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Differential diagnosis of pleomorphic adenoma by immunohistochemical means

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Abstract

Immunohistochemical study of major salivary gland tumours was performed on 60 pleomorphic adenomas, five basal cell adenomas and 10 adenoid cystic carcinomas to determine the diagnostic value of each antigen. Immunoreactivity examined were intermediate filaments (keratin, vimentin, desmin and glial fibrillary acidic protein [GFAP]) and related substances (actin, S-100 protein and secretory component). In pleomorphic adenomas, there was positive immunoreactivity for GFAP which was not observed in normal tissue or other neoplastic tissues. Immunoreactivity of GFAP was closely related to myxomatous and early chondromatous differentiation in pleomorphic adenoma. It is considered that GFAP immunoreactivity should be assessed in the occasional differential diagnostic dilemma of pleomorphic adenoma *versus* adenoid cystic carcinoma and basal cell adenoma, because of its ability to show potential and definite myxochondromatous differentiation.

Introduction

Although a great number of immunohistochemical studies of major salivary gland tumours have been published, their clinical and diagnostic implications are not fully apparent. We have performed basic immunohistochemical investigations of pleomorphic adenoma, basal cell adenoma and adenoid cystic carcinoma to determine the diagnostic value of intermediate filaments (keratin, vimentin, desmin and glial fibrillary acidic protein [GFAP]) and related substances (S-100 protein, actin and secretory component).

Materials and methods

Paraffin embedded tissue blocks from 75 primary major salivary gland tumours were collected from the files of Kanazawa University Hospital and Toyama Central Prefectural Hospital between 1980 and 1989. The tumours consisted of 60 pleomorphic adenomas (parotid gland, 48; submandibular gland, 12), 5 basal cell adenomas (parotid gland, 5), 10 adenoid cystic carcinomas (parotid gland, 4; submandibular gland, 6). The diagnostic criteria were based on World Health Organization (WHO) classification (Thackray and Sobin, 1972).

Sections were cut at three micrometer intervals from paraffin blocks of tumour tissue fixed with 10 per cent formalin, and immunohistochemical study (Hsu *et al.*, 1981) was performed. The series of primary antibodies and their working dilutions were as follows: anti-keratin monoclonal antibody which reacts with keratin polypeptides of 56 kilodaltons (KL-1; Immunotech, Luminy-Marseille, France), 1,200; anti-keratin monoclonal antibody which reacts to cytokeratins 8 and 18 according to Moll's (1982) classifications (CAM 5, 2; Becton Dickinson, Mountain View, California), 1:50; anti-vimentin monoclonal antibody (V9; Dakopatts, Denmark), 1:50; anti-desmin polyclonal antibody (Bioscience, Switzerland), 1:500; anti-glial fibrillary acidic protein (GFAP) polyclonal antibody (Dakopatts, Denmark), 1:500; anti-S-100 protein polyclonal antibody (Dakopatts, Denmark), 1:1,000; anti-muscle actin monoclonal antibody (HHF 35; Enzo Diagnostics, New York); and anti-secretory component (S.C.) polyclonal antibody (Dakopatts, Denmark), 1:500.

After dewaxing and hydration, the sections were treated with 0.3 per cent hydrogen peroxide in absolute methanol for 30 minutes at room temperature to block endogenous peroxidase activity. After they were immersed in phosphate buffered saline (PBS, pH 7.4) and treated with 10 per cent normal goat or horse serum for about 10 minutes to reduce background staining, they were incubated with primary antibody overnight at 4°C. After washing with PBS three times, the sections were incubated with biotinylated horse anti-mouse antibody (Vector Laboratories, Burlingame, California) for monoclonal primary antibody or biotinylated goat anti-rabbit antibody (Vector Laboratories) for polyclonal primary antibody at dilutions of 1:200 for 30 minutes at room temperature. After washing three times with PBS, they were treated with biotin-streptavidin-peroxidase complex (Dako Corporation, Santa Barbara, California) for 30 minutes at room temperature. After a further

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TABLE I
IMMUNOREACTIVITY OF PLEOMORPHIC ADENOMA

Site	KL-1	CAM 5.2	Vim.	Des.	S-100	GFAP	S.C.	Actin
I.T.C.	93%	88%	0%	0%	8.3%	0%	93%	0%
(n=60)	(+++)	(+++)	(−)	(−)	(+)	(−)	(+++)	(−)
O.T.C.	75%	75%	73%	0%	95%	63%	12%	67%
(n=60)	(+)	(+)	(++)	(−)	(++)	(+)	(+)	(+)
S.E.C.	89%	11%	0%	0%	0%	0%	0%	0%+
(n=45)	(+++)	(+)	(−)	(−)	(−)	(−)	(−)	(−)
Hyaline	42%	58%	84%	0%	90%	58%	5.3%	0%
(n=19)	(+)	(+)	(++)	(−)	(++)	(+)	(+)	(−)
Leiomyo.	6.9%	10%	74%	0%	83%	53%	0%	55%
(n58)	(+)	(+)	(++)	(−)	(++)	(+)	(−)	(+)
Myxo.	0%	17%	90%	0%	100%	100%	0%	45%
(n=60)	(−)	(+)	(+++)	(−)	(+++)	(+++)	(−)	(+)
Chond.	0%	0%	92%	0%	100%	70%	0%	0%
(n=39)	(−)	(−)	(+++)	(−)	(+++)	(++)	(−)	(−)

Vim., vimentin; Des., desmin; S.C., secretory component; I.T.C., inner tubular cells; O.T.C., outer tubular cells; S.E.C., squamous epithelial cells; Hyaline, hyaline cells; Leiomyo., leiomyomatous cells; Myxo., myxomatous cells; Chond., mature and immature chondroid cells.
%, number of positive case/total number of cases × 100. (−), negative immunoreactivity; (+), mean score is ranged under 1.0; (++), mean score is ranged from 1.0 under 2.0; (+++), mean score is ranged from 2.0 to 3.0.

TABLE II
IMMUNOREACTIVITY OF BASAL CELL ADENOMA

Site	KL-1	CAM 5.2	Vim.	Des.	S-100	GFAP	S.C.	Actin
Basal	0%	0%	100%	0%	20%	0%	0%	60%
(n=5)	(−)	(−)	(++)	(−)	(+)	(−)	(−)	(+)
I.T.C.	100%	20%	0%	0%	0%	0%	100%	0%
(n=5)	(+++)	(+)	(−)	(−)	(−)	(−)	(+++)	(−)
Trans.	100%	0%	60%	0%	100%	0%	0%	0%
(n=5)	(++)	(−)	(+)	(−)	(++)	(−)	(−)	(−)

%, (−), (+), (++), (+++), Vim., Des., S.C., same as Table I; Basal, basal cell located in periphery of cell nest and outer tubular cell; I.T.C., inner tubular cell; Trans., transitional cell.

TABLE III
IMMUNOREACTIVITY OF ADENOID CYSTIC CARCINOMA

Site	KL-1	CAM 5.2	Vim.	Des.	S-100	GFAP	S.C.	Actin
I.T.C.	90%	30%	0%	0%	0%	0%	90%	0%
(n=10)	(+++)	(+)	(−)	(−)	(−)	(−)	(++)	(−)
O.T.C.	10%	0%	20%	0%	0%	0%	0%	20%
(n=10)	(+)	(−)	(+)	(−)	(−)	(−)	(−)	(+)
Cyst	0%	0%	30%	0%	0%	0%	0%	40%
(n=10)	(−)	(−)	(+)	(−)	(−)	(−)	(−)	(+)
Indiff.	60%	20%	50%	0%	60%	0%	10%	60%
(n=10)	(+)	(+)	(+)	(−)	(+)	(−)	(+)	(+)

Vim., Des., S.C., %, (−), (+), (++), (+++), same as Table I; I.T.C., inner tubular cell; O.T.C., outer tubular cell; Cyst, cyst lining cell; Indiff., indifferent cell.

washing, they were incubated in diaminobenzidine with 0.005 per cent hydrogen peroxide in Tris buffer (pH 7.6), and then counterstained with Mayer's haematoxylin.

Normal salivary gland tissues without obvious atrophy and inflammation around tumours in 20 cases (parotid gland, 15; submandibular gland, 5) were used as control sections.

The sections were examined under light microscope. Total number of cells forming specific histological structures (Thackray and Lucas, 1974) and number of positively stained cells were counted at five points under x200 magnification. Then score (0, negative immunoreactivity; 1, positively stained cells were under 3 per cent; 2, positively stained cells were from 3 per cent under 50 per cent; 3 positively stained cells were over 50 per cent) was given.

Results

The results of immunohistochemical staining of tissues are summarized in Tables I, II and III. Some details are described below.

Normal salivary gland

Positive immunoreactivity for keratin of both KL-1

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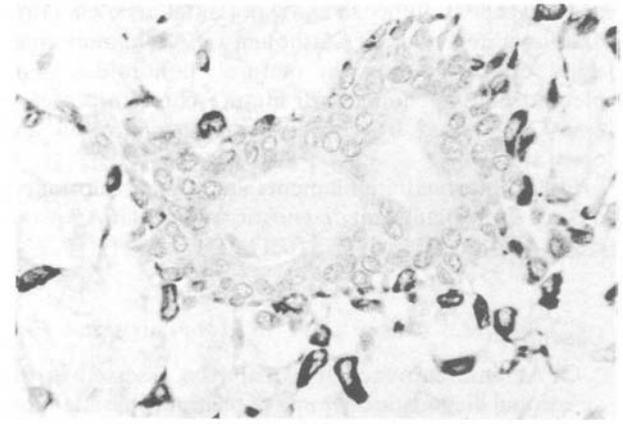

FIG. 1.
Outer tubular cells and their neighbouring epithelial cells in pleomorphic adenoma are positive for vimentin (ABC method, counterstained with hematoxylin, X340).

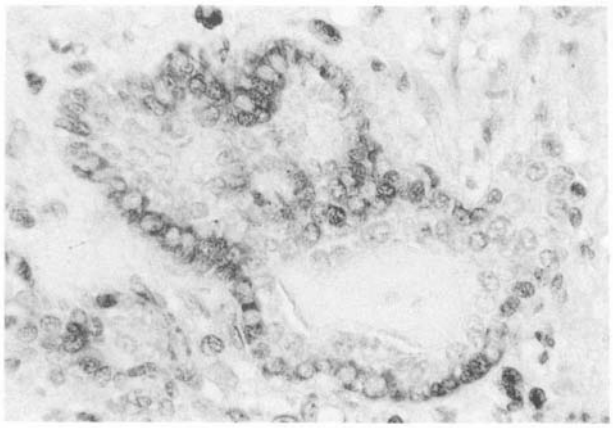

FIG. 2.
S-100 protein immunoreactivity in epithelial region of pleomorphic adenoma is almost same as vimentin except for a few inner tublar cells are positive for S-100 protein (ABC method, counterstained with hematoxylin, X340).

and CAM 5,2 was present in ductal epithelial cells from the intercalated ducts to the interlobular ducts. Spindle-shaped myoepithelial cells were strongly positive for actin; these were located basally around acini, intercalated ducts and distal striated ducts. Ductal epithelial cells were also positive for secretory component. Vimentin, desmin, GFAP and S-100 protein were negative in normal glands.

Pleomorphic adenomas

These tumours contained various types of cells in their epithelial and mesenchymal components. The proportion of epithelial and mesenchymal regions varied between tumours. All tumours had epithelial and myxomatous areas, and 65 per cent (39 cases) of tumours had an area of chondroid differentiation.

Inner tubular cells and squamous epithelial cells were intensely positive for keratin of KL-1. Ductal and the other epithelial cells and myxomatous cells were positive for keratin of CAM 5,2. Vimentin immunoreactivity was present in almost all tumour cells of epithelial or mesenchymal differentiation, except for inner tubular cells and squamous epithelial cells (Fig. 1). The immunoreactivity of S-100 protein was almost the same as that of vimentin, although a few inner tubular cells were positive for S-100 protein (Fig. 2). GFAP also showed the same immunoreactivity as vimentin and S-100 protein (Fig. 3), but GFAP immunoreactivity was significantly reduced in the mature chondroid cells with lacunar formation present in mature chondromatous tissue (Fig. 4) where vimentin (Fig. 5) and S-100 protein immunoreactivity was preserved.

Actin was positive in some of the outer tubular cells, and leiomyomatous cells in the epithelial regions, and S.C. was positive in inner tubular cells. Desmin-positive tumour cells were not observed.

Basal cell adenoma and adenoid cystic carcinoma

In basal cell adenomas (basal type, 3; tubular type, 1; trabecular type, 1) and adenoid cystic carcinoma (tubular type, 3; cribriform type, 5; solid type, 2), actin-positive spindle shaped cells were observed in relation to tubular structures, as in the pleomorphic adenomas. In both tumour types, all tumour cells were positive for keratin of both KL-1 and CAM 5,2. Some tumour cells in nests were also positive for vimentin. Although S-100-

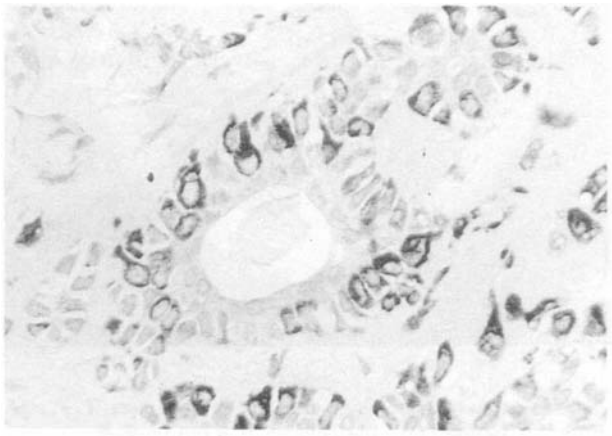

FIG. 3.
GFAP immunoreactivity in epithelial region of pleomorphic adenoma is similar to vimentin (ABC method, counterstained with hematoxylin, X340).

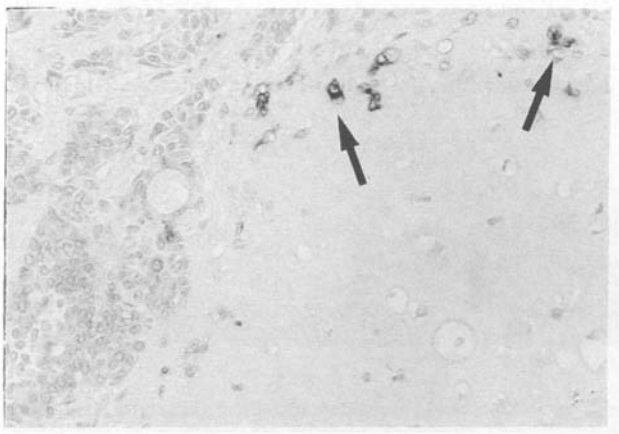

FIG. 4.
Although peripheral immature chondroid cells in mature chondroid tissue are positive for GFAP (arrows), mature chondrocytes are negative in pleomorphic adenoma (ABC method, counterstained with hematoxylin, X170).

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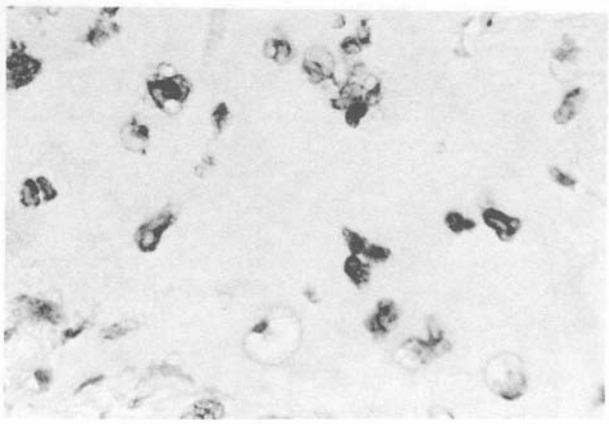

FIG. 5.
Mature and immature chondroid cells in pleomorphic adenoma are positive for vimentin (ABC method, counterstained with hematoxylin, X340).

positive cells were observed in tumour cell nests, GFAP and desmin immunoreactivity was absent in both tumour types.

Discussion

This study demonstrates that GFAP immunoreactivity in salivary gland tumour is closely related to myxomatous and early chondromatous differentiation in pleomorphic adenoma. Since its heterotopic expression in pleomorphic adenoma was reported first (Nakazato *et al.*, 1982), its morphological implication had been debated. Recently, GFAP immunoreactivity in pleomorphic adenoma showed early chondroid differentiation was confirmed by an immuno-electron microscopic procedure (Anderson *et al.*, 1990). In this study, it is disclosed that even outer tubular and other epithelial cells have potential mesenchymal (myxomatous and early chondromatous) differentiation by immunohistochemical procedure.

Occasionally, differential diagnosis between pleomorphic adenoma with scant myxochondromatous area and well differentiated adenoid cystic carcinoma with predominant tubular formations give a challenge to even experienced pathologists (Thackray and Sobin, 1972). In such a case, immunohistochemical examination of GFAP immunoreactivity may give a great help, because of its excellent ability to show potential myxochondromatous differentiation. Although GFAP immunoreactivity does not exist in mature chondroid region, pleomorphic adenoma with mature chondroid area is easily diagnosed by conventional haematoxylin and eosin stain.

Other intermediate filaments and related substances did not show significant diagnostic value as GFAP in our study.

Conclusion

GFAP immunoreactivity should be assessed in the occasional diagnostic dilemma of pleomorphic adenoma versus adenoid cystic carcinoma and basal cell adenoma, because of its excellent ability to show potential and definite myxochondromatous differentiation.

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