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INTERMEDIATE FILAMENT AND CARCINOEMBRYONIC ANTIGEN EXPRESSION IN SALIVARY GLAND PLEOMORPHIC ADENOMA

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ABSTRACT: In recent years immunohistochemical studies have become of relevance in the discovery of significant antigenic structures in human cells. Filamentous protein structures with a diameter between that of microtubules and microfilaments have recently been described with the name of intermediate filaments. These structures play an important role in certain cellular functions such as cytokinesis and chromosome movements during cell division. The immunohistochemical expression of these filaments in tumoral disease is identical to that seen in normal cells of the type the tumor arises in. We describe the expression of some of these filaments and of carcinoembryonic antigen in an attempt to clarify the complex origin of pleomorphic adenoma of the salivary glands.

Key words: Intermediate filaments, carcinoembryonic antigen, pleomorphic adenoma, salivary glands

INTRODUCTION

Despite the frequency with which pleomorphic adenomas (PA) occur in the oral cavity, the origin of this type of tumor remains obscure. The so-called mesenchymal component (chondroid, myxoid and cartilaginous tissue) has been the most common target of controversy in studies of the origin of this neoplasm. The term "mixed tumor," first used in 1859, was meant to underline the presence of both epithelial and mesenchymal components. However, histological, ultrastructural and immunohistochemical studies have led most modern researchers to favor an epithelial, or more specifically, a myoepithelial origin (1-7) — hence the term "mixed tumor," coined by Willis (8). A few workers still advocate a truly mixed origin for these tumors (9,10).

So as to better characterize the distribution of metaplasic epithelial ductal and myoepithelial cells in the mesenchymal component of PA, immunohistochemical techniques with highly speci-

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fic monoclonal antibodies (MAb) have recently been developed. The MAb are used mainly to detect the presence and distribution of tissular antigens. Of these latter, the intermediate filaments (IF) have aroused growing interest in recent years.

Intermediate filaments are protein structures which form part of the cytoskeleton. Measuring approximately 8-10 nm in diameter, these filaments, as their name implies, are intermediate in size between microfilaments and microtubules. Five types of IF have been identified to date:

1. Cytokeratins are a complex group comprising 19 polypeptides ranging in molecular weight from 40 to 70 kDa (11). These filaments are found mainly in epithelial cells. Of considerable significance is the fact that they show identical patterns of distribution patterns on epithelial tumors and on normal tissues from which these tumors are derived (12,13).

2. Vimentin is an IF made up of a simple 58 kDa polypeptide subunit, and is found in a wide range of mesenchymal cells in vivo and in some cell lines in vitro (14,15).

3. Desmin is a filamentous 52 kDa protein characteristic of muscle cells (16,17).

4. Glial fibrillary acidic protein (GFAP), with a molecular weight of 51 kDa (18-21), appears to be specific to the central nervous system, but has also been detected in the peripheral nervous system (22).

5. Neurofilaments are a group of three IF (molecular weight 210, 160 and 70 kDa) located in neurons of both the CNS and the peripheral nervous system (23).

Carcinoembryonic antigen (CEA) is an oncofetal antigen of glycoproteic nature, with a molecular weight of 200 kDa. This antigen is apparently related to the glandular function of tumoral cells (24).

We set out to determine the immunohistochemical distribution of IF and CEA in pleomorphic adenomas of the salivary glands, in an attempt to elucidate the complex origin of these tumors.

MATERIALS AND METHODS

A total of 9 specimens of PA from the parotid, submaxilary and sublingual glands were paraffin embedded and sectioned. Control sections were stained with hematoxylin and eosin, and sections for immunohistochemical analysis of FI and CEA were processed for peroxidase-antiperoxidase staining. Deparaffinized sections were quenched either for 30 min in 0.3% hydrogen peroxide in PBS, or for 15 min in 1% periodic acid, to remove endogenous peroxidase activity. The sections were then treated with either 0.0025% pronase in Tris buffer (pH 7.6) for 4 min, or with pepsin in HCI buffer (pH 2.5) for 30 min. The tissue sections were washed several times in PBS, and then incubated with the appropriate suppressor serum for 20 min. The suppressor serum was drained off and the sectins incubated with 50 μl of MAb (AE1-3 cytokeratins) or polysera (prekeratin and vimentin — DAKO, and S-100 protein) overnight at 4°C. Tissue sections were washed several times in PBS and then rinsed with 0.05 *M* Tris buffer, 0.1 *M* NaCl, at pH 8. For the final reaction, 5 mg diaminobenzidine (DAB) tetrahydrochloride was dissolved in 10 ml Tris buffer and 100 μl 0.3% hydrogen peroxide was added. The DAB solution was filtered and development of the peroxidase reaction performed immediately by incubating tissue sections for periods of 6-12 min. The DAB-treated sections were washed with distilled water, counterstained with hematoxylin, and mounted in permount.

RESULTS

The expression of cytokeratins by salivary gland PA is summarized in Table 1. This type of IF was expressed by cells from myxoid areas in 82% of all tumors analyzed, although staining was weak in 5 out of 9 cases. In contrast cells from epithelial areas as well as ductal cells were positive for cytokeratins in 100% of the tumors tested. Areas of squamous metaplasia were consistently negative for cytokeratins, while zones of cartilaginous metaplasia expressed this type of IF in 22% of the tumors.

Vimentin expression was seen in myxoid area cells in 55% of the tumors. Epithelial cells were weakly positive for this IF in a single case.

Ductal and periductal cells as well as areas of squamous metaplasia were negative, whereas vimentin was expressed by cartilaginous metaplastic cells in 66% of the PA studied.

Table 1. Expression of cytokeratins in pleomorphic adenoma of the salivary glands.

Case	ME	EA	DC	PC	SM	CM
1	+/-	+/-	+	-	NP	NP
2	+/-	+	+	-	-	-
3	+	+	+	-	NP	NP
4	-	+/-	+	-	NP	-
5	+	+/-	+	-	-	+
6	+	+/-	+	-	NP	NP
7	+/-	+/-	+	+/-	NP	+/-
8	+/-	+	+	+/-	NP	NP
9	+/-	+	+	+/-	-	-
%	82.2	100	100	33.3	0	22.2

ME, myxoid area; EA, epithelioid area; DC, ductal cells; PC, periductal cells; SM, squamous metaplasia; CM, cartilaginous metaplasia.
NP, not present.

Table 2. Expression of vimentin in pleomorphic adenoma of the salivary glands.

Case	ME	EA	DC	PC	SM	CM
1	-	-	-	-	NP	NP
2	-	+/-	-	-	-	-
3	+/-	-	-	-	NP	NP
4	-	-	-	-	NP	-
5	+/-	-	-	-	-	+
6	-	-	-	-	NP	NP
7	+	-	-	-	NP	+/-
8	+	-	-	-	NP	+
9	+	-	-	-	NP	+
%	55.5	11.1	0	0	0	66.6

ME, myxoid area; EA, epithelioid area; DC, ductal cells; PC, periductal cells; SM, squamous metaplasia; CM, cartilaginous metaplasia NP, not present.

As shown in Table 3, areas of squamous metaplasia expressed CEA in all tumors analyzed. On the other hand, all of the different cellular components of PA were negative for desmin antigen expression.

DISCUSSION

Our findings lead us to consider PA of the major salivary glands as a purely epithelial tumor. The positive expression of cytokeratins and vimentin by ductal and periductal cells as well as by those from epithelial areas support this conclusion. Pleomorphic adenoma may arise as a result of the metaplastic transformation of epithelial area cells (ductal or periductal). These metaplastic cells may in turn give rise to the myxoid zones and areas of squamous and chondroid metaplasia. In further support for this hypothesis, Welsh and Meyer (25) were unable to find evidence of a direct transformation of epithelial cells into mesenchymal cells.

Clearly, more extensive studies based on a

Table 3. Expression of carcinoembryonic antigen in pleomorphic adenoma of the salivary glands.

Case	MA	EA	DC	PC	SM	CM
1	-	-	-	-	NP	NP
2	-	-	-	-	+	-
3	-	-	-	-	NP	NP
4	-	-	-	-	NP	-
5	-	-	-	-	+	-
6	-	-	-	-	NP	NP
7	-	-	-	-	NP	-
8	-	-	-	-	NP	NP
9	-			-	NP	-
%	0	0	0	0	22	0

ME, myxoid area; EA, epithelioid area; DC, ductal cells; PC, periductal cells; SM, squamous metaplasia; CM, cartilaginous metaplasia.
NP, not present.

combination of microscopic and immunohistochemical techniques will be needed to determine with certainty the origin of pleomorphic adenoma in the salivary glands.

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