IMMUNOHISTOCHEMICAL EXPRESSION OF S-100 PROTEIN IN REACTIVE AND NEOPLASTIC MYOEPIHELIAL CELLS OF VARIANT SALIVARY PLEOMORPHIC ADENOMAS

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Immunohistochemically detectable S-100 protein in 76 salivary pleomorphic adenomas was found in two types of myoepithelium derived neoplastic cells; spindle-shaped anastomosing cells and round cells. Spindle-shaped tumor cells displayed a strong presence of S-100 protein-like immunoreactive substance (LIS), and they were usually located in the outer layer of duct-like structures and showed a continued proliferation into the stroma. Round tumor cells abundantly gave a S-100 protein LIS staining, and they were distributed in the outer layer of duct-like structures. Chondroid cells and chondrocytes in hyalinous and myxomatous tissue were also reactive for S-100 protein LIS. In rare cases, stromal connective tissue fibers exhibited an intense S-100 protein LIS staining. Peripheral nerve fibers and myoepithelial cells in the affected salivary glands were also positive for S-100 protein LIS.

The S-100 protein originally isolated from brain (8) belongs in a class similar to that of Ca^{2+} -binding protein which include calmodulin, troponin C, and intestinal calcium-binding protein. Consequently, S-100 protein containing cells probably participate in Ca-mediated processes. Immunohistochemical localization of S-100 protein has been reported in the central and peripheral nerve tissues (1, 11, 20, 21), Langerhans cells (12), melanoma and nerve cells (12, 19), chondrocytes (3, 13), as well as in myoepithelial cells of the salivary glands (6, 14). Recently, Hara *et al.* (6) have called attention to the fact that S-100 protein is present in myoepithelial cell and its related spindle-like neoplastic cells of salivary gland tumors.

Histologically, pleomorphic adenomas of the salivary glands are composed of two different cells types; one is an epithelium-derived tumor cell found in tubular, glandular, and ductal structures; and the other is a myoepithelial cell-derived tumor cell, which may transform to myofibrocytes or cells having mucus-secreting nature (4, 5). Tumorigenesis of pleomorphic adenoma is subsequently evident to proliferate to either epithelial components or myoepithelial cells having both biologic properties of smooth muscle-like and mesenchyme (2, 7, 18). In this regard, both clear cell adenoma of monomorphic adenoma and adenoid cystic carcinoma of salivary glands have a peculiar differentiation from the myoepithelial cells (15, 17), and mucoid secretion or hyaline stroma in pleomorphic adenoma is also from the myoepithelium-derived cells (4).

The present paper describes the distribution of S-100 protein in salivary pleomorphic adenomas and compares it with the localization of filament proteins in the same specimens (9, 10).

MATERIALS AND METHODS

Materials A total of 76 cases of pleomorphic adenoma were used. The tissue obtained immediately after surgery was cut into small pieces and fixed in 10% formalin for 12 hr at room temperature. Paraffin sections at 4 μ m were made for immunohistochemical detection of S-100 protein as well as for H-E routine stainings. Specimens were also treated for the demonstration of intermediate filament proteins such as keratin, desmin, vimentin, myosin and actin.

Methods Deparaffinized sections were rinsed with 0.01 M phosphate-buffered saline (PBS), pH 7.0 and immersed in 0.3% H₂O₂ methanol solution for 30 min to inactivate endogenous peroxidase. The sections were then rinsed with PBS, and treated according to the following steps:

- 1) Immersion in normal swine serum (1 : 20 dilution) for 30 min.
- 2) Reaction with rabbit anti-S-100 protein (1 : 100) for 60 min.
- 3) Reaction with swine anti-rabbit immunoglobulins (1 : 20) for 30 min.
- 4) Reaction with rabbit peroxidase anti-peroxidase complexes (PAP, 1 : 100) for 60 min.

Finally, they were rinsed well and reacted with 0.05 M Tris buffer containing 0.3% diaminobenzidine (DAB) and 0.05% H₂O₂ for 10 min. All reactions were done at room temperature (20°C).

According to documentation of Dakopatts, S-100 protein was purified from ox brain.

Normal rabbit serum was used instead of the first antibody for the control, and cells positive for S-100 protein were never observed in any of the controls.

Normal swine serum, rabbit anti-S-100 protein, swine anti-rabbit immunoglobulins and PAP were all purchased from Dakopatts, Copenhagen, Denmark.

RESULTS

Histopathology of Salivary Pleomorphic Adenomas

In order to describe the immunohistochemical localization of S-100 protein in neoplastic cells of pleomorphic adenomas, the various histologic types of epithelial and myoepithelial elements in this tumor were noted.

1. *Tubular, duct and gland-like structures*: Most cases of pleomorphic adenomas showed this type of growth pattern. Epithelial proliferation was generally exhibited as tubular, ductal, and glandular growths comprising of single, double or multiple layers of epithelial cells. The outermost layer of tumor cells of these structures occasionally showed a continuously outward proliferation with the formation of long, anastomosing and fibrillar structures. Such patterns of proliferation suggested the neoplastic transformation of myoepithelium.

2. *Solid epithelial structures*: In this type, squamous epithelial cells proliferated in groups or as a single mass, resulting in a solid pattern. The cellular shape resembled

a squamous cell or fibroblast. This type showed a squamous metaplasia with varying degrees of keratin pearl formation.

3. *Chondroid and osteoid structures*: Some parts of hyalinous or myxomatous stroma revealed chondroid, osteochondroid and osteoid tissues containing chondrocytes and/or osteo-chondroidal cells (Fig. 1d).

4. *Spindle-like or long anastomosing cell structure*: (Fig. 2) Cellular proliferation occurred as scattered growths and/or interconnections among spindle-shaped or anastomosing fibrillar tumor cells which were continuously connected to the outer zone of duct- or gland-like epithelial structures. Neoplastic cells were divided into small fibrillar cells and rather large anastomosing cells, both of which were usually located in hyalinous or myxomatous structures.

5. *Papillary growth pattern*: Single-, double- or multi-layered cells in a papillary pattern were not typical in the pleomorphic adenomas examined. Intraductal proliferating forms existed in single or two row arrangements with scant stromal connective tissue. Tumor cells in such epithelia revealed comparatively dark eosinophilic cytoplasm and compact basophilic nuclei.

6. *Multicystic pattern*: Small or large cystic cavities were distributed in solid tumor masses or in stromal connective tissues. The cyst-forming epithelial cells, irrespective of their sizes, originated from duct-like structures, and generally displayed a flat or very thin shape and were usually lacking at the luminal surface (Figs. 2c, 3a).

7. *Clear cell adenoma structure*: This tumor was composed of polyhedral cells or small, anastomosing triangular cells with well-delineated cytoplasmic borders, and it occurred in duct-like epithelial foci which showed a continuous proliferation in an outward direction (Fig. 4c). Many authors have suggested that the clear cell adenoma is probably of myoepithelial origin (15).

S-100 Protein Distribution

1. Normal salivary glands:

S-100-protein like immunoreactive substance (LIS) was limited to the stromal fibers (Fig. 1a). Fat cells in the glands also stained positive for S-100 protein LIS.

2. S-100 protein positive stroma:

In rare cases stromal fibers displayed an intense S-100 protein LIS staining. Most cases were of the typical tubular and ductal types of pleomorphic adenoma, and almost all interductal stroma were found to be filled with S-100 protein LIS positive fibers (Figs. 1b, 1c). No specific histologic features were obtained in stromal fibers on this case.

3. S-100 protein positive cells in chondroid and osteoid structures:

Chondroid cells or chondrocytes indicated a positive reaction for S-100 protein LIS (Fig. 1d), whereas osteoid and osteofibrous cells in hyalinous lesions lacked a S-100 protein LIS staining.

4. S-100 protein positive spindle cells: (Fig. 2)

Almost all of the spindle-shaped cells of the pleomorphic adenoma stained conspicuously for S-100 protein LIS. Histologically the cells could be divided into small and large cells and also could be classified as fine fibrillar, spindle-like, and

FIG. 1a. S-100 protein in normal submandibular gland. Stromal fibers in the gland show a strong S-100 protein LIS reaction. $\times 100$

FIG. 1b. S-100 protein LIS in pleomorphic adenoma. Interstitial stromal fibers of pleomorphic adenoma (duct-like and tubular type) indicate strong positive S-100 LIS proteins. $\times 100$

FIG. 1c. S-100 protein LIS in pleomorphic adenoma. Reactive stromal fibers in some of pleomorphic adenoma are rarely positive in S-100 protein LIS staining. $\times 100$

FIG. 1d. S-100 protein LIS in chondroid structure of stroma within pleomorphic adenoma. Chondroid cells and spindle tumor cells are characterized by marked positivity of S-100 protein LIS staining. $\times 100$

FIG. 2a. S-100 protein LIS in pleomorphic adenoma. Spindle tumor cells and long projecting cells which interconnect from peripheral zone of epithelial foci are highly reactive to S-100 proteins LIS. This S-100 protein LIS staining is the most common pattern in pleomorphic adenoma. $\times 100$

FIG. 2b. S-100 protein LIS stromal area of pleomorphic adenoma. Spindle tumor cells, large and small in size, show the highest staining for S-100 protein LIS. $\times 100$

FIG. 2c. S-100 protein LIS in pleomorphic adenoma. Spindle tumor cells and fibrillar tumor cells in the stroma, and outer located tumor cells of duct-like structure display an intense S-100 protein LIS staining. $\times 100$

FIG. 2d. S-100 protein LIS in pleomorphic adenoma. A solid neoplastic focus in pleomorphic adenoma are intermingled to the highest positive tumor cells and negative or very slightly positive tumor cells. $\times 100$

FIG. 3a and 3b. S-100 protein LIS in pleomorphic adenoma. Duct-like structures of pleomorphic adenoma usually contain S-100 protein LIS positive cells abundantly in the outer layer. However, the outer layered cells of duct-like structures are not always strongly positive for S-100 protein LIS. $\times 100$

FIG. 3c. S-100 protein LIS in round tumor cells and plasmotoid tumor cells in pleomorphic adenoma. S-100 protein LIS strong positivity is found in round oval and plasmotoid tumor cells distributing in glandular structure. $\times 100$

FIG. 3d. S-100 protein LIS in round tumor cells in pleomorphic adenoma. Round tumor cells scattered in duct-like structure of pleomorphic adenoma show the highest staining for S-100 protein LIS. The epithelial cells of duct-like structure are devoid of S-100 protein LIS staining. $\times 100$

FIG. 4a. Pleomorphic adenoma with myoepithelial type. HE staining. $\times 100$

FIG. 4b. S-100 protein LIS in the same specimen of Fig. 4a. The round tumor cells exhibit the strongest staining for S-100 protein LIS. $\times 100$

FIG. 4c. Pleomorphic adenoma with clear cell variant. This clear cell area is probably of myoepithelial origin. HE staining. $\times 100$

FIG. 4d. S-100 protein LIS in the same specimen of Fig. 4c. Clear tumor cells show a marked S-100 protein LIS staining. $\times 100$

long anastomosing cells, which had interconnections via their fibrillar processes with accompanying epithelial structures (Figs. 2a, 2b). Many S-100 protein LIS-positive spindle cells were attached to the outer side of tubular and ductal structures (Fig. 2c). In solid structures, except for the components of glandular epithelial cells, there was a mixture of S-100 protein LIS highly positive cells whose cellular shapes were spindle-like or stellate (Fig. 2d).

5. *S-100 protein positive round cells:* (Fig. 3)

The most intensely stained cells for S-100 protein LIS, having round, oval, or cuboidal shapes, were scattered throughout ductal or cystic epithelial structures, mainly in the outer zone. Such round cells proliferated to form solid masses or gland-like structures, or were scattered as intraductal growths (Figs. 3a–d). In many cases, there were mixed patterns of round and spindle cells strongly positive for S-100 protein LIS in the same duct-like structure. The epithelial cells in the central part of duct-like structures were usually negative or slightly positive for S-100 protein LIS, whereas they were strongly positive for keratin (12, 13).

6. *S-100 protein in solid epithelial structure:* (Fig. 4)

Round, oval, or squamous epithelioid cells, whose cellular arrangements were usually solid without any ductal or glandular organization, gave a marked S-100 protein LIS staining (Figs. 4a, 4b). The solid or medullary growth profiles could be classified into large groups: one in which there was large solid mass without any connection to duct-like and glandular structures, and the other in which small clusters of solid masses were mixed with glandular or duct-like structures (Figs. 4c, 4d). In both types, the cells, which were laden with S-100 protein LIS and formed solid structures, originated from the peripheral zone of the ductal or glandular structures.

Comparison between S-100 protein positive cells and tumor histology

1. Spindle-shaped tumor cells were generally strongly positive for S-100 protein LIS, irrespective of cellular size, shape, or location in pleomorphic adenomas (Fig. 2).
2. Round, oval and squamous-like tumor cells were occasionally positive for S-100 protein LIS. These rounded cells were distributed in the outer zone of duct-like and glandular structures (Fig. 3).
3. Distinct epithelial cells from ductal, tubular, glandular, cystic and solid structures indicated a negative reaction for S-100 protein LIS staining (Figs. 1b, 1c).
4. Chondrocyte-differentiating cells in the tumor stroma showed a positive reaction for S-100 protein LIS (Fig. 1d).
5. Stromal connective tissue fibers were generally negative for S-100 protein LIS; however, peripheral nerve fibers and fibers of an undistinguishable nature, which were surrounded by duct-like structures, rarely displayed a positive staining for S-100 protein LIS (Figs. 1b, 1c).

DISCUSSION

The progenitor cells of pleomorphic adenomas of human salivary glands have been suggested to be either ductal epithelial cells or modified myoepithelial cells (2, 4, 5, 7, 15–18). In the case of the former, the most prospective origin are those

of the intercalated duct cells. For the latter, the myoepithelial cells are thought to transform into cells having both epithelial and mesenchymal properties. Ultrastructural studies of myoepithelial cell-derived tumors have demonstrated that the spindle-shaped cells in them contain myofilamentous elements (2), and that the plasmacytoid type cells thin filaments (17), the modified or transformed myoepithelial cells in them contain also myofibrils, and that their ergastoplasm indicates the production of proteins secreted into the interstitium (7). Since it is a useful marker of normal myoepithelial cells and transformed myoepithelial cells (20), more recently Nakazato *et al.* (14) reported that S-100 protein existed in salivary gland neoplasms. From our investigation, S-100 protein positive tumor cells of myoepithelial origin in salivary glands were classified into two types: spindle tumor cells interconnected with anastomosing processes and rounded tumor cells. The spindle-shaped tumor cells, including many histologic variants were, without a doubt, derived from myoepithelial cells, as has been described. This type of spindle cell with long anastomoses proliferated from the peripheral zone of duct- and glandular-like structures into the hyalinous or myomatous areas. The fact that these cells appeared to proliferate only from duct-like or tubular structures, which usually consisted of a double or more layers of cells, was a most interesting finding in terms of S-100 protein histochemistry. The spindle-shaped cells in pleomorphic adenomas also gave slight staining keratin and desmin and a moderate-to-strong reaction for vimentin, as detected immunohistochemically (9, 10). These features suggest that tumor spindle cells have co-expressed phenotypes of both epithelial and mesenchymal cells. Also, the neoplastically transforming myoepithelial cells may undergo some changes in their cytoskeleton.

S-100 protein-positive round cells were unique tumor cells in pleomorphic adenoma, and the occurrence of such cells has been reported previously by Sciubba *et al.* (17) as the plasmacytoid cell. The round cells strongly positive for S-100 protein were located in the outer layer of duct-like structures and in papillary-like epithelial growths, and they were scattered, grouped, or merged together as massive growths. The histochemical properties of this type of possibly myoepithelium-derived cell have not been reported yet. These round or squamous tumor cells staining intensely for S-100 protein are assumed to also be of myoepithelial origin. Thus, the present study has demonstrated two types of neoplastic myoepithelium-derived cells with special reference to immunohistochemically detectable S-100 protein.

Ordinarily, it is of biologic and histochemical significance that normal or modified myoepithelial cells have both epithelial and smooth muscle in nature. However, neoplastically transformed myoepithelial cells seem to exist more mesenchymal features. Because spindle tumor cells produced or synthesized mucoid materials they were transformed to chondroid and osteochondroidal tissues. Chondrocytes in pleomorphic adenomas were shown to be positive for S-100 protein, as has already been noted in cartilage tissue (3, 13). Spindle tumor cells also contained keratin, desmin, and vimentin (10). Round tumor cells showing strongly positive for S-100 protein probably indicate myoepithelial origin, and this type of tumor cell could not be discriminated by routine histology.

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