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Intermediate filament expression in normal parotid glands and pleomorphic adenomas

B.F. Burns [1,2], **I. Dardick** [1,2], **and W.R. Parks** [1]

[1] Department of Laboratory Medicine, Ottawa Civic Hospital, 1053 Carling Avenue, Ottawa, Ontario, Canada, K1Y 4E9
[2] Department of Pathology, University of Ottawa, Ottawa, Ontario, Canada

Summary. A comparative immunohistochemical study of intermediate filament expression in normal parotid glands and pleomorphic adenomas (PA) was performed using material fixed in a modified methacarn fixative. The normal myoepithelial cells of acini stained only with monoclonal antibodies 312C8-1 (cytokeratin (CK) 14) and 4.62 (CK 19) while myoepithelial/basal cells of ducts also reacted with antibodies 8.12 (CK 13, 16), 8.60 (CK 10, 11, ±1), and PKK1 (CK 7, 8, 17, 18). Normal duct luminal cells showed a different CK profile, reacting consistently with ECK, a polyclonal antibody to epidermal prekeratin (CK 3,6), and monoclonal antibodies 4.62, PKK1 and 8.60. In PA, tumour cells at the periphery of ducts, in solid areas, and at the edge of myxoid regions all had CK profiles similar to normal myoepithelial/basal cells except that antibody 4.62 was generally negative. Vimentin and glial fibrillary acidic protein (GFAP) were uniformly negative in normal parotids but showed variable (often strong) reactivity with some cells in chondroid, myxoid and solid areas of PA. A surprising feature of most PA was the variability of CK subtype expression not only from one case to another but also within morphologically similar areas of the same specimen. These results suggest that the morphology of PA is the result of diversity of tumour cell differentiation rather than the processes implicit in a reserve cell histogenetic model.

Key words: Parotid gland – Parotid neoplasms – Intermediate filaments – Morphogenesis

Introduction

Pleomorphic adenomas represent one of the most consistent and characteristic examples of multipotential cellular differentiation in the field of human neoplasia. As such, they have provoked the curiosity of a diverse group of investigators over the years, leading to a number of theories regarding their histogenesis and morphological development. The precursor cell usually proposed for the origin of these tumors is the intercalated duct reserve cell (Eversole 1971; Regezi and Batsakis 1977). Recently, a co-ordinated proliferation of duct luminal cells and modified myoepithelial cells, coupled with an organized synthesis of extracellular materials, has been suggested as the basic mechanism for the innumerable morphological patterns of pleomorphic adenoma (Dardick et al. 1983; Erlandson et al. 1984; Dardick and van Nostrand 1987). Ultrastructural studies have shown a variability in the number of intermediate filaments and microfilaments, as well as the form of the myoepithelial cell component in the cellular, myxoid and chondroid areas of pleomorphic adenomas (Dardick et al. 1983). The extent of cytological variation possible in neoplastic myoepithelial cells is also apparent in other salivary gland tumours (Batsakis et al. 1983; Dardick and van Nostrand 1985).

The intermediate filament cytoskeleton of normal salivary gland has been studied in frozen section material using a variety of monoclonal antibodies to cytokeratins (Geiger et al. 1987). Using the cytokeratin (CK) nomenclature of Moll et al. (1982), the duct luminal cells express CKs 18 and 19; myoepithelial cells around acini are reactive with antibodies to CKs 4, 5, and 6 and to CK 18 as are the basal cells of excretory duct which are additionally positive for CKs 13 and 16 (Geiger et al. 1987). Another study (Caselitz et al. 1981), also found prekeratin and/or vimentin reactivity of myoepithelial/basal cells in normal glands and the tumour cells in the myxoid areas of pleomorphic adenomas using polyclonal antisera raised against bovine hoof. Recently, antibodies to CK 14

Offprint requests to: B.F. Burns

have been shown to stain the myoepithelial/basal cell components of acini and salivary ducts with equal intensity (Dairkee et al. 1985; Caselitz et al. 1986; Dardick et al. 1987).

Studies examining the contractile filament component of normal myoepithelial cells have shown bundles of microfilaments which react with antibodies to smooth muscle myosin (Palmer 1986) and to actin (Kahn et al. 1985).

In this study we have selected twelve pleomorphic adenomas to examine the intermediate filament profile of these tumours and the adjacent normal salivary gland. These tumours were all fixed in a modified methacarn fixative which we and others (Mitchell et al. 1985; Palmer et al. 1985) have found to result in more reliable detection of intermediate filaments than formalin fixation. We sought to define the patterns of intermediate filament expression in the various morphological regions of these tumours and to determine if they resembled the pattern of expression in the differentiated cells of normal salivary gland.

Materials and methods

Twelve parotid pleomorphic adenomas were obtained over an 18 month period, fixed initially in a modified methacarn fixative (60% methanol/30% chloroform/10% glacial acetic acid) and subsequently embedded in paraffin. Nine of these cases also had sufficient normal parotid tissue, which was methacarn-fixed, for assessment of specific normal cell types.

The antibodies used in this study with their specificities and sources are listed in Table 1. Sections were cut at 4 μm and floated onto Bondfast glue (Lepage, Bramalea, Ontaris, Canada) coated slides, dried overnight at 37° C. They were then deparaffinized in 3 washes of toluene followed by 3 washes in ethanol. Sections to be stained with rabbit polyclonal antibodies (ECK, vimentin and GFAP) were blocked first with 3% hydrogen peroxide for 30 min followed by a 1:10 Tris buffer dilution of normal swine serum. The primary antibody was incubated for 30 min at 20° C then overnight at 4° C. After 2 Tris buffer washes they were incubated with 1:100 swine anti-rabbit Ig for 30 min, washed and then incubated with rabbit peroxidase-anti peroxidase (1:100). After washing, slides were incubated with 0.05% DAB followed by 0.5% copper sulfate and finally counterstained with Mayer's hematoxylin.

The mouse monoclonal antibodies (PKK1, 8.60, 8.12, 312C8-1 and 4.62) were treated in the same way initially, substituting 1:10 normal horse serum for the swine serum before a 30 min primary antibody incubation. Following this the sections were developed with the Unistain detection kit (Becton Dickinson, Mountain View, CA), a goat anti-mouse Ig peroxidase method. Negative controls substituted normal rabbit serum for the first stage polyclonal antibodies or phosphate buffered saline for the monoclonal antibodies. Appropriate positive controls have been described previously (Dardick et al. 1988).

Stained sections were then examined microscopically and each antibody was scored as being negative, variably positive (that is less than 75% of cells of a certain morphological type being positive, while the remainder were negative) or consistently positive (greater than 75% of cells positive).

Table 1. Antibodies employed and their specifications

Antibody	Specificity
ECK (polyclonal)[a]	CK 3, 6; 56/64 KD
PKK1 (monoclonal)[b]	CK 7, 8, 17, 18
8.60 (monoclonal)[c]	CK 10, 11 ± 1
8.12 (monoclonal)[c]	CK 13, 16
312C8-1 (monoclonal)[d]	CK 14
4.62 (monoclonal)[c]	CK 19
Vimentin (polyclonal)[e]	Vimentin
Glial fibrillary acid protein (GFAP) (polyclonal)[a]	GFAP

[a] Dako, Santa Barbara, CA
[b] LabSystems, Helsinki, Finland
[c] ICN Biomedicals, Montreal, Quebec
[d] A kind gift of Dr. S.H. Dairkee, Peralta Cancer Research Inst., Oakland, CA
[e] Euro Diagnostics BV, Holland

The areas assessed in the normal parotid included the myoepithelial/basal cells surrounding the striated/excretory ducts and those around acini and intercalated ducts, the intercalated, striated, and excretory duct lining cells and acinar cells. In the pleomorphic adenomas, stellate and spindle cells of the myxoid areas were scored, as well as cells in the chondroid areas. The cells of cellular areas with limited or no apparent intercellular matrix were divided into those at the myxoid interface and the more central solid areas. Finally areas exhibiting duct differentiation were scored with respect to their peripheral and lining cells.

Results

The reactivity patterns of the nine normal parotid glands are shown in Table 2 and summarized graphically in Fig. 1. As can be seen the pattern of intermediate filament expression varies considerably from one cell type to another. The luminal cells of the intercalated, striated and excretory ducts have similar patterns of reactivity with antibodies ECK, PKK1 and 4.62 (Fig. 2), while less consistent staining with 8.60 and 8.12 (Fig. 3) is seen. The myoepithelial/basal cells of striated and excretory ducts are readily distinguished from the duct luminal cells if luminal cells are not also uniformly strongly positive; the latter result tends to obscure the minor basal cell population. This distinction is not so readily made between myoepithelial cells and luminal cells of the intercalated duct except with 312C8-1 where luminal cells are negative while myoepithelial cells stain positively. Therefore myoepithelial cells around the acinus are scored separately from the myoepithelial/basal cells of the striated and excretory ducts (the latter two show similar patterns). As seen in Fig. 4, numerous triangularly shaped, basally located cells of a large excretory duct stain positively with anti-

Table 2. Staining patterns to intermediate filament antibodies in normal parotid gland

Antibody (cytokeratin (CK) reactivity)	Cell type				
	Myoepithelial/basal cells		Duct luminal cells		Acinar
	Striated/ Excretory ductal (neg/var/pos)*	Acinar	Intercalated	Striated/Excretory	Cell
ECK (CK 3, 6)	2/2/5	7/1/1	0/2/7	0/1/8	9/0/0
PKK1 (CK 7, 8, 17, 18)	3/2/4	7/1/1	1/1/7	0/1/8	9/0/0
8.60 (CK 10, 11 +1)	2/5/2	9/0/0	1/7/1	1/1/7	8/1/0
8.12 (CK 13, 16)	0/3/6	9/0/0	2/5/2	0/7/2	9/0/0
312C8-1 (CK 14)	0/2/7	0/4/5	9/0/0	9/0/0	9/0/0
4.62 (CK 19)	0/0/7	1/2/5	0/2/6	0/1/7	7/0/0
Vimentin	8/1/0	8/1/0	9/0/0	9/0/0	9/0/0
GFAP	9/0/0	9/0/0	9/0/0	9/0/0	9/0/0

* reactivity patterns are scored as follows: negative (neg) – no antigen detected by the antibody; variable (var) – positive but less than 75% of cells stained; positive (pos) – greater than 75% cells stained

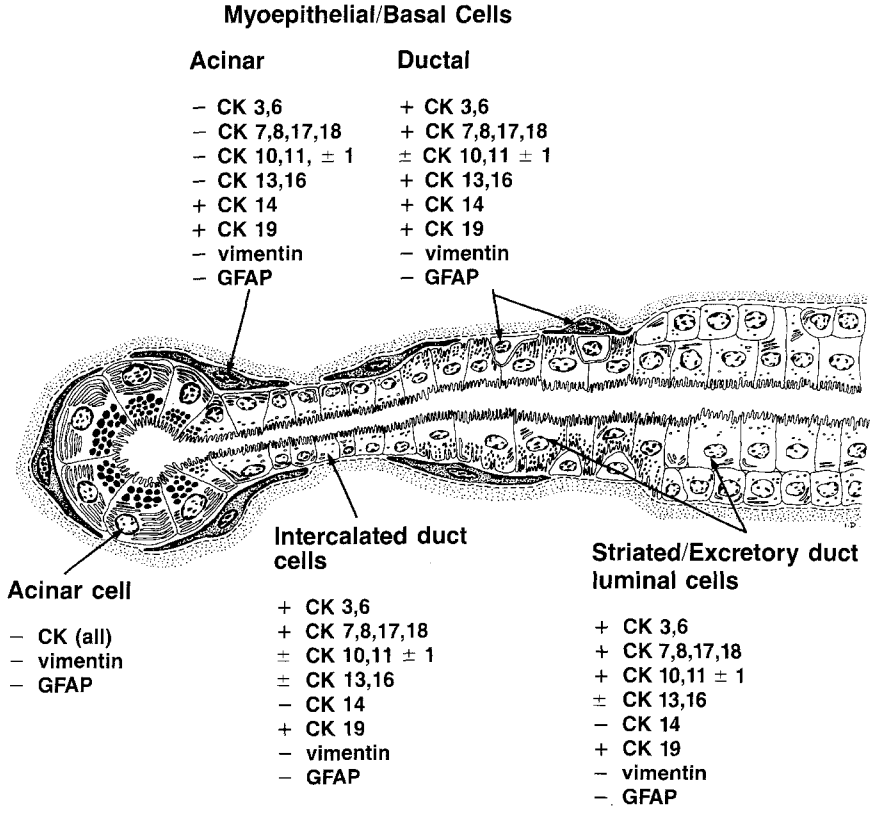

Fig. 1. Intermediate filament profile of parotid gland cells. The results of Table 2 are summarized schematically in this hypothetical acinar-ductal unit. The symbols +, ±, and – represent the most frequently encountered pattern of reactivity scored amongst all the cases. See Table 2 for explanation of symbols

bodies 312C8-1 and 4.62; these antibodies also react consistently with most acinar associated myoepithelial cells. Antibody 312C8-1 is negative in striated and excretory duct luminal cells, while 4.62, ECK and PKK1 stain most such cells (Table 2). Acinar cells show no convincing staining with any of the cytokeratin antibodies, vimentin or GFAP. The latter filaments are not detectable in any epithelial cell populations although vimentin can be demonstrated in fibroblasts and endothelial cells.

Table 3 and Fig. 5 show numerically and schematically the patterns of intermediate filament expression in the various morphological regions of 12 pleomorphic adenomas. The most striking finding is the variability in cytokeratin staining not

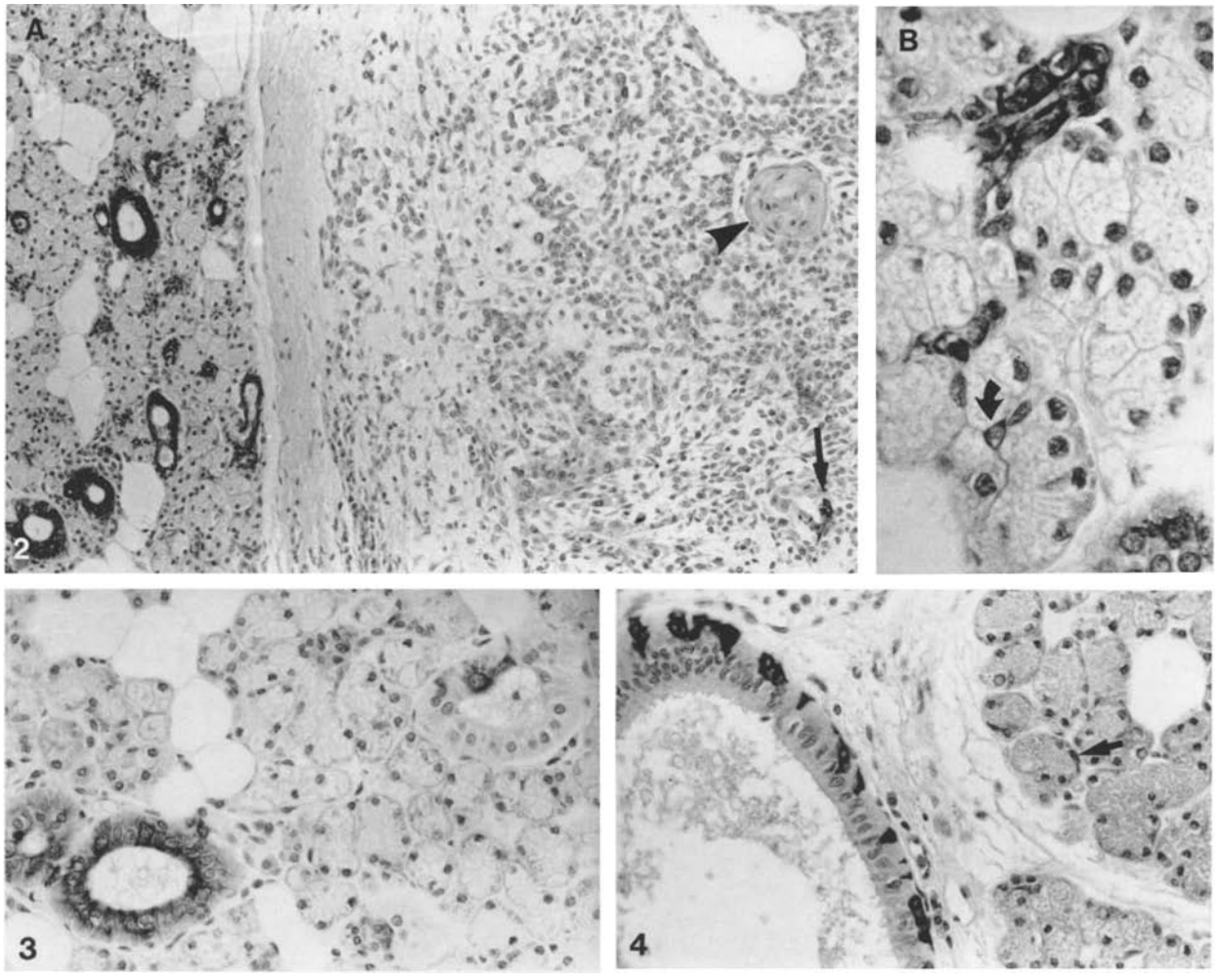

Fig. 2. **A** Normal parotid gland (*left*) and pleomorphic adenoma (*right*). The duct luminal cells of striated and intercalated ducts strongly express CK 19 while the adjacent tumour is negative except for a single small group of cells forming part of a duct-like structure (*arrow*). A focus of squamous metaplasia is also negative for CK 19 (*arrowhead*). **B** Normal parotid at higher magnification showing strongly staining intercalated duct near top and acinar myoepithelial cells (*curved arrow*). (Immunoperoxidase, monoclonal antibody 4.62, **A** ×330, **B** ×900

Fig. 3. Normal parotid gland. The striated duct luminal cells in the duct on the left are consistent in their expression of CKs 13, 16 while in the duct on the right only one luminal cell is positive, the rest being negative. (Immunoperoxidase, monoclonal antibody 8.12, ×600)

Fig. 4. Normal parotid gland showing consistent reactivity of myoepithelial/basal cells of an excretory duct (*left*) and around an acinus (*arrow*) to cytokeratin 14. (Immunoperoxidase, 312C8-1, monoclonal antibody, ×600)

only from one tumour to another but between similar type cells within the same tumour (Fig. 6). This tendency is more pronounced in the tumours than in the normal parotid gland. Cells at the extremes of the differentiation spectrum, namely duct luminal cells and chondroid cells are more consistent in their intermediate filament profiles (Table 3 and Fig. 5). Duct luminal cells, when identifiable in pleomorphic adenomas, usually react with antibodies ECK, 8.60 and 8.12 and slightly less consistently with PKK1 (Fig. 7). Reactivity with 312C8-1 is highly variable from case to case with some showing convincing negativity of the duct luminal cells in contrast to those cells immediately peripheral to them (Fig. 8). In others the duct luminal cells are positive and cannot be distinguished from other positive cells.

As shown in Fig. 5 cells in the center of solid areas and those at the interface with myxoid areas show similar cytokeratin profiles. However a progressive loss of cytokeratin reactivity can be detected as solid cellular/interface areas progress to

Table 3. Staining patterns to intermediate filament antibodies in pleomorphic adenomas

Antibody (cytokeratin reactivity)	Morphologic area in tumour (see Fig. 3 diagram)					
	Myxoid (neg/var/pos)*	Chondroid	Interface	Solid	Duct	
					peripheral	luminal
ECK (CK 3, 6)	7/ 5/0**	5/0/0	1/11/0	0/10/2	0/7/0	0/5/5
PKK1 (CK 7, 8, 17, 18)	7/ 5/0	4/1/0	3/ 9/0	1/ 9/2	1/6/0	0/5/4
8.60 (CK 10. 11, ±1)	9/ 2/0	5/1/0	8/ 2/1	6/ 3/2	4/2/1	3/2/4
8.12 (CK 13, 16)	3/ 8/1	3/2/0	0/ 7/5	0/ 6/6	0/3/4	0/2/8
312C8-1 (CK 14)	5/ 7/0	4/1/0	5/ 6/1	5/ 6/1	2/2/3	3/3/3
4.62 (CK 19)	10/ 1/0	6/0/0	9/ 2/0	8/ 2/1	6/1/0	4/3/2
Vimentin	0/10/2	0/3/2	0/ 9/3	0/ 9/3	0/6/1	6/3/1
GFAP	0/10/2	1/3/1	0/10/2	4/ 8/0	1/6/0	7/2/0

* reactivity patterns are scored as follows: Negative/Variable/Consistent Positive as in Table 2
** Many cases did not contain all morphological areas, especially chondroid areas, thus some subtotals are less than 12

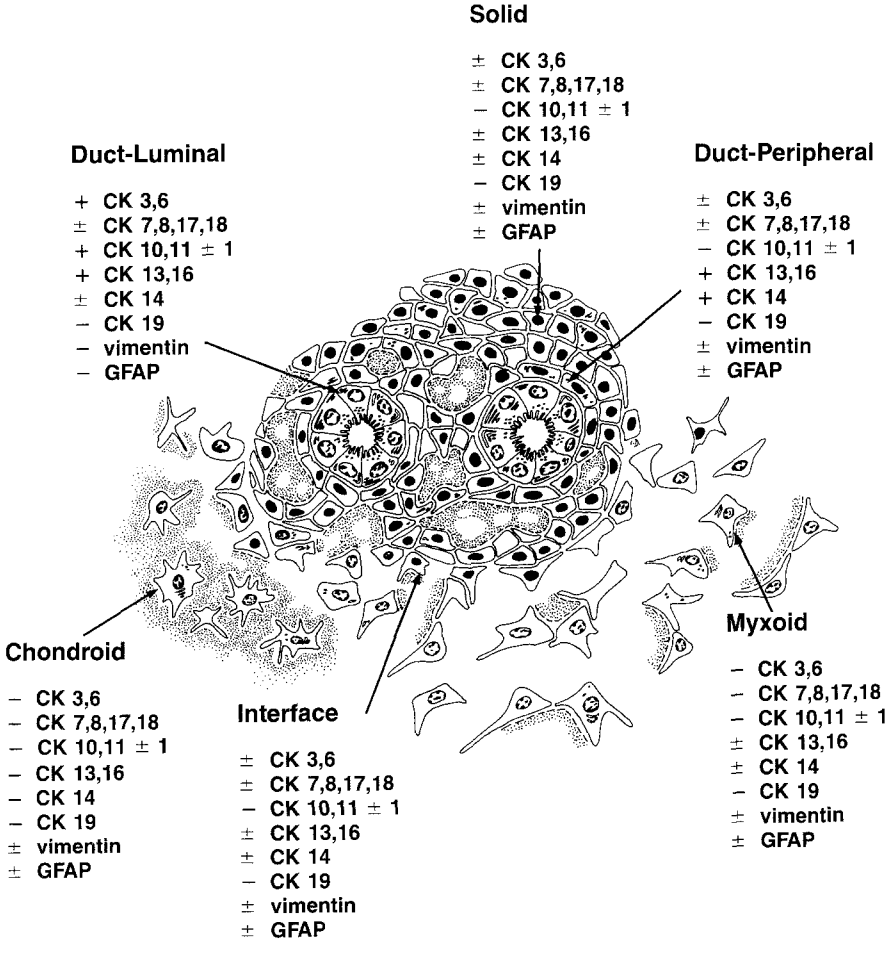

Fig. 5. Intermediate filament profile of pleomorphic adenoma. The results of Table 3 are summarized schematically in this diagramatic representation of the various morphological cell types found in pleomorphic adenomas. The reactivity patterns of +, ±, and – were determined as explained in Fig. 1

myxoid/chondroid cells. The cytokeratin profile of normal parotid duct myoepithelial/basal cells (Table 2 and Fig. 1) is most faithfully reproduced in those tumour cells in pleomorphic adenoma lying immediately peripheral to neoplastic ducts structures (Table 3 and Fig. 5). Both show consistent reactivity with antibodies 8.12 (Fig. 9) and 312C8-1, but less consistent staining with antibodies ECK and PKK1. Interestingly, while normal duct myoepithelial/basal cells are almost uniformly negative for vimentin and GFAP (one exception with vimentin staining only myoepithelial cells), the tu-

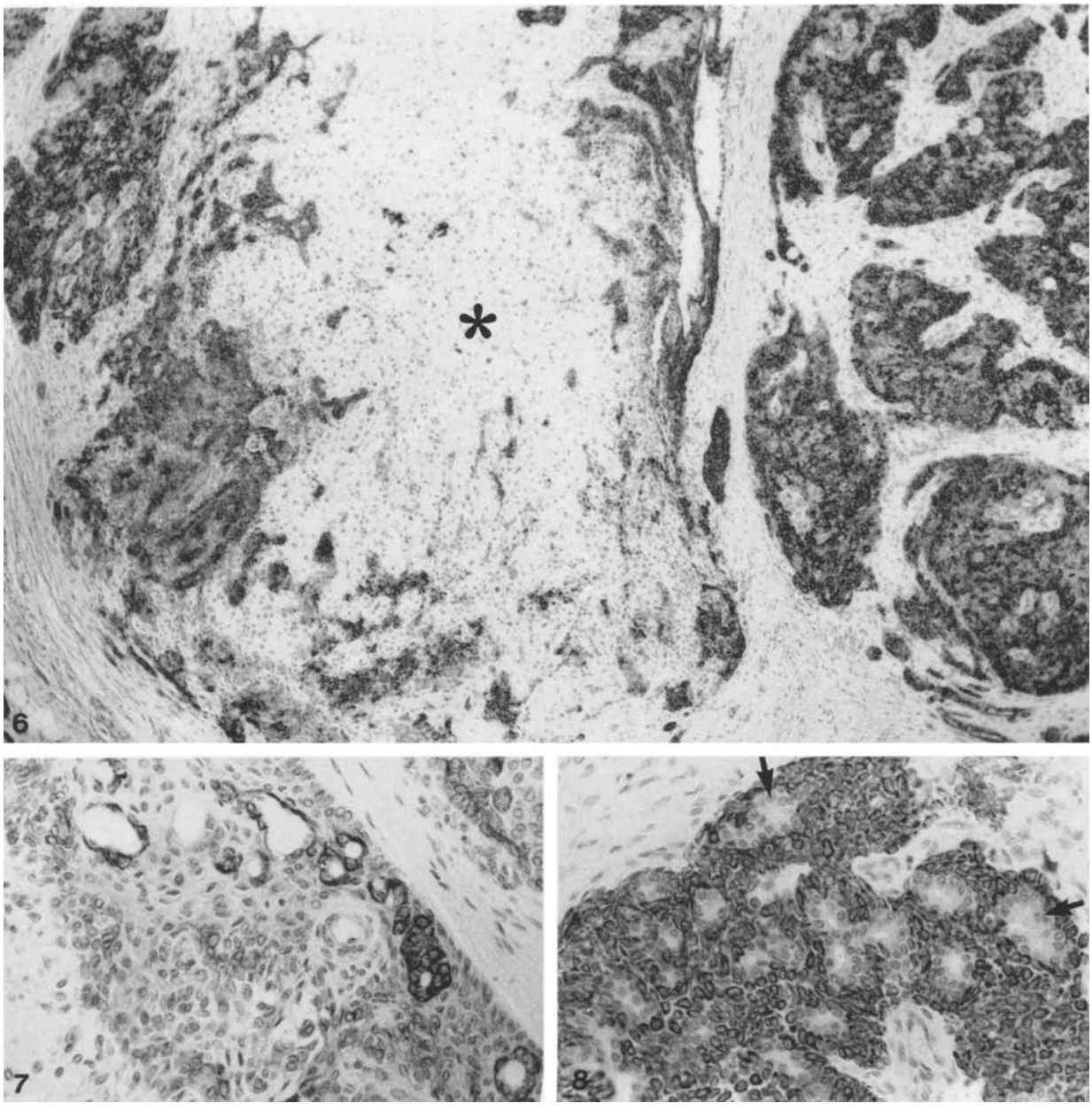

Fig. 6. Pleomorphic adenoma. Regional differences in cytokeratin 13, 16 expression are seen in this low power view of tumour nodules. The myxoid area (*asterisk*) is generally negative, while the more cellular areas show cell to cell variability. (Immunoperoxidase, monoclonal antibody 8.12, ×190)

Fig. 7. Pleomorphic adenoma. Duct luminal cell differentiation in this case is associated with expression of CKs 7, 8, 17, 18 while adjacent solid and myxoid cells are negative. (Immunoperoxidase, monoclonal antibody PKK1, ×600)

Fig. 8. Pleomorphic adenoma. Cells lining duct structures (*arrows*) are negative for cytokeratin 14 while those immediately peripheral to the luminal cells and in the solid and interface areas stain positively. Myxoid cells at the edge are negative. (Immunoperoxidase, monoclonal antibody 312C8-1, ×600)

mour cells peripheral to duct lining cells share with cells of other morphologic areas a variable, or in some tumours consistent, pattern of reactivity to these two non-epithelial intermediate filaments (Table 3).

Myxoid cells reproduce the pattern of cytokeratin expression of myoepithelial cells less faithfully than those tumour cells in the solid areas, while chondroid cells are almost uniformly devoid of CK (Table 3). Myxoid and chondroid cells stain

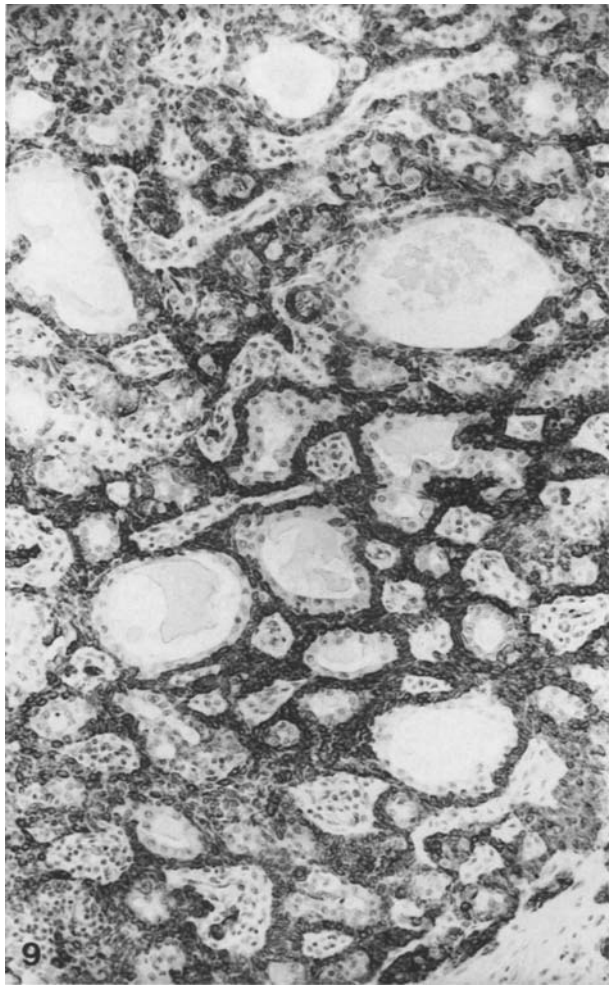

Fig. 9. Pleomorphic adenoma. Pattern of CKs 13, 16 expression is similar to that of cytokeratin 14 in this tumour (same case as Fig. 6). (Immunoperoxidase, monoclonal antibody 8.12, ×240)

strongly, although again inconsistently, with vimentin and GFAP.

Discussion

The discovery of the group of cytoskeletal proteins collectively called intermediate filaments and their ability to be identified immunologically and biochemically has resulted in the cataloging of characteristic intermediate filament profiles for virtually all normal cells (Moll et al. 1982; Osborn and Weber 1983; Quinlan et al. 1985; Ramaekers et al. 1983). These profiles are especially diverse in the epithelial tissues where the cytokeratin (CK) intermediate filaments can be divided into 19 different acidic or basic subtypes on the basis of size and isoelectric point. Monoclonal and polyclonal antibodies have been raised which recognize one or more of these subtype CKs, enabling researchers to characterize the different patterns of CK expression in epithelia and in tumours derived from these cells (Moll et al. 1982; Quinlan et al. 1985).

In the salivary glands these patterns of CK expression are particularly complex because of the numerous different types of specialized epithelial cells. Previous studies have shown that broadly reactive polyclonal antisera to cytokeratins stained myoepithelial cells and luminal cells of intercalated, striated and excretory ducts (Caselitz et al. 1981; Kahn et al. 1985; Toto and Hsu 1985). The use of monoclonal antibodies and unfixed frozen section material has further refined the CK profile of the constituent normal salivary gland cells (Geiger et al. 1987). This study showed expression of CK 18 in all epithelial cell types including acinar cells while CK 19 was found in ducts but not acini. Myoepithelial cells of acini and basal cells of ducts were also found to react with antibodies to CKs 4, 5, and 6 while only the basal cells expressed CKs 13 and 16. Unfortunately the immunofluorescence method utilized made precise recognition of specific cell types difficult, especially those of the intercalated duct. Other studies have found variable expression of vimentin in myoepithelial cells (Kahn et al. 1985) while glial fibrillary acidic protein (GFAP) was negative in normal salivary glands (Nakazato et al. 1985).

One of the major problems experienced in assessing intermediate filament expression is the loss of reactivity due to formalin fixation (Cooper et al. 1985). This has been resolved in our study by fixing all salivary gland tumours in a modified methacarn fixative (methanol/chloroform/acetic acid), which has been shown to give superior reactivity for a variety of cytoplasmic antigens including the CKs (Mitchell et al. 1985). This fixative has been used successfully on salivary glands and their tumours to demonstrate that basal cells of striated and excretory ducts but not acinar, myoepithelial cells or intercalated ducts express a CK defined by a monoclonal antibody, CK 16a (Palmer et al. 1985). Using the modified methacarn fixative and the inherently better morphological definition possible with paraffin embedded rather than frozen sections, we have extended these findings using a panel of antibodies to intermediate filaments, including a range of CK subtypes.

The patterns of intermediate filament expression in normal parotid glands are summarized in Table 2 and illustrated diagramatically in Fig. 1. In common with the study of Geiger et al. (1987), we find CK 19 expression to be present in luminal cells at all levels of the salivary ducts but also note

it in most myoepithelial cells of acini and ducts. Similarly, antibodies reactive with CKs 13 and 16 (our 8.12, and the KS 8.58 antibody of Geiger et al. (1987)) appear to separate the population of basal cells in striated and excretory ducts (positive) from the myoepithelial cells of acini (negative). There are, however, a group of basal cells in the striated/excretory ducts that, in common with acinar myoepithelia cells, strongly express CK 14, as shown by reactivity with 312C8-1. This monoclonal antibody, raised against a ductal adenocarcinoma of breast, has been shown to react strongly with breast (Dairkee et al. 1985) and salivary gland (Dardick et al. 1987) myoepithelium. This reactivity of myoepithelial cells contrasts with the absence of staining in the intercalated, striated, and excretory duct luminal cells. Expression of intermediate filaments other than CK is generally not found in normal salivary glands. Although other investigators have demonstrated CK 18 in acinar cells using frozen sections (Caselitz et al. 1986; Geiger et al. 1987) we could not see convincing staining of these cells in this study. This may reflect a small loss of antigenity for CK 18 due to fixation which puts these cells below the threshold for detection. Variable expression of vimentin has been noted in myoepithelial cells in another study (Kahn et al. 1985), but we find this in only one case. Glial fibrillary acidic protein (GFAP) is not seen in any normal parotids.

One point of difficulty found in this study was that of assessing reactivity of an antibody in the minor basal cell population when the predominant duct luminal cells were also strongly positive. This was the case for ECK, PKK1, 4.62 and to a lesser extent 8.60. We scored both basal and luminal cell populations as positive of there were no gaps in the reactive cells along the outer aspects of the ducts. Thus the larger ducts are generally consistent in their expression of CKs 3 and 6 (ECK) and CKs 7, 8, 17 and 18 (PKK1) although further dissection within these groupings is not possible. Variability of intermediate filament expression in similar cell types of normal parotid is not as prominent as in the pleomorphic adenomas. However, we did note some examples of this, particularly in luminal cells of striated ducts. Such variability has been noted previously in myoepithelial and basal cells of striated and excretory ducts, using both immunologic and ultrastructural methods of detection (Dardick et al. 1987).

Studies of pleomorphic adenomas have tended to focus on relating similarities of light microscopic or ultrastructural features or antigenic characteristics of these tumours to normal salivary glands in an effort to determine their histogenesis (Caselitz et al. 1981; Dardick et al. 1982, 1983; Erlandson et al. 1984; Kahn et al. 1985; Lam 1985; Palmer et al. 1985; Regezi and Batsakis 1977; Toto and Hsu 1985). The emerging consensus from these studies is that a considerable proportion of the neoplastic cells of pleomorphic adenomas share many features with myoepithelial cells that have undergone varying degrees of structural modification. However, neoplastic ductal and acinar cells are also differentiated, and the organization of the ductal and modified myoepithelial cells reflects that of the normal salivary gland (Dardick and van Nostrand 1987; Erlandson et al. 1984). The modified myoepithelial cells may display cytoplasmic actin microfilaments (Erlandson et al. 1984) and remnants of basal lamina (Dardick et al. 1983; Erlandson et al. 1984). Smooth muscle myosin, found consistently in normal MEC is rarely found in pleomorphic adenomas (Palmer et al. 1985).

Studies of intermediate filament expression and particularly CK profiles in cells from the varying morphological areas of pleomorphic adenomas have followed the emergence of specific antibodies. Caselitz et al. (1981) examined the pattern of reactivity in normal parotid and pleomorphic adenomas using polyclonal antibodies to prekeratin, vimentin and desmin. They found prekeratin in duct-type and myxoid cells in the tumours, while vimentin was found in cells "similar in morphology to the prekeratin positive cells", suggesting dual expression of these filaments. Combined expression of vimentin and CK in the tumor cells of pleomorphic adenomas has since been confirmed (Krepler et al. 1982; Erlandson et al. 1984). Using a similar polyclonal CK antibody, others have found preferential expression of CK in cells showing duct differentiation and in periductal cell groupings with only weak CK expression in myxoid and chondroid areas (Erlandson et al. 1984). This study, however, hints at some variability in the staining pattern of the tumours. Another study, using mainly formalin-fixed material, found pleomorphic adenomas to have patterns of vimentin and Mallory body CK expression similar to normal myoepithelial cells except in the areas of duct differentiation (Kahn et al. 1985). Some variability in expression of CK 16a has been noted in the solid areas of pleomorphic adenomas, with most cells being negative (Palmer et al. 1985).

Our findings, shown in Table 3 and illustrated schematically in Fig. 5, extend these studies and highlight a variability of intermediate filament expression not emphasized previously. As we have illustrated, this instability of phenotype can be seen

either as overall differences in expression of certain CK subtypes between different tumours or as regional or even cell to cell variability within the same tumour. Certain patterns of expression, however, are discernible although exceptions are present in almost every instance. As noted previously by others (Kahn et al. 1985) in terms of cytokeratin polypeptide content, the cells in the solid areas of pleomorphic adenomas resemble normal myoepithelial cells more so than the cells of other regions in these tumours. Differentiation toward myxoid or chondroid features is associated with a general loss of CK expression and more pronounced expression of the mesenchymal and glial markers, vimentin and GFAP respectively, neither of which are found with any regularity in normal parotid glands. Similarly, the pattern of CK expression in the few areas where definite duct type dfifferentiation is present reflect a profile paralleling those of normal duct luminal cells rather than of normal myoepithelial cells.

The conclusions we have drawn from these findings are that histogenetic models of pleomorphic adenomas will be incomplete if they fail to reflect the expression of divergent differentiation seen in these tumours. As noted previously by one of us (Dardick et al. 1983; Dardick and van Nostrand 1987), from a morphogenetic standpoint the pleomorphic adenoma can be seen as a tumour reflecting the cellular differentiation potential of the entire ductal-acinar unit. Cytokeratin expression reflects these differentiation states but does not necessarily imply a specific histogenesis. Others have noted that synthesis of some CKs in cell culture can be modulated by growth and differentiated states (Cooper et al. 1985). Thus, the CK polypeptides are more correctly regarded as markers of differentiation pathways than of cell lineage. The considerable variability in the profile of intermediate filament expression in areas with a similar growth pattern, both within individual pleomorphic adenomas and between others, is a prime example of this biological tenet.

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References

Batsakis JG, Kraemer B, Sciubba JJ (1983) The pathology of head and neck tumors: the myoepithelial cell and its participation in salivary gland neoplasia. Head Neck Surg 5:222–233

Caselitz J, Osborn M, Seifert G, Weber K (1981) Intermediate sized filament proteins (prekeratin, vimentin, desmin) in the normal parotid gland and parotid gland tumors: Immunofluorescence study. Virchows Arch [Pathol Anat] 393:273–286

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

Cooper D, Schermer A, Sun T-T (1985) Classification of human epithelia and their neoplasms using monoclonal antibodies to keratins: Strategies, applications, and limitations. Lab Invest 52:243–256

Dairkee SH, Blayney C, Smith HS, Hackett AJ (1985) Monoclonal antibody that defines human myoepithelium. Proc Natl Acad Sci USA 82:7409–7413

Dardick I, Nostrand van AWP, Phillips MJ (1982) Histogenesis of salivary gland pleomorphic adenoma (mixed tumour) with an evaluation of the role of the myoepithelial cell. Human Pathol 13:62–75

Dardick I, Nostrand van AWP, Jeans MTD, Rippstein P, Edwards V (1983) Pleomorphic adenoma: I. Ultrastructural organization of "epithelial" regions. II. Ultrastructural organization of "stromal" regions. Human Pathol 14:780–809

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

Dardick I, Nostrand van AWP (1985) Myoepithelial cells in salivary gland tumors – revisited. Head Neck Surg 7:395–408

Dardick I, Nostrand van AWP (1987) Morphogenesis of salivary gland tumors. A prerequisite to improving classification. Pathol Annu 22 (pt 1) 1–53

Dardick I, Claude A, Parks WR, Hoppe D, Stinson J, Burns BF, Little J, Brown DL, Dairkee SH (1988) Warthin's tumor: An ultrastructural, immunohistochemical, and fluorescent study of basilar epithelium. Ultrastruct Pathol (In press)

Erlandson RA, Cardon-Cardo C, Higgins PJ (1984) Histogenesis of benign pleomorphic adenoma (mixed tumor) of the major salivary glands. An ultrastructural and immunohistochemical study. Am J Surg Pathol 8:803–820

Eversole LR (1971) Histogenetic classifications of salivary gland tumors. Arch Pathol 92:433–443

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

Kahn HJ, Baumal R, Marks A, Dardick I, Nostrand van AWP (1985) Myoepithelial cells in salivary gland tumors. An immunohistochemical study. Arch Pathol Lab Med 109:190–195

Krepler E, Denk H, Artlieb U, Moll R (1982) Immunohistochemistry of intermediate filament proteins present in pleomorphic adenomas of the human parotid gland: Characterization of different cell types in the same tumor. Differentiation 21:191–199

Lam RMY (1985) An electron microscopic histochemical study of the histogenesis of major salivary gland pleomorphic adenoma. Ultrastruct Pathol 8:207–223

Mitchell D, Ibraham S, Gusterson B (1985) Improved immunohistochemical localization of tissue antigens using modified methacarn fixation. J Histochem Cytochem 33:491–495

Moll R, Franke WW, Schiller DL, Geiger B, Krepler R (1982) The catalog of human cytokeratins: patterns of expression

in normal epithelia, tumours and cultured cells. Cell 31:11–24

Nakazato Y, Ishida Y, Takahashi K, Suzuki K (1985) Immunohistochemical distribution of S-100 protein and glial fibrillary acidic protein in normal and neoplastic salivary glands. Virchows Arch [Pathol Anat] 405:299–310

Osborn M, Weber K (1983) Tumor diagnosis by intermediate filament typing: A novel tool for surgical pathology. Lab Invest 48:372–394

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

Quinlan RA, Schiller DL, Hatzfeld M, Achtstatter T, Moll R, Jorcano JL, Magin TM, Franke WW (1985) Patterns of expression and organization of cytokeratin intermediate filaments. Ann N Y Acad Sci 455:282–306

Ramaekers FCS, Puto JJG, Moesker O, Kant A, Huysmans A, Haag D, Jap PHK, Herman CJ, Vooijs GP (1983) Antibodies to intermediate filament proteins in the immunohistochemical identification of human tumours: an overview. Histochem J 15:691–713

Regezi JA, Batsakis JG (1977) Histogenesis of salivary gland neoplasms. Otolaryngol Clin North Am 10:297–307

Toto PD, Hsu DJ (1985) Product definition of pleomorphic adenoma of minor salivary glands. J Oral Pathol 14:818–832

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