Immunohistochemical Study of the Distribution of S-100 Protein in Pleomorphic Adenomas of Minor Salivary Glands

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(Received 10 July and accepted 1 October 1991)

Key words: pleomorphic adenoma, minor salivary glands, immunohistochemistry, S-100 protein

Abstract

Twelve pleomorphic adenomas of minor salivary gland origin were examined for the distribution of S-100 protein, detected using the peroxidase-antiperoxidase (PAP) method. Strong S-100 protein immunoreactivity was noted in areas containing plasmacytoid cells, stellate and spindle cells against a myxochondroid or hyalinous stroma, and solid epithelial areas. Tubular and duct-like structures showed variable stainability. Stromal tissue and normal salivary glands were generally negative for S-100 protein. These findings were compared with those reported elsewhere.

Introduction

S-100 protein is an acidic calcium-binding protein first isolated by Moore[1] in 1965, and so named because of its solubility in 100% ammonium sulfate solution at neutral pH[1–3]. Early studies suggested that this protein was nervous tissue-specific and produced by glial cells[1,4–6]. Immuno-crossreactivity of S-100 protein present in the brain of a wide variety of species, from humans to reptiles, indicates that this protein is not species-specific[7].

Biochemically, S-100 protein consists of two subfractions, S-100a (α, β subunits) and S-100b (β, β subunits). The α and β subunits are composed of 93 and 91 amino acids, respectively. The molecular weights of these two subfractions are 20,907 (S-100a) and 21,014 (S-100b)[8–10].

Up to now, clinical and diagnostic applications of S-100 protein have been confined mainly to tumors of nervous tissue origin. In recent years, studies of S-100 protein have been extended to human non-neurogenic tumors including malignant melanomas[11] and salivary gland tumors[12–14]. The aim of the present investigation was to study in detail the distribution of S-100 protein in pleomorphic adenomas of minor salivary glands and to compare these findings with those reported elsewhere.

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Materials and Methods

Twelve pleomorphic adenomas of minor salivary glands were studied. The clinical details of these selected cases are summarized in Table 1. Normal salivary gland tissue adjacent to these tumors, judged normal by light microscopic examination, was also studied.

**Light microscopy**

All samples were fixed in 10% formalin and sections 4 μm thick were prepared using standard laboratory techniques. The sections were stained with hematoxylin and eosin, alcian blue, periodic acid-Schiff and Southgate's mucicarmine, and studied routinely to determine the histopathology of the pleomorphic adenomas.

**Immunohistochemical method**

Immunohistochemical localization of S-100 protein was studied by Sternberger's peroxidase-antiperoxidase (PAP) method[15].

Deparaffinized sections were immersed in 0.3% methanolic H2O2 solution for 30 min to inactivate endogenous peroxidase, and were then rinsed well in 0.05 M Tris buffer and treated according to the following steps:

1. Immersion in normal swine serum (1:5 dilution) for 20 min
2. Reaction with rabbit anti-S-100 protein (1:200) for 30 min
3. Rinsing in Tris buffer 3 times for 10 min
4. Reaction with swine anti-rabbit immunoglobulin (1:40) for 30 min
5. Rinsing in Tris buffer 3 times for 10 min
6. Reaction with horseradish peroxidase rabbit antiperoxidase (PAP) (1:100) for 30 min
7. Rinsing in Tris buffer 3 times for 10 min
8. Immersion in 0.05 M Tris buffer containing 0.005% 3,3-diaminobenzidine tetrahydrochloride (DAB) with 0.03% H2O2 solution for 15 min
9. Rinsing in water, counterstaining with hematoxylin, dehydration and

<table>
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<th>Case no</th>
<th>Age</th>
<th>Sex</th>
<th>Race</th>
<th>Onset</th>
<th>Site</th>
<th>Clinical Presentation</th>
<th>Treatment</th>
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<td>M</td>
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<td>palate</td>
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<td>enucleation</td>
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<td>mobile lump</td>
<td>enucleation</td>
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<td>18</td>
<td>F</td>
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<td>1yr</td>
<td>(R) buccal sulcus</td>
<td>firm mass</td>
<td>excision</td>
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<td>M</td>
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<td>20</td>
<td>M</td>
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<td>1mth</td>
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<tr>
<td>6</td>
<td>26</td>
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<td>4yr</td>
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<tr>
<td>7</td>
<td>52</td>
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<tr>
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<td>3yr</td>
<td>(l) cheek</td>
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According to specifications supplied by Dakopatts, the S-100 protein used had been purified from ox brain.

Normal rabbit serum was used instead of the first antibody for the controls, and no cells positive for S-100 protein were observed in any of them. Normal swine serum, rabbit anti-S-100 protein, swine anti-rabbit immunoglobulins, PAP and normal rabbit serum were all purchased from Dakopatts, Copenhagen, Denmark.

Results

Positive staining for S-100 protein was evident in all twelve cases of pleomorphic adenoma at the following sites:

1. **Plasmacytoid or hyaline cell component**
   This was the component stained most intensely for S-100 protein. The cells were round, oval or cuboidal, with homogeneous eosinophilic cytoplasm and eccentric nuclei (Fig. 1a). Such cells appeared as solid masses (Fig. 1b), periductal growths (Fig. 1c), or as loosely-cohesive cells in a myxoid stroma (Fig. 1d).

2. **Stellate and spindle cell components**
   These mesenchymal-like tumor cells, occurring as scattered groups in a myxomatous or chondroid stroma (Figs. 2a and b), or as intercon-

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Fig. 1a Pleomorphic adenoma with plasmacytoid-type myoepithelial cells (Hematoxylin and eosin stain, ×198)

Fig. 1b Sheets of plasmacytoid cells showing strong S-100 protein immunoreactivity (PAP, ×198)
Fig. 1c  S-100 protein positivity of periductal and plasmacytoid cells (PAP, $\times$198)

Fig. 1d  S-100 protein-positive plasmacytoid cells in a myxoid stroma (S-100 protein negative) (PAP, $\times$198)

Fig. 2a  Stellate and spindle-shaped cells showing S-100 protein positivity in a myxomatous stroma (PAP, $\times$198)
Fig. 2b  S-100 protein deposit outlining the bodies and dendritic processes of stellate cells in a chondroid stroma (PAP, ×396)

Fig. 2c  S-100 protein-positive stellate cells occurring as anastomosing cellular proliferations in a connective tissue stroma (PAP, ×39.6)

Fig. 3  S-100 protein-positive periductal cells (PAP, ×198)
Fig. 4b  Nests and solid epithelial masses showing strong S-100 protein uptake (S) (PAP, ×39.6)

Fig. 4a  Pleomorphic adenoma consisting of solid sheets of myoepithelial cells (Hematoxylin and eosin stain ×39.6)

Fig. 5a  S-100 protein staining of intercalated ducts in a minor salivary gland (PAP, ×198)
necting areas of cellular proliferation in other types of connective tissue stroma (Fig. 2c), showed consistent and fairly intense staining for S-100 protein. A dark brownish deposit was found in the nuclei and cytoplasm of these stellate cells, distinctly outlining the cell bodies and their processes (Fig. 2b).

3. **Tubular and duct-like structures**
   The periductal cells surrounding these structures demonstrated S-100 protein positivity, but not always strongly (Fig. 3).

4. **Solid epithelial areas**
   These consisted of round, oval or squamous epithelioid cells arranged in solid sheets, nests or clusters with little or no glandular differentiation (Fig. 4a). Marked staining for S-100 protein was also noted in these areas (Fig. 4b).

Stromal connective tissue fibers were generally negative for S-100 protein.

In the normal salivary gland tissue there was positive immunostaining of acinar cells and epithelial cells of the intercalated ducts in some but not all cases (Figs. 5a and b).

No immunostaining of neoplastic and normal tissue was seen in sections incubated with normal rabbit serum.

![Fig. 5b Acinar cells and striated duct (arrow) are S-100 protein-negative (PAP, ×198).](image)

**Discussion**

The present findings regarding the distribution of S-100 protein in pleomorphic adenomas are in good agreement with those of previous studies\[12-14,16-19\]: tumor cells in myxomatous and chondroid areas, periductal epithelial cells, and cells in epithelial sheets or solid cords were stained positively by the PAP method using anti-S-100 protein. Duct-lining cells and stromal connective tissue fibers were, in contrast, S-100 protein-negative.

The results of immunostaining of normal salivary gland tissue were variable in all the cases studied here, and this was also reflected in other reports\[12,13\]. **Hara et al.**[13] and **Takahashi et al.**[20] found no S-100 protein in epithelial cells of intercalated ducts, acini, striated ducts and excretory ducts. However, myoepithe-
lial cells surrounding acini and intercalated ducts were specifically positive for S-100 protein\textsuperscript{[19–21]}. On the other hand, NAKAZATO et al.\textsuperscript{[12]} and ERLANDSON et al.\textsuperscript{[22]} demonstrated that acini cells were S-100 protein-positive in some of the cases they examined, and other reports also described S-100 protein immunoreactivity in intercalated ducts\textsuperscript{[11,12,14,18,19]}. The variation in findings among these studies may be ascribed to the differing sensitivities of the immunoperoxidase methodologies employed by various laboratories, different primary antibody sources, poor specimen fixation and differences in sample size\textsuperscript{[18,19]}. Another possible reason is that the S-100 protein immunolocalization is often nuclear, and may have been masked by the hematoxylin counterstain in some cases\textsuperscript{[19]}. Furthermore, NAKAZATO et al.\textsuperscript{[12]} observed that not only was the immunostaining of normal salivary glands variable, but that the intensity of the reaction was far weaker than that in tumors. This led to the suggestion that S-100 protein was actively synthesized by adenoma cells during the course of tumor development\textsuperscript{[12]}.

According to KAHN et al.\textsuperscript{[21]}, the presence of S-100 protein in tumors represents a characteristic of tumor stem cells inherited from their corresponding non-transformed progenitors, and is not a consequence of dedifferentiation of tumor cells during clonal expansion. Demonstration of S-100 protein may therefore be used to confirm the histogenesis of tumors. There is general agreement that the progenitor cells of pleomorphic adenomas of human salivary glands are either ductal epithelial cells or modified myoepithelial cells\textsuperscript{[17,23–25]}. There is also a general consensus that the various neoplastic cells in pleomorphic adenomas that express S-100 protein represent cells of myoepithelial lineage\textsuperscript{[12]}. However this concept is marred by the fact that myoepithelial cells may induce salivary duct cells to express S-100 protein, as shown in a recent study involving co-culture of the two cell types\textsuperscript{[24]}.

The presence of S-100 protein in cells of extraneural tissues including pleomorphic adenomas has not been satisfactorily explained. One interpretation is that adenoma cells may not be related to neural tissue, but may synthesize the neural system-specific protein as a result of their multidirectional differentiation\textsuperscript{[12]}. Another explanation proposes that S-100 protein is a nervous system-associated, but not nervous system-specific, protein\textsuperscript{[12]}.

The usefulness of S-100 protein alone as a diagnostic marker in distinguishing pleomorphic adenomas from other salivary gland tumors is limited by the fact that numerous other types of salivary gland tumor are also S-100-positive. Positive consistent staining for S-100 protein has been reported in monomorphic adenomas (canalicular, salivary duct, clear cell and dermal analogue type) and polymorphous low-grade adenocarcinomas\textsuperscript{[18]}. Inconsistent positive staining has been noted in adenoid cystic carcinomas, carcinoma ex pleomorphic adenomas, clear cell carcinomas and nonclassified adenocarcinomas\textsuperscript{[18]}. Rare or no staining has been found in oxyphilic adenoma, basal cell adenoma, Warthin's tumor, mucocoeplidermoid carcinomas and acinic cell carcinomas\textsuperscript{[13,18]}. However interdigitating cells identified as Langerhan's cells, which stained positive for S-100 protein, have been found in the lymphoid component of Warthin's tumor\textsuperscript{[13,20]}. 
Acknowledgments

We are indebted to H. Govindan for technical assistance, Victoria for preparation of the typescript, and W.K. Wong for photographic assistance. We are grateful to Dr. M. Jegathesan, Director, Institute for Medical Research, for his encouragement and support. This study was funded by a University of Malaya research grant Vote PJP 44/87 and 336/89 (to S. C. H).

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