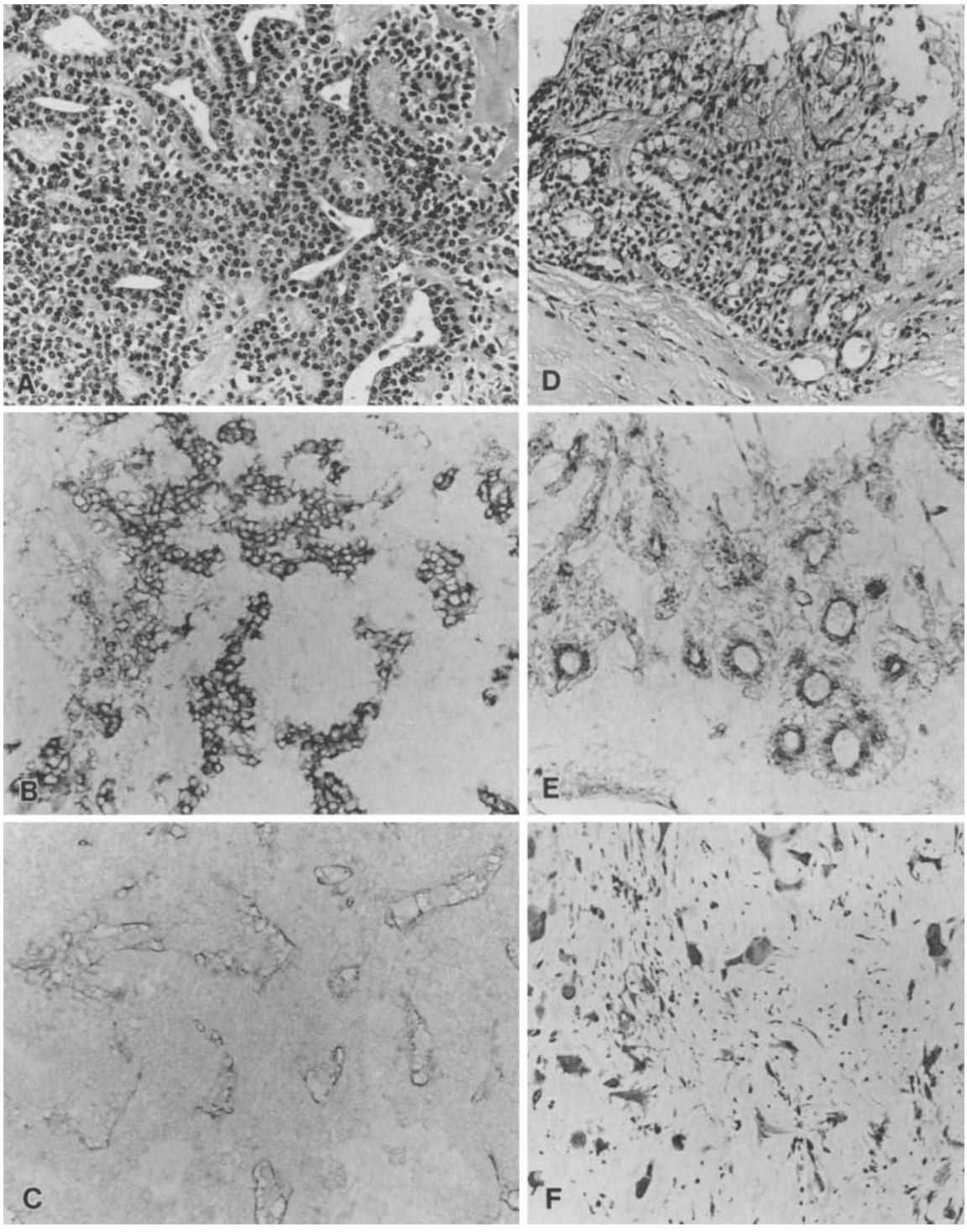


Fig. 4. (**A–C**) Pleomorphic adenomas × 100. (**A**) Pleomorphic adenoma is histologically composed of irregular sized duct-like structures consisting two cell layers. (**B**) MoAb 67D11. Immunostaining of 67D11 antibody is limited to luminal tumour cells. (**C**) MoAb 115F5. Luminal borders of duct-like structures reveal slight 115F5 staining. (**D**) Clear cell variant of pleomorphic adenoma. (**E**) MoAb 115F5. Luminal tumour cells of clear cell foci show positive 115F5 staining, whereas outer tumour cells not. (**F**) MoAb 115F5. Chondroidal changed cells in hyalinous structures stain with 115F5 antibody

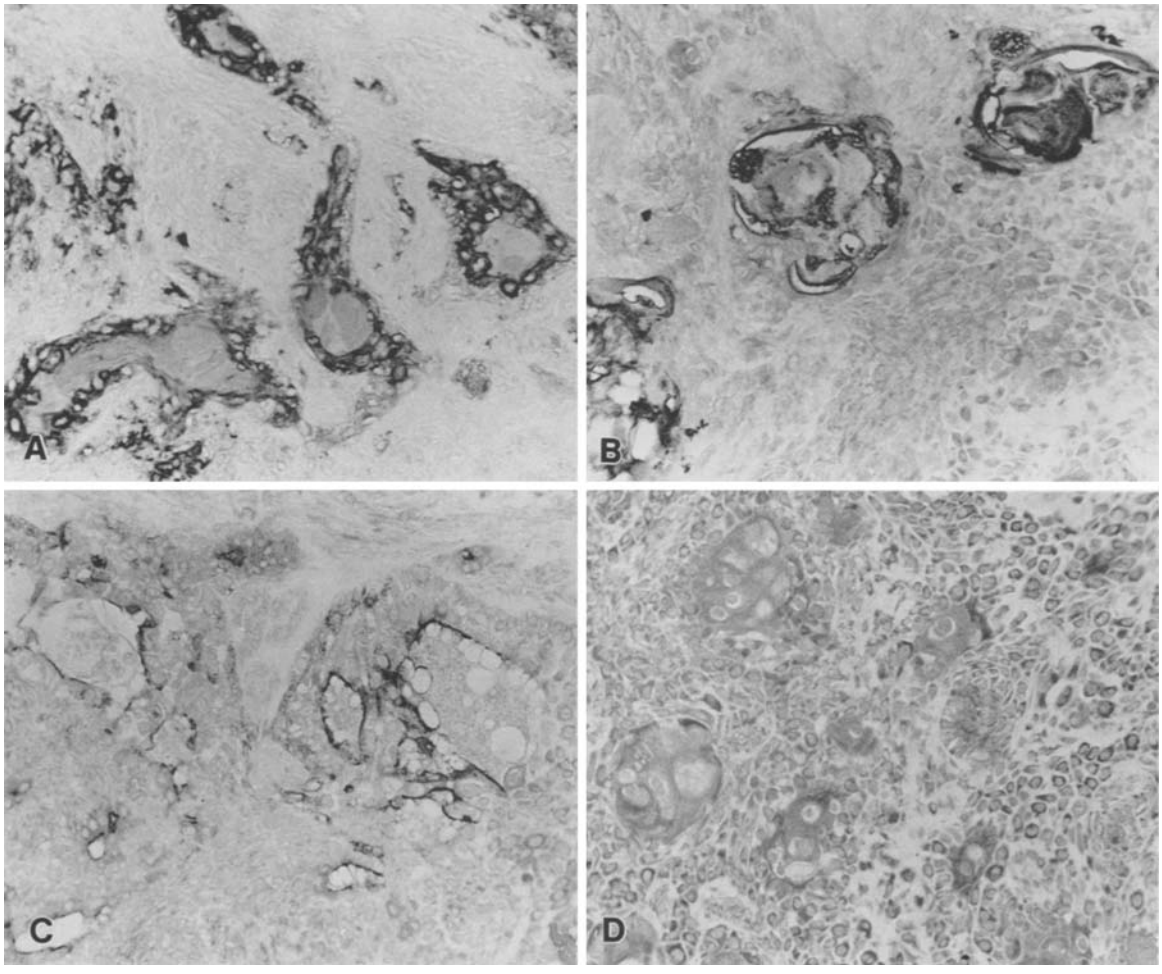


Fig. 5. Squamous metaplastic type of pleomorphic adenoma ×100. (**A**) MoAb 67D11. Squamous metaplastic cells in pleomorphic adenoma show intensely positive staining to 67D11 antibody. (**B**) MoAb 115G3. Squamous metaplastic tumour cells indicate high staining of MAM-3 antigen. (**C**) MoAb 115D8. Immunohistochemical staining is limited to border zones between squamous metaplastic epithelium and highly keratinized tissue. (**D**) MoAb 115F5. No marked staining reaction of 115F5 is found in tumour tissue, and a very weak reaction is restricted is modified myoepithelial cell foci

(Fig. 3F). Positive staining reactions with MoAb's 115D8 and 115F5 were evident in luminal borders or the luminal side of luminal tumour cells, and the intensity of MoAb 115D8 staining was generally higher than that of MoAb 115F5 (Figs. 3C, D, G, H, 4C). Outer tumour cells of the structures were devoid of any staining for MAM-3 and MAM-6 antigens.

Irrespective of the presence or absence of clear tumour cells, MAM-3 and MAM-6 antigens were manifested on the luminal surfaces of tubular structures in clear cell variants (Fig. 4E). Outer clear tumour cells and other clear tumour cells were negative with all the MoAb's.

Chondroidal cells scattered in hyalinous or myxomatous tissues gave positive reactions for MAM-3 and MAM-6 antigens with varying intensities (Fig. 4F).

Table 4. Frequency of positive staining for MAM-3 and MAM-6 antigens in salivary gland tumours

	Total cases	MAM-3		MAM-6	
		67D11	115G3	115D8	115F5
Pleomorphic adenoma	67	54	36	59	48
Adenolymphoma	5	5	4	5	2
Mucoepidermoid carcinoma	3	3	1	3	3
Sialoadenocarcinoma	6	5	3	6	3

Metaplastic squamous cells in pleomorphic adenomas revealed strong staining with MoAb's 67D11 and 115G3, and border staining of epidermoid cells. Completely keratinized cells was seen with MoAb 115D8. Staining of squamous metap-

Table 5. Distribution patterns of MAM antigens in salivary gland tumours

	MAM-3		MAM-6	
	67D11	115G3	115D8	115F5
Pleomorphic adenomas				
Transforming areas				
Proliferating duct-like epithelial cells	0	0–1	0–4	0–4
Atrophic acinar cells	0	0–3	4 (LB)	1–4 (LB)
Tubulo, duct-like structures				
luminal tumour cells	3–4	0–4	3–4 (LB)	2–4 (LB)
outer spindle tumour cells	0	0	0	0
Duct-like structures				
luminal tumour cells	2–4	0–3	2–4 (LB)	2 (LB)
outer tumour cells	0	0	0	0
Clear cell variant				
luminal clear cell	2	2	3 (LB)	2 (LB)
outer clear cells	0	0	0	0
Modified myoepithelial cell	1–4	1–2	2 (LB)	2 (LB)
Chondroidal cells	2–3	±	3	3
Squamous metaplastic cells	3–4	3–4	4 (BKC)	
Adenolymphoma				
Basal tumour cells	0	0	2–3	2–4
Luminal tumour cell	0–4	0–4	0 (4: LB)	0
Mucoepidermoid carcinoma				
Mucous tumour cells	1	0–4	0–1	0–2
Epidermoid cells			2 (4: LB)	3
Sialadenocarcinomas	0–4	0–2	2	2

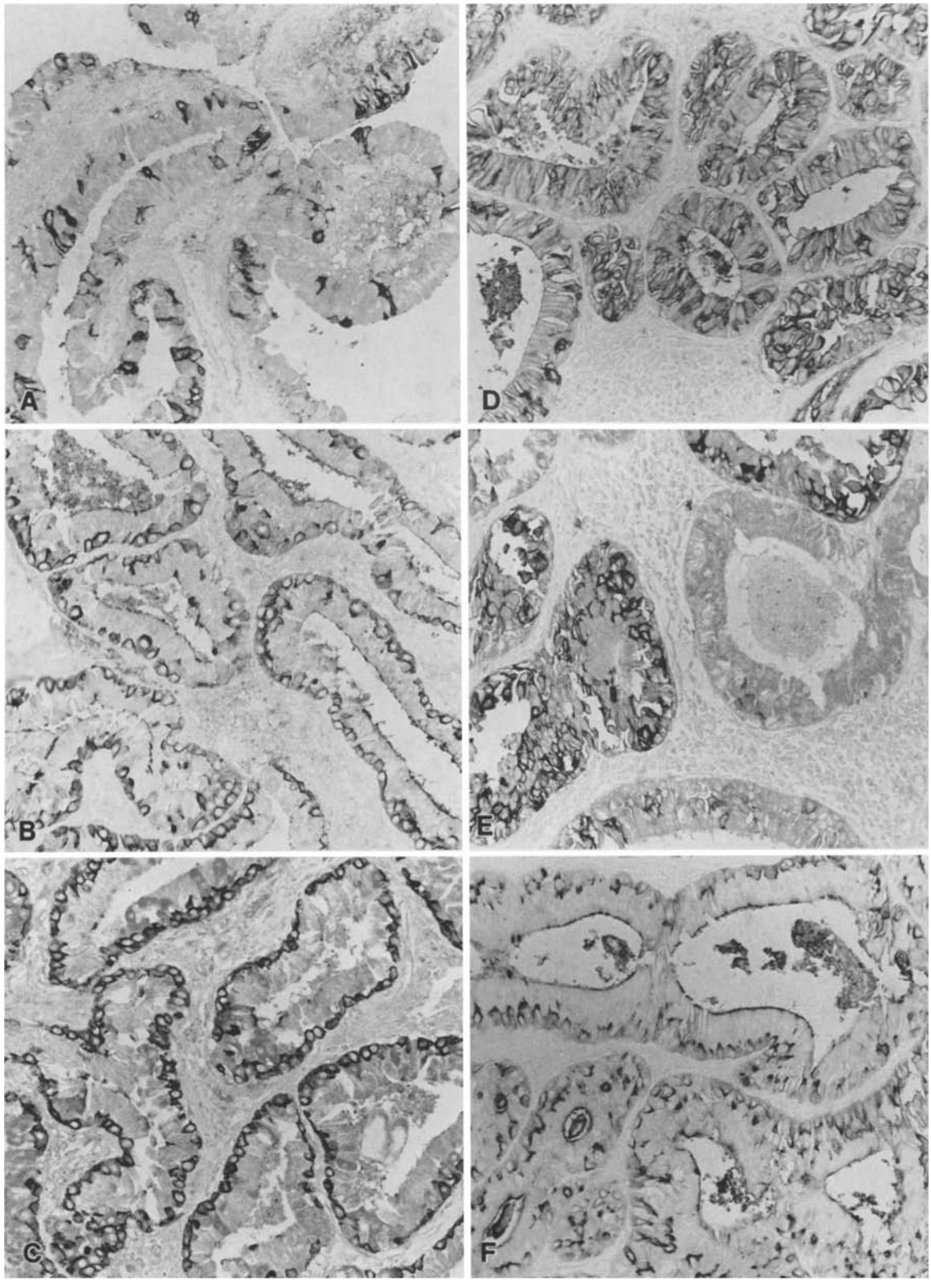
LB: luminal border, BKC: border of keratinizing cell, 0: negative, ±: trace, 1: slight, 2: moderate, 3: strong, 4: most strong

lastic cells was negative or trace with MoAb 115F5 (Fig. 5A, B, C, D).

The frequency of positive staining for MAM-3 and MAM-6 antigens is shown in Table 4, and expression of the antigens in pleomorphic adenomas and other tumours is summarized in Table 5.

In adenolymphomas tumour epithelia were characterized by the presence of positive staining for MAM-3 antigen in occasional luminal tumour cells and for MAM-6 antigen in basal tumour cells. Immunostaining with MoAb 67D11 was strong in a limited number of tumour cells located in the luminal epithelium (Fig. 6A, D), and that with MoAb 115G3 was irregularly positive in some foci and negative in other foci (Fig. 6E). Moderate MoAb 115D8 staining was evident in basal tumour cells, and strong in luminal surfaces (Fig. 6B, F). Expression of MoAb 115F5 (MAM-6) was confined to basal tumour cells of epithelial structures

Fig. 6. (**A–C**) Epithelial cells of adenolymphoma are consisted of two type tumour cells; single basal cell and columnar (apical) tumour cell. ×100. (**A**) MoAb 67D11. Immunostaining of 67D11 is localized in some tumour cells of columnar epithelial zones. (**B**) MoAb 115D8. Basal tumour cells indicate positive 115D8 staining, and luminal borders of tumour epithelia indicate positive staining. (**C**) MoAb 115F5. Strong 115F5 staining is limited to basal tumour cells of adenolymphoma. Basal tumour cells contain higher amounts of MAM-6 antigens. (**D–F**) Serial sections of adenolymphoma ×100. Tumour epithelium of adenolymphoma. No consisted of basal or parabasal tumour cells, and high columnar apical cells. (**D**) MoAb 67D11. Immunostaining to MoAb



67D11 in tumour epithelium is distributed irregularly both basal tumour cells and columnar tumour cells, but most reaction product of 67D11 is localized at apical columnar cells. (**E**) MoAb 115G3. Histochemical deposition of 115G3 is irregular in epithelial foci and some epithelial masses are strongly positive, whereas other foci negative. (**F**) MoAb 115D8. Luminal surface of tumour epithelial foci is intensely positive, and basal and parabasal tumour cells are also strongly stained to 115D8

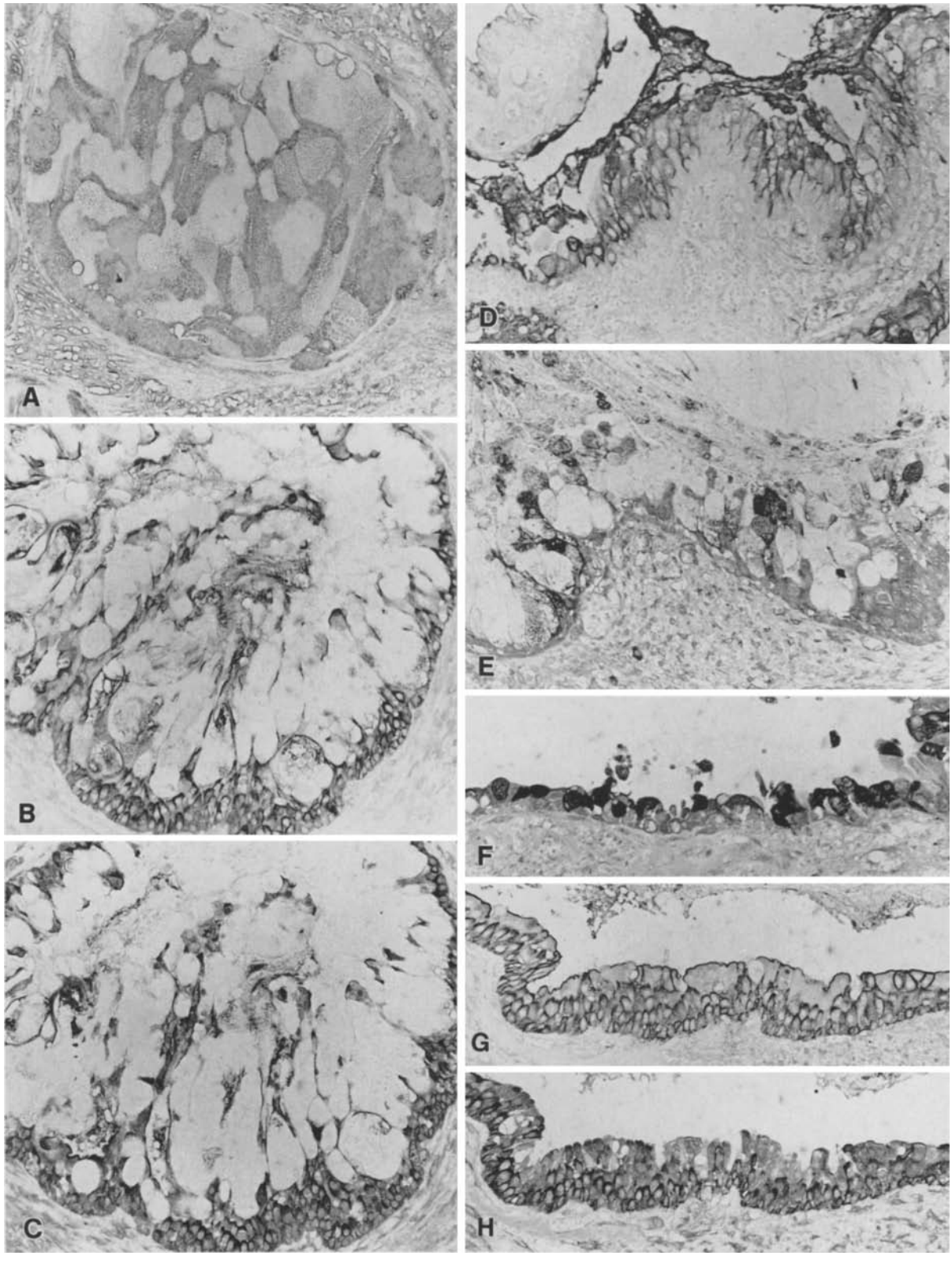


Fig. 7. (**A–C**) Serial sections of mucoepidermoid carcinoma (G-I). (**A**) MoAb 67D11. Some mucous secreting cells stain weakly to 67D11 antibody. (**B**) MoAb 115G8. Epidermoid tumour cells stain moderately, whereas mucous cells unstain. (**C**) MoAb 115F5. Epidermoid tumour cells stain strongly to 115F5 antibody. Epidermoid cells in mucoepidermoid carcinoma are usually positive to MAM-6 antigens. (**D–E**) Serial sections of mucoepidermoid carcinoma (G-II). (**D**) MoAb 67D11. Epidermoid tumour cells

(Fig. 6C). In the case of high columnar epithelium, parabasal tumour cells also contained MAM-6 antigen.

In mucoepidermoid carcinomas the tumour tissue consisted of epidermoid, mucous-secreting and intermediate cells. Immunohistochemical features were different in epidermoid and mucous cells. MAM-3 antigen was lacking or low in both epidermoid and mucous tumour cells (Fig. 7A, D, E), though mucous-secreting tumour cells were stained with variable intensity by MoAb 115G3 (Fig. 7E, F). Epidermoid tumour cells expressed positive staining with MoAb's 115D8 and 115F5 for MAM-6 antigen (Fig. 7B, C, G, H), and the luminal surface of mucous-secreting cells manifested strong MoAb 115D8 staining (Fig. 7G).

Sialoadenocarcinomas tumour cells showed variable staining for MAM-3 and MAM-6 antigens. Frequency of positive cases, and of MAM-3 and MAM-6 antigens are shown in Tables 4 and 5 respectively.

Discussion

Epithelial membrane antigen (EMA) derived from human milk fat globule membrane has been examined immunohistochemically in many types of human neoplastic lesions (Hilkens et al. 1984; Pinkus et al. 1985; Sloane et al. 1981, 1982; Zotter et al. 1985; Tron et al. 1987; Marshall et al. 1984). MoAb's raised against EMA, which detect two types of antigens, designated MAM-3 and MAM-6, were produced in the Netherlands Cancer Institute (Hilkens et al. 1984a, b), and were employed in the present study to evaluate the distribution of MAM-3 and MAM-6 antigens in salivary glands tumours as well as in normal salivary glands. Immunohistochemical localizations of antigens MAM-3 and MAM-6 have already been described in mammary glands (Hilkens et al. 1984), skin adexal tumours (Tsubura et al. 1987), and endometrial and endocervical cancers (Tsubura et al. 1985). Antiserum to EMA has also been used to detect EMA in normal salivary glands and tumours or obstructive sialadenitis (Gusterson et al. 1982; Tatemoto et al. 1987a, b). The present study has examined the immunohistochemical distribution of two antigens, MAM-3 and MAM-6, in various types of salivary gland tumours, and compared them.

Immunohistochemical expression of MAM-3 and MAM-6 antigens was different in acinar cells and ductal segments of the normal glands. Distribution of MAM-3 and MAM-6 in salivary glands resembled relatively that of the antigens in sweat glands (Tsubura et al. 1987). Staining with MoAb 115D8 was confined to luminal and lateral borders of acinar cells and ducts, and this luminal border-positive type of 115D8 distribution was the same as seen in the staining with MoAb to EMA (Tatemoto et al. 1982, 1987) and CEA (Tsukitani et al. 1985; Sumitomo et al. 1987). Thus, the epitope recognized by 115D8 might be somewhat similar to the determinant recognized by EMA and CEA.

Histogenesis of pleomorphic adenomas is suggested to involve intercalated duct cells and myoepithelial cells, and MoAb 67D11 characteristically stained the intercalated duct cells of normal glands and luminal tumour cells of pleomorphic adenomas. Thus, the luminal tumour cells of tubuloductal structures may have arisen from intercalated duct cells. Except for a few cases, MoAb's 115D8 and 115F5 (MAM-6 antigen) were limited to luminal borders of pleomorphic adenomas. Squamous metaplasia was occasionally occurred in pleomorphic adenomas, and squamous metaplastic cells particularly expressed MAM-3 antigens (MoAb's 67D11 and 115G3). MAM-3 antigen has also been detected in squamous cell carcinoma cells in uterine cervix (Tsubura et al. 1985). Existence of MAM-3 antigen in pleomorphic adenoma cells indicates that the antigen might be a characteristic feature of squamous metaplasia, as well as of squamous cell carcinomas.

Immunohistochemical expression of MAM-6 antigen was quite comparable between basal tumour cells of adenolymphomas and ductal basal cells in the normal gland. Recently, ductal basal cells in normal salivary glands have been described as reacting with MoAb against keratin (Takai et al. 1988) and MoAb against KM-231 (lung carcinoma antigen) (Tsujii et al. 1989). Most of the studies related to keratin expression in ductal basal cells in the normal salivary glands and in basal tumour

located at upper zone are stained to 67D11, whereas those at basal layer are devoid of the staining. (**E**) MoAb 115G3. Some of mucous secreting cells indicate the strongest staining to 115G3, and epidermoid tumour cells in the basal layer show weak staining. (**F–H**) Cyatic change in mucoepidermoid carcinoma (G-II). (**F**) MoAb 115G3. Mucous secreting cells located in superfacial zone indicate the strongest 115G3 staining. (**G**) MoAb 115D8. Histochemical staining of 115D8 is limited to surface border and is localized in epidermoid tumour cells. Mucous cells are devoid of the staining. (**H**) MoAb 115F5. Immunostaining to 115F5 is distributed in epidermal cells, and is stained slightly in mucous secreting cells

cells of adenolymphoma have focused on the question as to whether ductal basal cells or reserve cells are the same or not (Caselitz et al. 1986; Born et al. 1987; Gusterson et al. 1982; Orito et al. 1989). Previous papers noted that ductal basal cells of the normal salivary glands were stained positively with MoAbs against keratins such as, KS8.58 (Geiger et al. 1987), PKK1, K8.12, CKB1 (Palmer et al. 1985); 312C8-1 (Dardick et al. 1988), and LICR-LON16a (Knight et al. 1985). Adenolymphomas expressed different keratin staining in two types of tumour epithelium; tumour basal cells were characteristically decorated with antibodies specific keratins (PKK1, K8.12), which is the same keratin distribution in ductal basal cells (Orito et al. 1989). The present result showing similarity of MAM-6 antigen expression between normal ductal basal cells and basal tumour cells of adenolymphomas, along with the findings obtained with MoAb's against keratin proteins, suggest that basal tumour cells of adenolymphomas probably originate from normal ductal basal cells.

Mucoepidermoid carcinomas consist of benign mucous-secreting tumour cells, and epidermoid cells that are categorized as malignant. MoAb 115G3 staining was particularly strong in some mucous tumour cells, whereas MoAb 67D11 did not react much with most mucous cells and not at all with epidermoid tumour cells. It is interesting to note that MoAb's against MAM-3 antigen have shown different reactivities with mucous-secreting cells of mucoepidermoid tumours. Although immunohistochemical localization of MAM-6 antigen was confined to luminal borders of glandular tumour tissues, positive reactions in epidermoid tumour cells might be heterogeneous and alternative expressions.

References

Born IA, Schweheimer K, Maier H, Otto HF (1987) Cytokeratin expression in normal salivary glands and in cystadenolymphomas demonstrated by monoclonal antibodies against selective cytokeratin polypeptides. Virchows Arch [A] 411:583–589

Caselitz J, Walther B, Wustrow J, Seifert G, Weber K, Osborn M (1986) A monoclonal antibody that detects myoepithelial cells in exocrine glands, basal cells in other epithelia and basal and supra basal cells in certain hyperplastic tissues. Virchows Arch [A] 409:725–738

Caselitz J, Osborn M, Hamper K, Wustrow J, Rauchfuß A, Weber K (1986) Pleomorphic adenomas, adenoid cystic carcinomas and adenolymphomas of salivary glands analysed by a monoclonal antibody against myoepithelial/basal cells. Virchows Arch [A] 409:805–816

Caselitz J, Osborn M, Wustrow J, Seifert G, Weber K (1986) Immunohistochemical investigations on the myoepithelial islands in lymphoepithelial lesions. Use of monoclonal keratin antibodies. Lab Invest 55:427–432

Dardick I, Rippstein P, Skimming L, Boivin M, Parks WR, Dairkee SH (1987) Immunohistochemical and ultrastructure of myoepithelium and modified myoepithelium of the ducts of human major salivary glands: Histologenetic implications for salivary gland tumors. Oral Surg 64:703–715

Dardick I, Parks WR, Little J, Brown DL (1988) Characterization of cytoskeletal proteins in basal cells of human parotid salivary gland duct. Virchow Arch [A] 412:525–532

Geiger S, Geiger B, Leitner O, Marshak G (1987) Cytokeratin polypeptides expression in different epithelial elements of human salivary glands. Virchow Arch [A] 410:403–414

Gusterson BA, Lucas RB, Ormerod MG (1982) Distribution of epithelial membrane antigen in benign and malignant lesions of the salivary glands. Virchow Arch [A] 397:227–233

Hilkens J, Buijs F, Hilgers J, Hageman Ph, Calafat J, Sonnenberg A, Van der Valk M (1984) Monoclonal antibodies against human milk fat globule membranes detecting differentiation antigens of the mammary gland and its tumors. Int J Cancer 34:197–206

Hilkens J, Hilgers J, Buijs F, Hageman Ph, Schol D, van Doornewaard G, van den Tweel J (1984) Monoclonal antibodies against human milk fat globule membranes useful in carcinoma reaction. In: Peerers H (ed) Protides of the Biological Fluids, vol. 31. Pergamon Press, Oxford/New York, pp 728–735

Knight J, Gusterson B, Jones RR, Landells W, Wilson P (1985) Monoclonal antibodies specific for subsets of epidermal keratins: Biochemical and immunocytochemical characterization. Applications in pathology and cell culture. J Pathol 145:341–354

Leoncini P, Cintorino M, Vindigni C, Leoncini L, Armellini D, Bugnoli M, Skalli O, Gabbiani G (1988) Distribution of cytoskeletal and contractile proteins in normal and tumour bearing salivary and lacrimal glands. Virchows Arch [A] 412:329–337

Marshak G, Leitner O (1987) Cytokeratin polypeptides in normal and metaplastic human salivary gland epithelium. J Oral Pathol 16:442–449

Marshall RJ, Herbert A, Braye SG, Jones DB (1984) Use of antibodies to carcinoembryonic antigen and human milk fat globule to distinguish carcinoma, mesohelioma, and reactive mesothelium. J Clin Pathol 37:1215–1221

Orito T, Shinohara H, Okada Y, Mori M (1989) Heterogeneity of keratin expression in epithelial tumor cells of adenolymphoma in paraffin sections. Pathol Res Pract 84:600–608

Palmer RM (1986) The identification of myoepithelial cells in human salivary glands. A review and comparison of light microscopical methods. J Oral Pathol 15:221–229

Palmer RM, Lucas R, Knight J, Gusterson B (1985) Immunocytochemical identification of cell types in pleomorphic adenoma, with particular reference to myoepithelial cells. J Pathol 146:213–220

Pinkus GS, Kurtin PJ (1985) Epithelial membrane antigen – a diagnostic discriminant in surgical pathology. Immunohistochemical profile in epithelial, mesenchymal and hetmatopoietic neoplasms using paraffin sections and monoclonal antibodies. Hum Pathol 16:929–940

Sloane JP, Ormerod MG (1981) Distribution of epithelial membrane antigen in normal and neoplastic tissues and its value in diagnostic tumor pathology. Cancer 47:1786–1795

Sloane JP, Ormerod MG, Carter RL, Gusterson BA, Foster CS (1982) An immunocytochemical study of the distribution of epithelial membrane antigen in normal and disordered squamous epithelium. Diag Histopathol 5:11–17

Sumitomo S, Kumasa S, Mitani H, Mori M (1987) Comparison of CEA distribution in lesions and tumors of salivary glands as determined with monoclonal and polyclonal antibodies. Virchow Arch [B] 53:133–139

Takai T, Yamada Y, Shinohara Y, Orito T, Tsukitani K, Mori M (1988) Monoclonal antibodies against keratins bind to intercalated duct and ductal basal cells of normal salivary glands in paraffin sections. Acta Histochem Cytochem 21:573–584

Tatemoto Y, Kumasa S, Watanabe Y, Mori M (1987) Immunohistochemical expression of monoclonal antibody against epithelial membrane antigen in salivary gland tumors. Acta Histochem Cytochem 20:113–124

Tatemoto Y, Kumasa S, Watanabe Y, Mori M (1987) Epithelial membrane antigen as a marker of human salivary gland acinar and ductal cell function. Acta Histochem 82:219–226

Taylor-Papadimitriou J, Peterson JA, Arklie J, Burchell J, Ceriani RL, Bormer WF (1981) Monoclonal antibodies to epithelium-specific component of human milk fat globule membrane: Production and reaction with cells in culture. Int J Cancer 28:17–21

Tron V, Wright JL, Churg A (1987) Carcinoembryonic antigen and milk-fat globule protein staining of malignant mesothelioma and adenocarcinoma of the lung. Arch Pathol Lab Med 111:291–293

Tsubura A, Morii S, Hilkens J, Hilgers J (1985) Expression of MAM-3 and MAM-6 antigens on endometrial and endocervical carcinomas. Virchow Arch [A] 407:59–67

Tsubura A, Morii S, Ueda S, Sasaki M, Zotter St, Watzig V, Mooi W, Hageman PhC, Hilkens J, Van der Tweel J, Meijer C, Hilgers J (1987) Immunohistochemical demonstration of MAM-3 and MAM-6 antigens in normal human skin appendages and their tumors. Arch Dermatol Res 279:550–557

Tsuji T, Shinozaki F, Yamada Y, Mori M (1989) Immunohistochemical detection of human lung and gastric cancer antigen in human salivary gland tumors. Anticancer Res (in press)

Tsukitani K, Kobayashi K, Murase N, Sumitomo S, Mitani H, Mori M (1985) Characterization of cell in salivary gland lesions by immunohistochemical identification of carcinoembryonic antigens. Oral Surg 59:595–599

Zotter S, Lossnitzer A, Kunze KS, Müller M, Hilkens J, Hilgers J, Hageman P (1985) Epithelial markers for paraffin-embedded human tissues. Immunohistochemistry with monoclonal antibodies against milk fat globule antigens. Virchow Arch [A] 406:237–251

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