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Immunohistochemical and ultrastructural correlates of muscle-actin expression in pleomorphic adenomas and myoepitheliomas based on comparison of formalin and methanol fixation

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Summary. The degree and range of differentiation of the cells referred to as myoepithelial-like in pleomorphic adenomas and the tumour cells of myoepitheliomas are not definitely established. This type of information is critical for establishing reliable diagnostic criteria, such as expression of muscle-specific actin and ultrastructural identification of myofilaments, in these and other salivary gland tumours. Pleomorphic adenomas (18) and myoepitheliomas (5), of which 10 cases were fixed only in formalin and 13 cases where tissues were fixed in both formalin and methanol/acetic acid, were studied. Each tumour and normal accompanying parotid was immunostained with two monoclonal antibodies for smooth muscle actin, HHF35 and MSA. Staining of myoepithelial cells was absent in certain samples of normal gland with both HHF35 (15%) and MSA (69%) when formalin-fixed tissue was used. Using formalin-fixed tissue from 15 pleomorphic adenomas/myoepitheliomas, 2 (14%) had focal positivity with HHF35, while 8 cases (57%) were positive with MSA. However, a certain degree of false positivity was suspected since in samples of normal parotid, both acinar and duct cells were frequently stained, particularly with MSA. With methanol/acetic acid-fixed tissue only 4 of 13 cases (31%) were positive with either MSA or HHF35 and 2 of these only had a minor proportion of the tumour cells expressing muscle-specific actin. Using alcohol-fixed tissue, myoepithelial cells were strongly stained in all examples of normal parotid gland with both anti-actin antibodies. In 5 cases examined by electron microscopy, there was no apparent correlation between immunohistochemical results and the presence or absence of cytoplasmic filament accumulation. The results indicate considerable tumour cell heterogeneity in muscle-specific actin expression and suggest that non-luminal cells in pleomorphic adenomas and the tumour cells in myoepitheliomas may differentiate as classical myoepithelial cells, as partially differentiated (i.e. modified myoepithelial cells) or as the counterpart of basal cells present in the intra- and interlobular ducts of normal salivary gland.

Key words: Salivary gland – Neoplasms – Myoepithelial cells – Monoclonal antibodies – Actin

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Introduction

In terms of cytoplasmic filaments, myoepithelial cells of the exocrine glands, sweat, salivary and breast, are probably the most unusual cells in the body. No other epithelial cell has been shown to contain, in addition to cytokeratin, both the isoform of actin associated with muscle cells (Tsukada et al. 1987a, b; Dardick et al. 1988b; Gugliotta et al. 1988) and myosin (Palmer et al. 1985). It is also likely that myoepithelial cells, at least in the normal parotid, contain glial fibrillary acidic protein (Achstätter et al. 1986; Stead et al. 1988; Gustafsson et al. 1989), an intermediate filament normally expressed in glial cells, the pituitary, and Schwann cells of peripheral nerves (Eng 1980). At least in acinar units undergoing atrophy in non-neoplastic tissue adjacent to pleomorphic adenomas (Zarbo et al. 1991), myoepithelial cells of major and minor salivary glands appear to have the capability of expressing glial fibrillary acidic protein. Some monoclonal antibodies to vimentin stain myoepithelial cells to varying degrees (Erlandson et al. 1984; Born et al. 1987; Gustaffson et al. 1989), while others do not (Burns et al. 1988; Yamada et al. 1988; Gustaffson et al. 1989). Desmin and neurofilament-type intermediate filaments have not been identified in myoepithelium of human glands (Gustaffson et al. 1989). Thus, myoepithelial cells contain or have the potential to express an unusual range of functional cytoplasmic filaments.

Controversy still centres on the role of myoepithelial cells in such tumours as pleomorphic adenoma and the

degree of their structural and functional modification in salivary gland tumours. One characteristic feature of pleomorphic adenoma is the presence of a discrete population of cells on the outer aspect of the duct or gland-like structures. An essential question regarding these cells has been the degree to which myoepithelial cell characteristics can be demonstrated within them, either immunohistochemically or ultrastructurally. It is also unclear to what extent muscle-specific actin is present in these and other tumour cells in pleomorphic adenoma.

The development of murine monoclonal antibodies to muscle-specific actin has allowed a more precise detection of myoepithelial cells in breast and salivary glands since both acinar and luminal duct cells are devoid of this isoform of actin (Tsukada et al. 1987a, b; Dardick et al. 1988a, 1989; Gugliotta et al. 1988; Srigley et al. 1990). Antibodies with this degree of specificity are essential in the study of pleomorphic adenomas in order to establish the extent and location of myoepithelial-like differentiation in these neoplasms; a few such studies have been performed on formalin-fixed tissue (Kahn et al. 1985; Leoncini et al. 1988; Hirano et al. 1990) and two have used methacarn and/or frozen fixed tissues (Draeger et al. 1990; Zarbo et al. 1990). Previous experience in our laboratory had suggested that formalin may denature antigenic determinants of interest. The present study therefore compared the staining patterns in normal human parotid gland and in pleomorphic adenomas and myoepitheliomas using two monoclonal antibodies directed against muscle-specific actin, and formalin- and alcohol-fixed tissue. In addition, samples of some of the tumours included in this study were examined by conventional electron microsocpy in order to assess the correlation between immunohistochemical detection of smooth muscle actin in these tumours and ultrastructural evidence for myofilament-type accumulations.

Materials and methods

Twenty-three cases of pleomorphic adenoma and myoepithelioma were selected for immunohistochemical assessment of the expression of muscle-specific actin. Of these, 10 cases had tumour and normal tissues fixed only in 10% buffered formalin, while 13 other cases had tumour and normal tissues fixed in both formalin and a modified Carnoy's fixative (90% methanol and 10% glacial acetic acid). Of these 13 cases, formalin-fixed tissue sections from 5 pleomorphic adenomas were immunostained with the monoclonal antibodies specified below, in addition to the 10 cases of salivary gland tumours with formalin-fixed tissues noted above. All of the methanol/acetic acid (MAA)-fixed cases were immunostained. The tissues were embedded in paraffin and sections from multiple blocks were stained with haematoxylin and eosin for review of the classification of the salivary gland tumors: 18 were typical pleomorphic adenomas and, using previously published criteria (Dardick et al. 1988a, 1989), 5 were classified as myoepitheliomas (2 spindle cell/myxoid, 1 plasmacytoid/solid and 2 reticular types).

Immunohistochemistry was carried out on 4-μm sections of formalin- and MAA-fixed tissue from pleomorphic adenomas and myoepitheliomas of parotid gland, and portions of adjacent salivary gland. Two monoclonal antibodies to muscle-specific actin and a two-step indirect immunoperoxidase technique were used. One murine monoclonal antibody, recognizing the alpha and gamma isotypes (42 kDa) of skeletal, cardiac and smooth muscle actin, was developed from clone HHF35 (Gown et al. 1985), marketed by Enzo Biochemical (New York, N.Y., USA; diluted 1:2,500). The second mouse monoclonal antibody, clone 011D, was produced from actin isolated from human myocardium, also recognizes skeletal, smooth and cardiac actin isoforms, and was used in the pre-diluted form provided by Biomeda Corporation (Foster City, Calif., USA).

Tissue sections were exposed to 2% fetal calf serum for 5 min to block non-specific protein-protein interactions. Sections were then treated with two changes of 3% hydrogen peroxide in absolute methanol for 10 min each, followed by three 2-min washes in phosphate-buffered saline (PBS). Primary antibodies were applied for $1^1/_2$ h at 37° C. Slides were then washed in PBS, three changes for 2 min each, prior to applying the secondary antibody, horseradish peroxidase-conjugated goat anti-mouse [F(ab)$_2$)] antibody (Jackson Laboratories, Minnesota; diluted 1:200), for 1.5 h at room temperature. The colour reaction was developed using 3,3′-diaminobenzidine tetrahydrochloride/hydrogen peroxide (100 mg DAB to 200 ml 0.05 M TRIS buffer (pH 7.6) plus 200 μl of 30% hydrogen peroxide) for 4–5 min. Subsequently, the sections were counter-stained with haematoxylin.

As a positive control, a separate block was prepared that contained both formalin- and methanol-fixed normal parotid tissue. Sections from this block were placed on every slide with a section from a tumour and the degree of staining of the myoepithelium, especially in the methanol-fixed parotid, was monitored. This procedure ensured the correct interpretation of negative staining in the tumours in which no normal parotid salivary gland was present. Blood vessels in both the tumour and normal tissue also served as internal controls.

Five cases of pleomorphic adenoma/myoepithelioma were used to compare the presence or absence of smooth muscle actin, as defined by the two monoclonal antibodies, with ultrastructural expression of cytoplasmic filaments. The tissues had been glutaraldehyde fixed and then post-fixed in osmium tetroxide and routinely processed for embedding in epon/araldite. Representative areas were selected from toluidine-blue-stained plastic sections and thin sections cut and stained with uranyl acetate and lead citrate prior to survey and photography at 60 kV in a Philips EM301 or 400 microscope.

Table 1. Immunostaining using antibodies to muscle-specific actin on formalin-fixed tissue of pleomorphic adenomas, myoepitheliomas and adjacent parotid salivary gland

Antibody	HHF35			MSA		
Tissue	Neg	1+	2+	Neg	1+	2+
Normal gland ($n=13$)						
Acini	12	1	0	6	5	2
Ducts	12	1	0	4	7	2
Myoepithelial cells	2	6	5	9	1	3
Blood vessels	0	3	10	0	7	6
Pleomorphic adenoma ($n=11$)						
Tumour cells	9	3	2[a]	5	3	3
Myoepithelioma ($n=4$)						
Tumour cells	4	0	0	2	2	0

HHF35, Anti-muscle actin antibody from Enzo Biochemical, N.Y., USA; MSA, anti-muscle-specific actin antibody from Biomeda Corp., Calif., USA; immunostaining was graded as: neg, no staining; 1+, weak to moderate staining; 2+, strong staining.

[a] Staining was focal in both cases with only approximately 5% of the tumour cell population positive

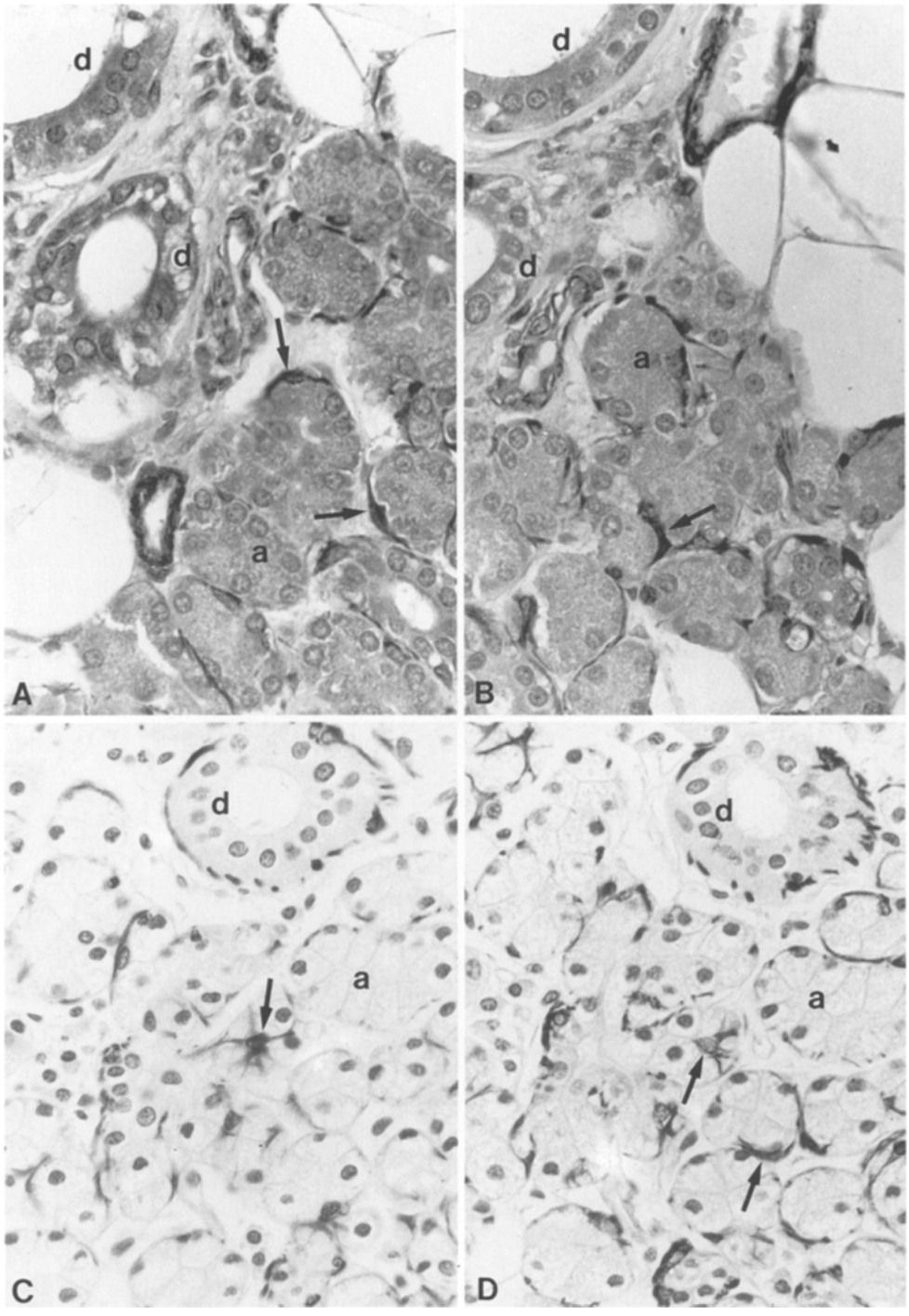

Fig. 1. Serial section comparison of immunostaining of normal parotid salivary gland using monoclonal antibodies MSA (**A, C**) and HHF35 (**B, D**). Background staining of acinar (*a*) and duct (*d*) cells is greater with formalin-fixed tissue (**A, B**) compared to MAA-fixed salivary gland (**C, D**), and myoepithelial cells (*arrows*) are better defined with the latter fixation (**C, D**). There also appear to be more myoepithelial cells and their processes with HHF35 (**D**) than with MSA (**C**) but both monoclonal antibodies stain myoepithelial cells associated with the striated duct (*d*) in **C** and **D**. Immunoperoxidase (IMP) with haematoxylin counterstain; **A–D** ×400

Results

Smooth muscle cells of blood vessels and myoepithelial cells of normal parotid gland, both containing muscle-specific actin, provided a useful internal control for comparing the two monoclonal antibodies (Table 1). With both anti-muscle-specific actin antibodies, blood vessel smooth muscle cells were stained to some degree in the 13 samples of normal human salivary gland. Myoepithelial cells in two examples were negative with HHF35, however, while myoepithelium was not immunostained in 9 cases using MSA. When myoepithelial cells were positive in formalin-fixed tissue, the degree and intensity of staining was comparable with MSA and HHF35 (Fig. 1 A, B). Although acinar and duct luminal cells, as expected, were negative with HHF35 in the majority of cases, using the MSA antibody only 6 of 13 samples of parotid gland had unstained acini, and only 4 of 13 had unstained ducts (Table 1). Using formalin-fixed tissue and the MSA antibody on normal salivary gland, therefore, produced some degree of background staining and abolished staining of myoepithelial cells.

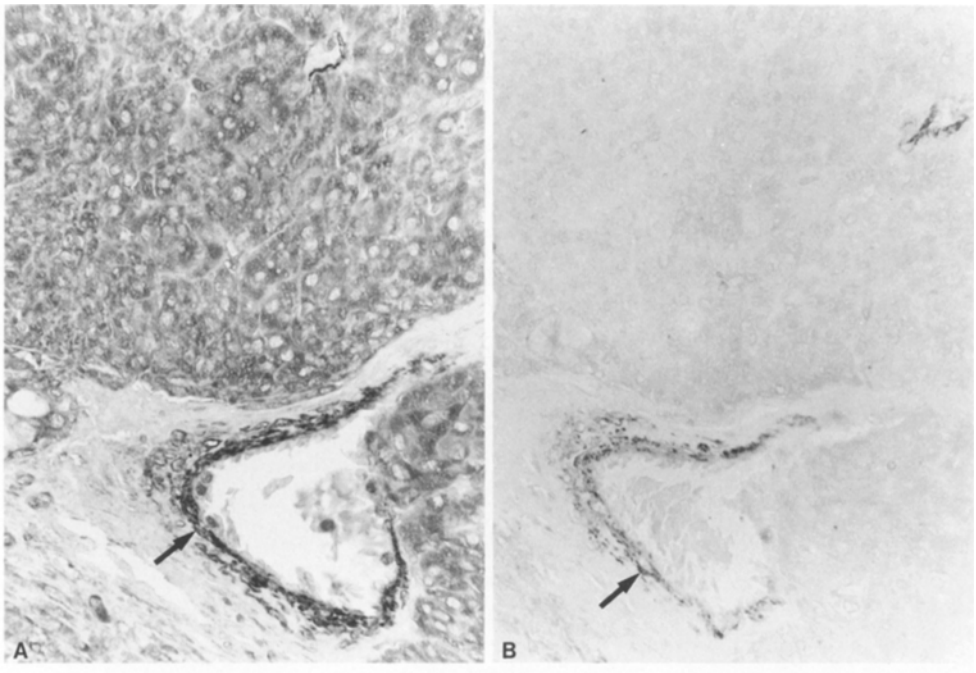

Fig. 2. Comparison of monoclonal antibodies MSA (**A**) and HHF35 (**B**) in serial sections of a formalin-fixed example of pleomorphic adenoma. Some cellular areas of this tumour are diffusely stained by MSA (**A**) but are negative with HHF35 (**B**); positive stained blood vessel (*arrows*) serves as an internal control. IMP with haematoxylin counterstain; **A, B** ×400

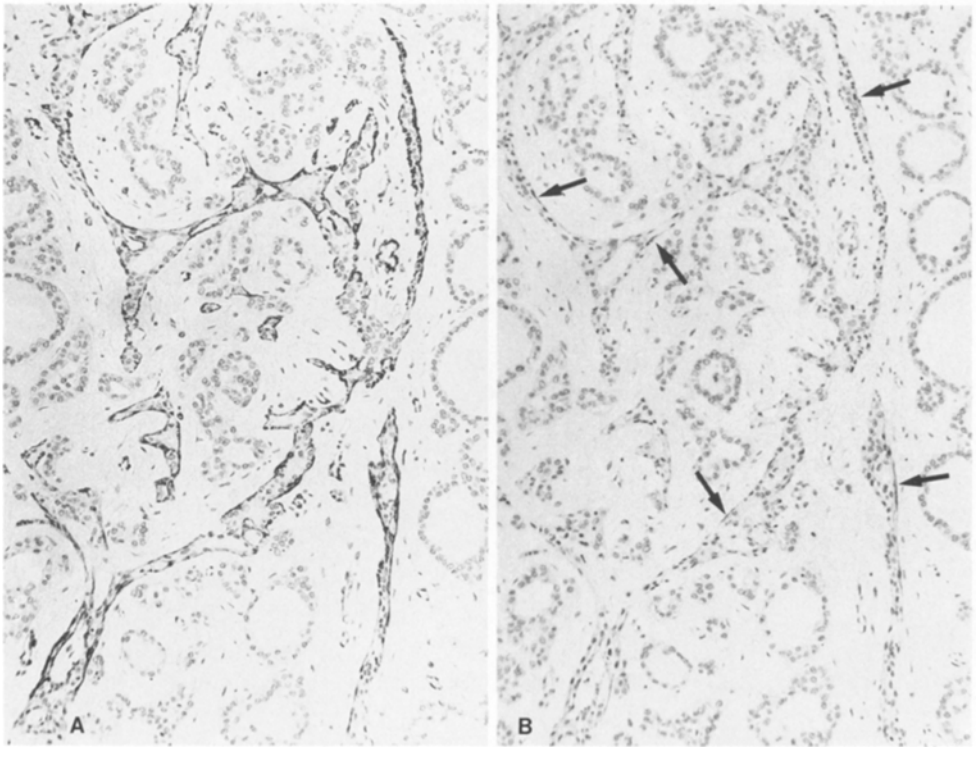

Fig. 3. Comparison of monoclonal antibodies MSA (**A**) and HHF35 (**B**) in an MAA-fixed pleomorphic adenoma (case 13 in Table 2). Strong staining of spindle-shaped, periductal cells occurred in a few isolated foci with MSA (**A**), but these cells were infrequently and only weakly stained (*arrows*) with HHF35 (**B**). Control parotid gland tissue on the same slides had strongly stained myoepithelial cells with both antibodies. IMP with haematoxylin counterstain; **A, B** ×160

Of the 15 tumours studied with formalin-fixed tissue, the tumour cells of all 4 myoepitheliomas and all but 2 pleomorphic adenomas (13%), in which staining was quite focal, were negative with HHF35 (Table 1). But 8 cases (53%), consisting of 6 pleomorphic adenomas and 2 myoepitheliomas, had some degree of staining with MSA (Table 1). Figure 2 shows the diffuse, moderate, cytoplasmic staining obtained in the cellular area of a pleomorphic adenoma with MSA and the negative staining with HHF35 on a serial section.

Compared to formalin-fixed samples (Fig. 1A, B), there was enhanced staining and better definition of the myoepithelium in all samples of normal parotid salivary gland and when immunostaining for muscle-specific actin was performed on methanol-fixed tissue (Fig. 1C, D; Table 2). In the case illustrated in Fig. 1, staining of serial sections suggested that more myoepithelial cells or their processes were identified with HHF35 than with MSA in MAA-fixed human parotid salivary gland. Myoepithelial cells associated with striated duscts were

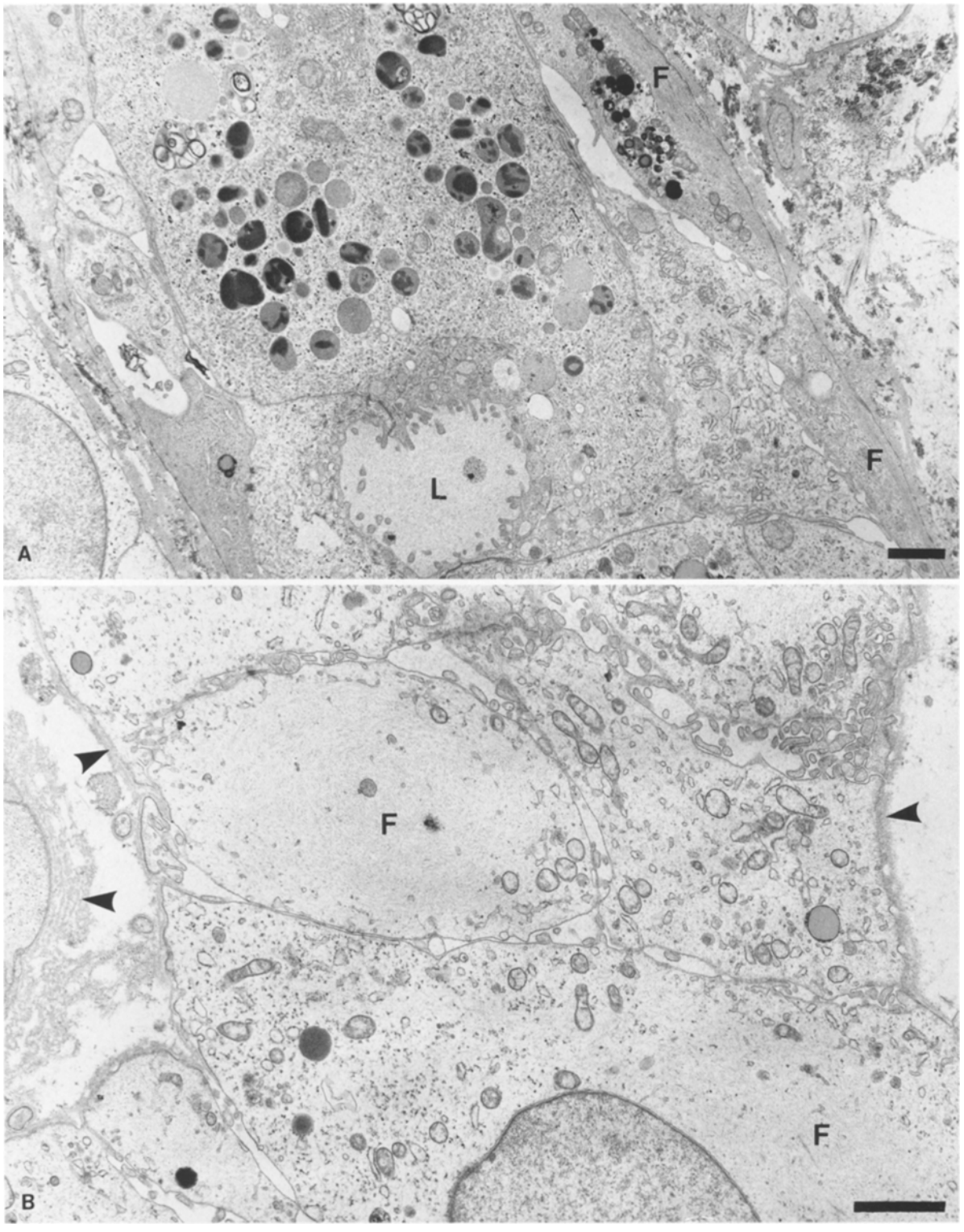

Fig. 4A, B. Pleomorphic adenoma (case 5 in Table 2). **A** Tumor cells with linear accumulations of filaments (*F*) are situated at the periphery of luminal cells forming an intercellular lumen (*L*). **B** Other tumour cells without specific differentiation have more centrally placed aggregates of intermediate-type filaments. Basal lamina (*arrowheads*), often reduplicated, coats the outer aspects of the tumour cell cords. Uranyl acetate and lead citrate (UA and LC). **A** ×9,900; **B** ×16,800, Bars = 1 μm

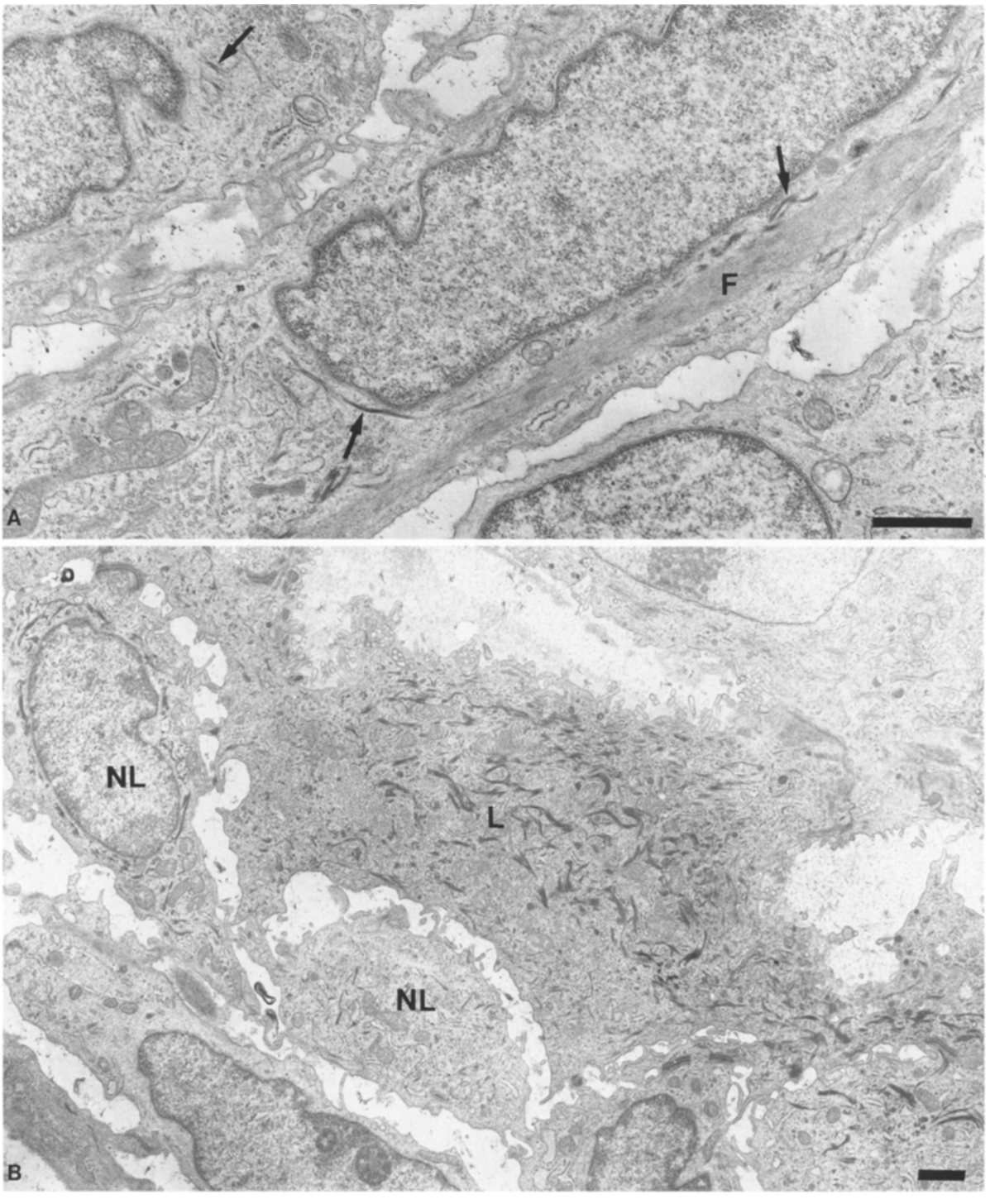

Fig. 5A, B. Pleomorphic adenoma. **A** Some non-luminal spindle cells have elongated bands of cytoplasmic filaments (*F*) containing multiple dense staining regions. Tonofilaments (*arrows*) are also present. **B** Luminal cells (*L*) are particularly rich in tonofilaments, but these are also present in non-luminal (*NL*) tumour cells. UA and LC. **A** ×18,000; **B** ×8,300; *bars* = 1 μm

identified with both antibodies (Fig. 1C, D). No background staining of the ductal and acinar cells was observed with either antibody (Table 2).

In the case of tumours with MAA-fixed tissues, the 2 myoepitheliomas (cases 3 and 7) were negative with both of the anti-smooth muscle actin antibodies (Table 2). However, 4 (cases 10 to 13) of the 11 pleomorphic adenomas (36%) had some proportion of the tumour cells stained with the two antibodies, but without any marked differences between them (Table 2). Figure 3, from case 13, reveals the difference in the number and intensity of tumour cells stained using HHF35 compared to MSA in the main region of this pleomorphic adenoma that was positive (Table 2). It is noteworthy that tumour cells positively stained by HHF35 and MSA were more spindle shaped (Fig. 3A).

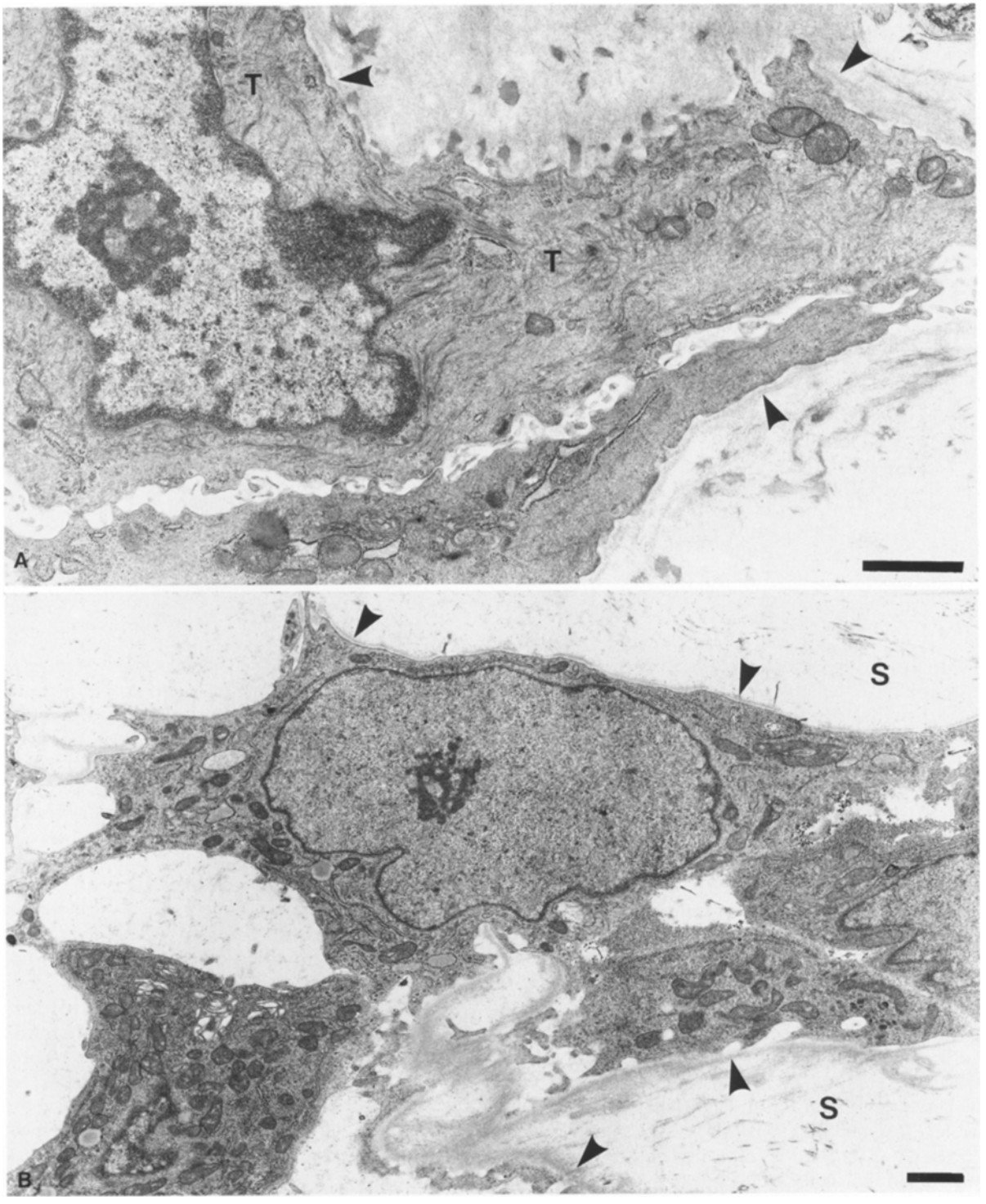

Fig. 6A, B. Pleomorphic adenomas (Table 2). **A** Case 12. Peripherally placed tumour cells (rimmed by basal lamina (*arrowheads*) on the stroma-associated aspect) have dispersed tonofilaments (*T*), but no evidence of myofilaments. **B** Case 13. In this pleomorphic adenoma, peripheral cells adjacent to the stromal tissues (*S*) and basal lamina (*arrowheads*) are devoid of obvious intermediate filaments and myofilaments. UA and LC. **A** ×18,000; **B** ×10,600; *bars* = 1 μm

Five representative cases of pleomorphic adenoma/myoepithelioma were examined by conventional electron microscopy. Cases 5, a pleomorphic adenoma, and case 7, a myoepithelioma, were negative immunohistochemically using both HHF35 and MSA on MAA-fixed tissue (Table 2). In case 5, some tumour cells peripheral to the duct luminal cells exhibited a band of filaments (at times containing short, linear densities) aligned parallel and adjacent to the cell membrane (Fig. 4A). A larger proportion of the tumour cells had a focal accumulation of cytoplasmic filaments with a random or whorled distribution resembling the intermediate types, especially

Table 2. Comparative immunostaining of adjacent sections of pleomorphic adenomas ($n=11$) and myoepitheliomas ($n=2$) and normal parotid gland ($n=13$) using two monoclonal antibodies to smooth muscle actin on methanol-fixed tissues

Antibody	Tumour[a]	HHF35		MSA	
Case		Intensity	Percent cells[b]	Intensity	Percent cells
1	PA	0	0	0	0
2	PA	0	0	0	0
3	MYO	0	0	0	0
4	PA	0	0	0	0
5	PA	0	0	0	0
6	PA	0	0	0	0
7	MYO	0	0	0	0
8	PA	0	0	0	0
9	PA	0	0	0	0
10	PA	1+	<5	1+	<5
11	PA	2+	50	1+	30
12	PA	2+	10	2+	20
13	PA	1+	<5	2+	15
Parotid gland					
Acini		0	0	0	0
Ducts		0	0	0	0
Myoepithelial cells		2+	–	2+	–
Blood vessels		2+	–	2+	–

[a] PA, Pleomorphic adenoma; MYO, myoepithelioma
[b] Percentage of cells stained in the two tumour types
Immunostaining was graded as: 0, no staining; 1+, weak to moderate staining; 2+ strong staining

vimentin (Fig. 4B). No myofilament-like accumulations were evident in the tumour cells of case 7. Another typical pleomorphic adenoma included in Table 1 that was negative with both HHF35 and MSA also exhibited linear cytoplasmic zones of filaments and densities with a parallel alignment in non-luminal tumour cells (Fig. 5A). The rich complement of intermediate filaments, in this case cytokeratins, that could be found in both luminal and non-luminal tumour cells in some pleomorphic adenomas is evident in Fig. 5B.

Cases 12 and 13 (Table 2) were 2 other pleomorphic adenomas in which some proportion of tumour cells were stained positively with both MSA and HHF35. Representative micrographs of case 12 revealed that tumour cells often contained many cytoplasmic filaments but that these were not aligned or distributed in the fashion of myofilaments seen in normal myoepithelial or smooth muscle cells (Fig. 6A). Obvious intermediate filaments or myofilaments were entirely absent in the portions of case 13 examined (Fig. 6B). In both of these examples, the absence of discernible myofilaments even applied to the non-luminal cells adjacent to the basal lamina separating tumour cells from stromal materials (Fig. 6A, B).

Discussion

In certain salivary gland tumours, differentiation of both luminal and myoepithelial-like cells can be clearly demonstrated (Batsakis et al. 1983; Dardick and van Nostrand 1985, 1987; Mori et al. 1987; Ogawa et al. 1990). However, the criteria for the identification of the tumour counterpart of normal myoepithelial cells remains incompletely defined. Originally, this required identification of myofilaments and other characteristics of normal myoepithelial cells at the ultrastructural level, and more recently the expression of muscle-type actins in myoepithelial cells of salivary glands using immunocytochemistry has become another standard. Developing reliable histological criteria for classifying salivary gland tumours necessitates establishing the degree to which tumour cells mimic their counterpart in normal tissues. Pleomorphic adenomas and myoepitheliomas, diagnosed on traditional criteria, serve as ideal models for this purpose; the former express both luminal and non-luminal (periductal or modified myoepithelial cells) tumour cells (Batsakis et al. 1983; Dardick and van Nostrand 1985; Mori et al. 1987), while the less frequent myoepitheliomas, as the designation indicates, differentiate as only a single cell-type (Dardick et al. 1989; Mori et al. 1989). The current investigation was designed to study the extent of expression of muscle-specific actin in such tumours and how muscle-specific actin contained in normal and neoplastic salivary gland tissues is affected by fixation.

The results indicate that in using monoclonal antibodies for muscle-specific actin, formalin fixation is less than ideal, and Table 1 provides evidence that for the detection of such antigens in normal parotid gland myoepithelium, formalin fixation cannot be used with confidence. Myoepithelial cells were unstained in certain specimens, while other cells not expected to stain, e.g. acinar and duct cells, were sometimes positive, particularly with the MSA antibody. There may be a number of explanations to account for the invariable staining of smooth muscle cells of blood vessels with both antibodies in formalin-fixed tissue even when normal myo-

epithelial cells are negative. Actin in smooth muscle cells may have different antigenic properties, such as a greater number of binding sites and less sensitivity to cross-linking by formalin, compared to that in myoepithelial cells or the concentration of muscle-specific actin in myoepithelial cells may be considerably lower than in smooth muscle cells so that formalin fixation leaves insufficient unaltered actin for detection immunocytochemically in the former cells but a positive result in the latter. It follows that precise and reliable interpretation of immunostaining in pleomorphic adenomas and myoepitheliomas is not possible using formalin-fixed tissue. Perhaps these effects of formalin fixation account for the relatively high proportion of positively stained tumours reported by Hirano and associates (1990). Using two other monoclonal antibodies against alpha-type smooth muscle actin, they noted staining in 60% of the 23 pleomorphic adenomas with one antibody and 65% with the other (Hirano et al. 1990). Other reports in the literature, also based on formalin-fixed tissue, suggest a considerable degree of actin expression in pleomorphic adenoma and myoepitheliomas (Mori et al. 1987, 1989; Morinaga et al. 1987; Leoncini et al. 1988; Anderson et al. 1990). These results contrast with those obtained using methanol-fixed or frozen tissues from salivary gland tumours both in this report and in others (Draeger et al. 1990; Zarbo et al. 1991).

Using the HHF35 antibody on frozen and methanol-fixed samples, Zarbo and associates (1991) detected muscle-specific actin in 50% of 26 pleomorphic adenomas but commented that in most cases the muscle actin expression was very focal, comprising less than 5% of the tumour cell population. In the current series, muscle-specific actin was observed immunohistochemically in 4 of 13 pleomorphic adenomas/myoepitheliomas (31%) using methanol-fixed tissue. In 2 of these cases, 30–50% of the tumour cells stained for muscle-specific actin, while less than 5% stained in the remaining 2 cases. In another series of 12 pleomorphic adenomas studies immunohistochemically by Draeger and colleagues (1990) using frozen sections, no actin-positive tumour cells were noted. Even in myoepitheliomas of salivary gland, only 1 of 5 had tumour cells positive (in this particular case more than 50% of the population) for muscle-specific actin using methanol-fixed tissue (Dardick et al. 1988a). Such results show the variable differentiation of non-luminal tumour cells in pleomorphic adenomas and of the cell population in myoepitheliomas, and the need to re-examine the diagnostic criteria for these tumour cells. Perhaps, based on current information, it is no longer necessary to require the entire characteristics of normal myoepithelial cells in non-luminal tumour cells of pleomorphic adenomas and other salivary gland tumours in order to refer to such tumour cells as modified myoepithelial cells. In salivary gland tumours, full differentiation into myoepithelial cells is seldom achieved.

Myoepithelial and basal cells form a continuous layer external to acinar and duct luminal cells of salivary glands (Dardick et al. 1987; Burns et al. 1988; Leoncini et al. 1988). Both actin-expressing and non-actin-expressing tumour cells might be organized to reflect the positioning of myoepithelial cells in the normal gland, but express the complete differentiation of these cells only in a subset of the neoplastic counterpart. The higher proportion of non-actin-containing tumour cells, however, could represent basal cells of excretory duct and the subpopulation of basal cells of striated duct that are immunohistochemically negative for muscle-specific actin with a number of different antibodies (Dardick et al. 1987, 1988b; Draeger et al. 1990; Zarbo et al. 1991). This interpretation could account for the lack of myofilaments within non-luminal tumour cells in ultrastructural examinations of some pleomorphic adenomas (Erlandson et al. 1984; Mackay et al. 1988; Anderson et al. 1990), their ready detection in a few others (Chaudhry et al. 1982; Mackay et al. 1988), and the features observed in this report, even when these tumour cells are otherwise morphologically similar in structure and location by both light and electron microscopy. In salivary gland tumours, the term modified myoepithelial cell, to include those non-luminal cells (i.e. the counterpart of the myoepithelial cell in normal salivary gland) that are positive or negative for muscle-specific actin and/or myofilaments, seems quite appropriate. This concept, however, may require expansion to include possible differentiation of basal cell-type tumour cells.

How reliable are ultrastructural observations of myofilament organization in pleomorphic adenomas and myoepitheliomas? Based on data in this report, it is inappropriate to draw conclusions that are too specific. Seemingly, there is limited correlation between immunohistochemical and ultrastructural findings. In the 2 cases examined by conventional electron microscopy that were negative with both HHF35 and MSA antibodies (case 5 in Table 2 and one case included in Table 1), myofilaments were suggested by electron microscopy. Whether such filaments are actually of intermediate type, or immunohistochemical techniques are insufficiently sensitive, requires further study especially by immunoelectron microscopy. Cases 12 and 13 in Table 2 were at least focally positive for muscle-specific actin, but no filaments were apparent ultrastructurally. This may be due simply to sampling or actin may not always be typically organized even in benign tumours. Perhaps it should not be surprising if the counterpart of myoepithelial cells in pleomorphic adenomas and the tumour cells in myoepitheliomas infrequently express muscle-specific actin. Structural and organizational characteristics of tumour cells frequently become dissociated from functional aspects. In human salivary gland tumours where the organization and differentiation is so complex compared to tumours of many other organs, failure to mimic faithfully the normal tissue is not unexpected. Since muscle-specific actin was detected to some degree in 4 of the 13 cases of pleomorphic adenoma/myoepithelioma with the more reliable methanol-fixed tissue, functional differentiation in the form of neoplastic myoepithelium can be demonstrated in certain tumours. The results of this and another study (Zarbo et al. 1991) indicate that the variation of expression of actin seen both within and between tumours is probably not artefactual if methanol-fixed tissues are utilized and suggests both morpho-

logical and functional tumour cell heterogeneity. Results obtained from studies using formalin-fixed tissue to demonstrate actin filament production in tumour cells of pleomorphic adenomas and myoepitheliomas should be interpreted with caution.

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