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IMMUNOHISTOCHEMICAL LOCATION OF S—100 AND GLIAL FIBRILLARY ACIDIC PROTEINS IN SALIVARY GLAND PLEOMORPHIC ADENOMAS

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INTRODUCTION

In recent years immunohistochemical studies have been aimed at tracing specific antigens with high--specificity. Knowledge of these antigens is of great benefit not only for the understanding of the normal ultrastructure of a.number of human tissues, but also to focus the diagnosis of many malignant diseases.

Like other parts of the body, the salivary glands have been subjected to numerous investigations designed to analyze the presesce and distribution of a wide range of antigens in normal and tumoral tissue (1,2). Recently, immunohistochemistry has been added to the list of methods used to investigated the immunohistochemical determination of the S-100 and glial fibrillar acid (GFAP) proteins (3).

Protein S-100 was isolated by MOORE from the bovine brain in 1965 (4). This low molecular weight acid protein is able to transport calcium, and is composed of two subunits, designated α and β. To date three different species of dimers have been isolated from mammalian brains: S-100 ao (α), S-100 a ($\alpha\beta$) and S-100 b($\beta\beta$) (5). These units have been found in a variety of cells and tissues, including neoplastic cells of the salivary glands (6-9).

Glial fibrillar acid protein, the largest of the glial filaments, is one of the five of intermediate filaments described thus far. This protein is found mainly in the astrocytes and ependymal cells of the brain (10), as well as in the glial cells of the rat myoenteral plexus (11). To date there is little published evidence for an extraneural distribution of GFAP (12).

This study analyzes the immunohistochemical distribution of these proteins in pleomorphic adenoma of the salivary glands, in an attempt to provide information on the complex genesis of this type of tumor.

MATERIALS AND METHODS

The tissue samples analyzed in this study were obtained from 9 patients with a pleomorphic adenomal affecting the parotid, submaxillary and sublingual glands. Three sections from each tissue specimen were used: one was stained with hematoxylin-eosin and used as control, and one each was used in immunohistochemical studies to detect S-100 protein and GFAP.

The immunohistochemical location of protein S-100 and FGAP were determined by the peroxidase (PAP) method. Anti-S-100 protein and anti-GFAP sera were used at dilutions of 1:1000, and the tissue sections were incubated overnight at 4°C. Anti-rabbit IgC and soluble PAP complex were added at dilutions of 1:20 followed by incu-

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bation for 1h at room temperature. Monospecific antibodies were used at a final concentration of 50μg/ml.

RESULTS

The data in Table 1 show that in 100% of the tumors, cells from the myxoid and from the epithelial areas (Fig. 1) expressed S-100 protein, although positivity was slight in one case. Eighty-two per cent of the tumors expressed S-100 on ductal cells, and periductal cells were positive in 55% of all tumors tested. It should be noted that although some tumors contained no periductal cells, this type of cell was consistently positive for S-100 protein in all neoplasm in which these cells were present.

Areas of squamous metaplasia, when present, were always negative for anti-S-100 antibody labelling, where as 80% of the tumors which showed cartilaginous differentiation expressed S-100 protein.

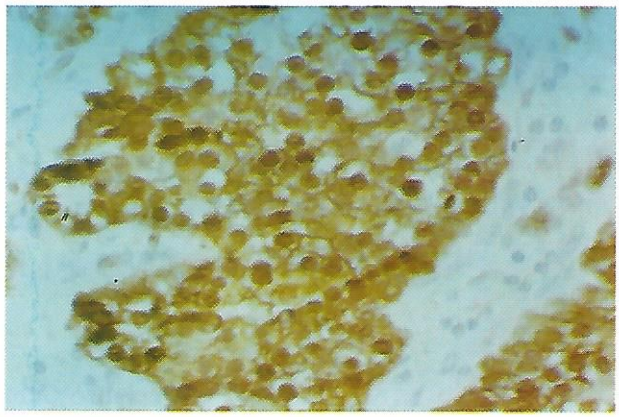

Fig. 1 — (Trabajo S-100 y GFAP)

Table 2 gives the results of anti-GFAP antibody labelling. In 100% of the tumors tested, from myxoid areas were positive for this antigen (Fig. 2), while the other cells as well as the areas of tumoral metaplasia failed to express this protein.

Table 1
Expression of S-100 protein 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	+	+	+/–	ND	NP	NP
7	+	+	+/–	+	–	–
8	+	+	+/–	+	NP	NP
9	+	+/–	–	+	NP	+
%	100	100	82	55	0	80

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

Table 2
Expression of glial fibrillary acidic protein in pleomorphic adenoma of the salivary glands

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

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

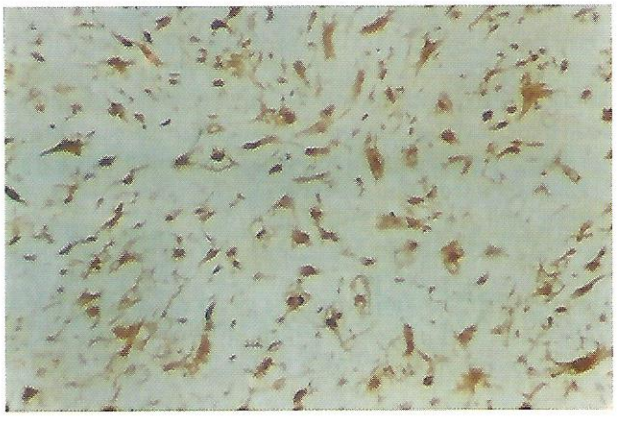

Fig. 2 — (Trabajo S-100 y GFAP)

DISCUSSION

Despite being the most frequent type of tumor in the salivary glands, the origin of pleomorphic adenomas has yet to be elucidated. Is it a neoformation derived from the metaplastic transformation of ductal cells, or do various types of cells give rise to this complex tumor?

Our results concur with those obtained by earlier investigators (13-15) with regard to the distribution of S-100 protein. However, normal salivary gland tissues does not stain for this protein; moreover, as mentioned above, S-100 protein has been frequently linked to neural structures. Hence our findings can be interpreted as support for any of three different hypotheses (16):

1. Pleomorphic adenoma may arise from neuroectodermal cells.

2. The cells comprising this tumor may not be related to neural tissues, although they are able to synthesize proteins specific to the nervous system as a result of multidirectional differentiation.

3. The S-100 protein, while not specific for the nervous system, may nevertheless be associated with it in some other as yet unknown manner.

We consider hypotheses 2 and 3 to be the more plausible, given the lack of evidence in support of a neuroectodermal origin for this tumor.

As with S-100 protein, no studies have reported the expression of FGAP by normal salivary gland tissue, a fact which suggests that the expression of this protein by pleomorphic adenomas results from its synthsis by tumoral cells. Moreover, a glial origin for this tumor is unlikely to explain GFAP antigen expression, given the lack of neural elements in this type of tumor (17,18).

ABSTRACT

The S-100 protein is a low molecular weight protein which has been isolated mainly in mammalian brain cells. Glial fibrillary acidic protein is one of the five types of intermediate filaments described to date. We used immunohistochemical methods to study the expression in this patterns of expression of these two proteins in pleomorphic adenoma of the salivary glands, in an attempt to explain their tumor and to elucidate the origin of this complex neoplasm of the salivary glands.

Key words: S-100 protein, glial fibrillary acid protein, pleomorphic adenoma, salivary glands.

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