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Distribution of S-100b protein in normal salivary glands and salivary gland tumors

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Summary. Immunohistochemical studies were performed for the presence of S-100b protein in non-neoplastic and neoplastic salivary gland tissues by the peroxidase anti-peroxidase (PAP) method. Some cases of pleomorphic adenoma were investigated by immuno-electron microscopy. S-100b protein could not be detected in epithelial cells of intercalated ducts, acini, striated ducts and excretory ducts of non-neoplastic salivary gland. However, myoepithelial cells surrounding the acini and intercalated ducts were specifically stained by S-100b protein. In pleomorphic adenomas, S-100b protein-positive cells could be mostly observed in the myxoid and chondroid areas, and the basal layer cells of the double-layered ductal cells were also positive. In clear cell adenoma, the clear cells were also S-100b protein positive. In adenoid cystic carcinomas, S-100b protein-positive cells could be found in trabecular areas, but not in tumor cells showing cribriform-pattern. In other tumors (Warthin's tumor, oxyphilic adenoma, basal cell adenoma, mucoepidermoid tumor and acinar cell carcinoma), S-100b protein positive cells were seldom observed. Immuno-electron microscopically, S-100b protein was diffusely distributed in the cytoplasm of myoepithelial cells as well as of tumor cells of pleomorphic adenoma, being distributed especially on the membrane of endoplasmic reticulum and the outer nuclear membrane.

Key words: S-100 protein – Myoepithelial cell – Salivary gland tumor

Introduction

S-100 protein was first isolated by Moore (1965) as a soluble protein, which was considered to be nervous tissue-specific and species nonspecific. An unusual degree of cross-reactivity to bovine S-100 protein was reported for brain extracts of mammals, birds, fishes and reptiles (Zomzely-Neurath

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and Walker; 1980). Recently, the presence of S-100 protein has been reported in adenohypophysis, chondrocytes, Langerhans cells and interdigitating reticular cells (Nakajima et al. 1980; Stefansson et al. 1982; Takahashi et al. 1981). Isobe et al. (1977) found that bovine brain S-100 protein was composed of two predominant components (S-100a and S-100b), both of which cross-reacted with anti-bovine S-100 protein serum provided by Moore. In order to study the distribution of S-100 protein in various kinds of tissues, it is necessary to purify subfractions of S-100 protein. In previous studies, we reported the isolation and purification of S-100b protein (Endo et al. 1981) and the presence of S-100b protein in adipose tissues by the immunohistochemical method (Hidaka et al. 1983). In the present study, we examined the presence of S-100b protein in various kinds of salivary gland tumor tissues by immunohistochemical and immuno-electron microscopical observations, and it was found that myoepithelial cells, neoplastic and non-neoplastic, were specifically S-100b protein positive. The significance of S-100b protein distributed in the cytoplasm of myoepithelial cells is briefly discussed.

Materials and methods

Preparation of antiserum

The bovine S-100b protein was prepared and monospecific anti-bovine S-100b protein antiserum was obtained from rabbits as previously described (Hidaka et al. 1983).

Immunoperoxidase staining for light microscopy

Blocks of formalin-fixed and paraffin-embedded specimens selected for this study included 14 cases of pleomorphic adenoma, 6 cases of monomorphic adenoma (Warthin's tumors (2) oxyphilic adenoma (1), basal cell adenoma (2) and clear cell adenoma (1)), 6 cases of mucoepidermoid tumor, 2 cases of acinar cell carcinoma, 7 cases of adenoid cystic carcinoma, normal human major salivary glands and normal rat submaxillary glands.

The peroxidase anti-peroxidase (PAP) method (Sternberger 1979) with slight modification was used. 5 μm thick sections were deparaffinized in xylol and treated with 0.3% H_2O_2 in methanol (30 min) to block endogenous peroxidase activity. The sections were washed with phosphate buffered saline (PBS, pH 7.2) and treated with normal swine serum (1:10, 15 min), after washing with PBS, the sections were incubated with rabbit anti-bovine S-100b protein antiserum (1:200, 30 min), washed with PBS and incubated with swine anti-rabbit IgG (DAKO; 1:20, 30 min), washed with PBS, incubated with PAP complex (DAKO: 1:50, 30 min). After washing with PBS, the sections were stained for 5 min with 3-3′-diamino-benzidine DAB, Sigma chemical company) (0.5 mg/ml) in 0.05 M Tris-HCl buffer (pH 7.6), containing 0.01% H_2O_2. Sections serial to those processed for immunoperoxidase staining, were stained with haematoxylin and eosin.

Immuno-electron microscopy

Fresh samples from 2 cases of human pleomorphic adenoma and submaxillary glands of adult rats weighing 200–250 g were fixed in 4% paraformaldehyde/0.1 M phosphate buffer (pH 7.4) for 16–18 h at 4° C on a shaker, washed overnight with 3 changes of 10% sucrose in PBS, and embedded in O.C.T. (Ames Co.) and quickly frozen in dry-ice acetone. 6 μm thick sections cut on a cryostat were mounted on albumin coated glass slides, stained by the indirect immunoperoxidase technique. The sections were incubated with normal swine serum (1:10, 15 min). After washing with PBS, the sections were incubated for 12 h with

anti-bovine S-100b protein antiserum (1:200) at 4° C. After washing with PBS, the sections were incubated for 3 h with HRPO-labelled swine anti-rabbit IgG (DAKO; 1:50) at 4° C, and then washed in PBS. The sections were incubated for 30 min in Karnovsky's diaminobenzidine solution without H_2O_2 and then incubated for 5 min in complete Karnovsky's solution containing H_2O_2 (0.01%) (Graham and Karnovsky 1966). The sections were washed in PBS, post-fixed with 2% OsO_4 for 1 h. After dehydration in graded ethanol solutions, the sections were embedded in EPON 812 by the inverted capsule method. Ultrathin sections were cut with L K B ultramicrotome, and unstained sections were examined with a Hitachi H 600 electron microscope.

Control of specificity of the immunohistochemical staining

Control experiments included the following,

1. Substitution of normal rabbit serum for the specific antiserum.
2. Anti-bovine S-100b protein antiserum was absorbed with bovine S-100b protein conjugated Sepharose 4B.

No enzyme reaction was found in these control sections.

Routine electron microscopy

Fresh samples of 2 cases of pleomorphic adenoma were fixed in 2% glutaraldehyde in phosphate buffer and post-fixed in 1% OsO_4 in phosphate buffer, and after dehydration in graded ethanols, embedded in Epon 812. Thin sections were stained with uranyl acetate followed by lead citrate.

Double immunodiffusion

Double immunodiffusion of crude extracts of rat brain, human brain, bovine brain, against anti-bovine S-100b serum was performed on a slide glass covered with 1% agarose containing 0.9% NaCl. The antiserum cross-reacted with crude extracts of rat brain and human brain, showing a single precipitin line.

Results

S-100b protein was observed only in the cytoplasm in frozen materials by the indirect immunoperoxidase method, but in paraffin-embedded materials by PAP method it was sometimes observed in both the cytoplasm and the nucleus.

Non-neoplastic salivary gland tissues

In human salivary glands, S-100b protein could not be observed in epithelial cells of intercalated ducts, acini, striated ducts and excretory ducts. However, myoepithelial cells surrounding acini and intercalated ducts were specifically positive for S-100b protein. Fat cells and peripheral nerves were also positive (Fig. 1). In the case of rat salivary glands, similar findings were observed.

Immuno-electron microscopically, S-100b protein was distributed diffusely in the cytoplasm of myoepithelial cells, especially on the membranes of endoplasmic reticulum and the outer nuclear membrane, and also associated with filamentous structures. It was, however, not found in the cisternae of endoplasmic reticulum, mitochondria and the nucleus (Fig. 2).

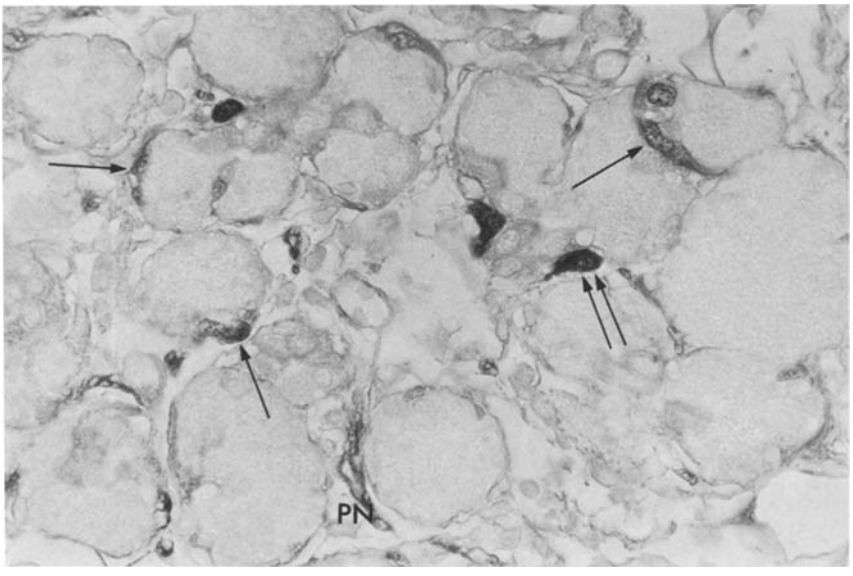

Fig. 1. Human parotid gland, immunoperoxidase staining for S-100b protein. S-100b protein is distributed in myoepithelial cells surrounding acini (*arrow*) and in the intercalated duct (*double arrow*). Peripheral nerve (*PN*) is also stained (× 200)

Pleomorphic adenoma

Light microscopically S-100b protein-positive cells were observed mostly in myxomatous and chondromatous areas. The myxoid cells, being spindle or star-shaped, and chondroid cells were intensely stained. S-100b protein was also observed in the basal layer cells of the double-layered duct epithelium, but not in inner layer cells or squamous cells (Figs. 3, 4). Electron microscopically, myoepithelial cells in tumor alveoli were readily identifiable by the presence of myofilaments and their distribution of the peripheral part of tumor alveoli. In addition, the cytoplasm of myoepithelial cells contained moderate numbers of mitochondria and a moderate amount of both smooth and rough endoplasmic reticulum. On the stromal side, the plasma membrane had many invaginations, pinocytotic vesicles and irregular basement membrane (Fig. 6).

Immuno-electron microscopically, the distribution of S-100b protein in the cytoplasm of positive tumor cells were essentially the same as those in non-neoplastic myoepithelial cells as described above (Figs. 5, 7 and 8).

Adenoid cystic carcinoma

Six cases of adenoid cystic carcinoma were examined light microscopically. S-100b protein-positive cells were seldom observed in solid or cribriform areas (Fig. 9). However in trabecular areas, S-100b protein positive cells were observed to be randomly located in tumor tissues (Fig. 10).

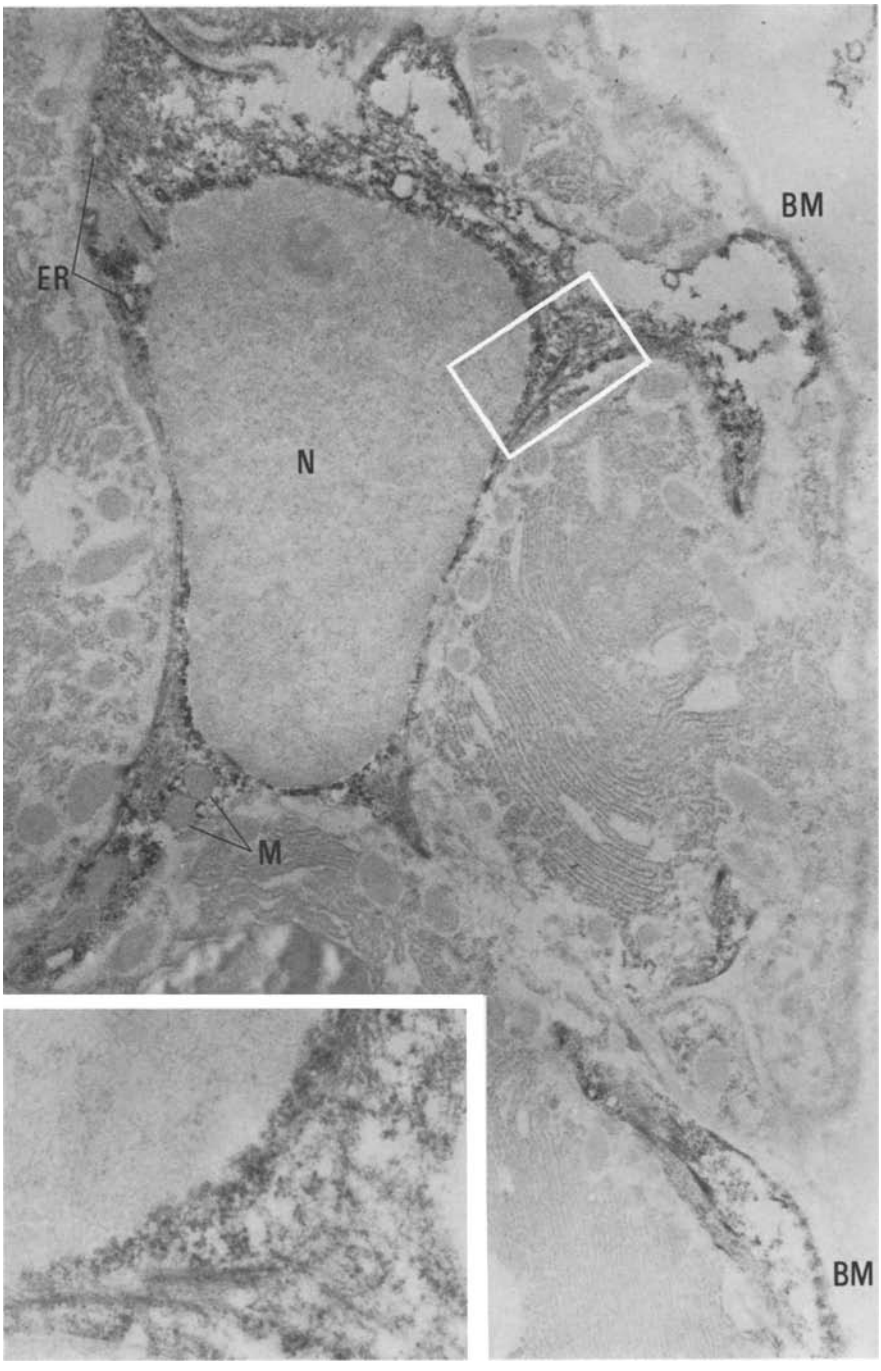

Fig. 2. Electron micrograph showing S-100b protein positive acinar myoepithelial cells of the rat submaxillary gland. Myoepithelial cells have basement membrane (*BM*). S-100b protein is diffusely distributed in the cytoplasm and on the membrane of endoplasmic reticulum (*ER*) and outer nuclear membrane, but absent from the cisternae of endoplasmic reticulum (*ER*), mitochondria (*M*) and the nucleus (*N*). S-100b protein is not observed in acinar epithelial cells (× 10,000). *Inset:* Enlargement of rectangle. S-100b protein is associated with filamentous structures similar to cytoskeletal components (× 30,000)

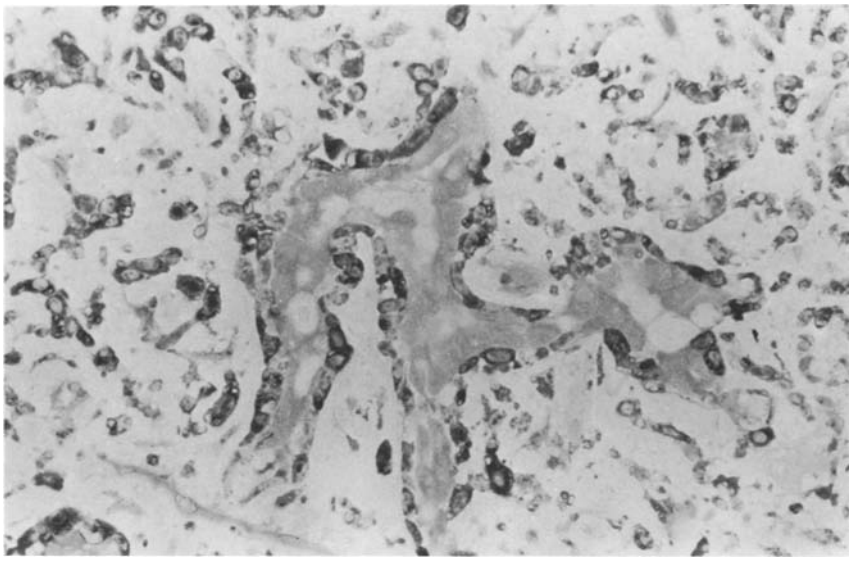

Fig. 3. Immunoperoxidase staining for S-100b protein in the pleomorphic adenoma. With the exception of inner duct lining epithelial cells, almost all tumor cells are S-100b protein positive (× 300)

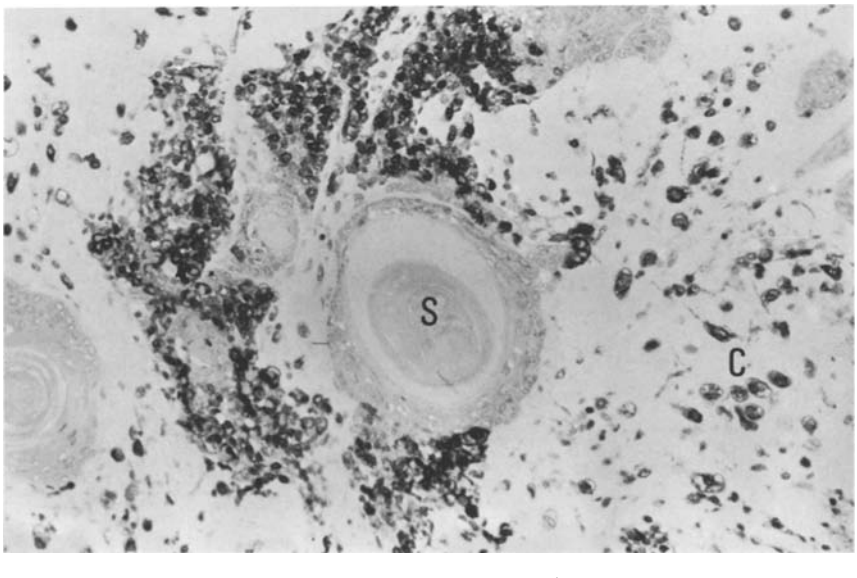

Fig. 4. Immunoperoxidase staining for S-100b protein in the pleomorphic adenoma. S-100b protein is absent from squamous epithelium (*S*). Chondroid cells (*C*) are S-100b protein positive (× 150)

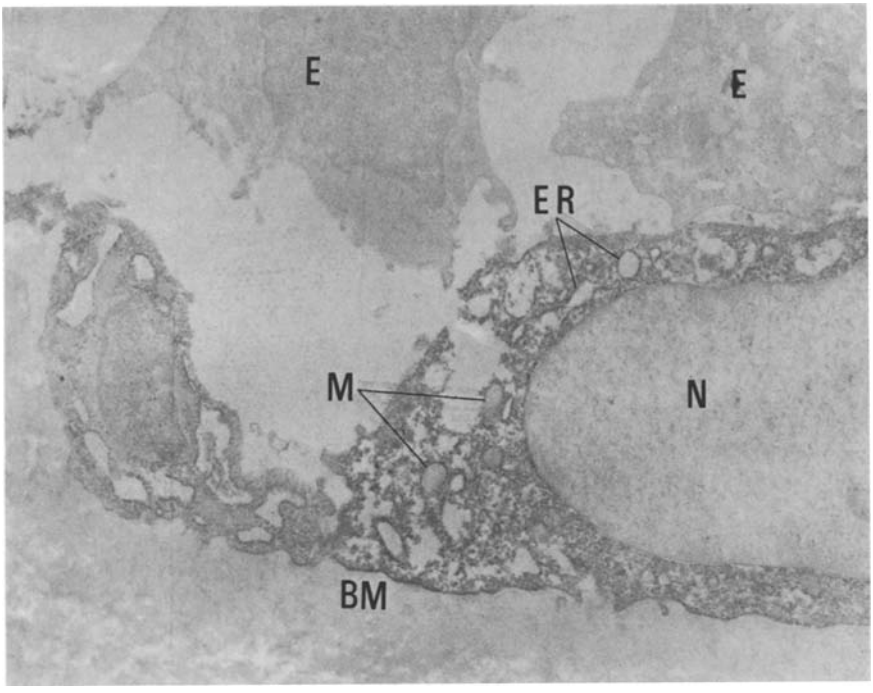

Fig. 5. Immuno-electron micrograph for S-100b protein in the pleomorphic adenoma. S-100b protein is distributed in the basal layer cells of double-layered duct epithelium. No reaction product is found in inner layer epithelial cells (*E*). *BM*, basement membrane; *ER*, endoplasmic reticulum; *M*, mitochondria; *N*, nucleus (× 8,700)

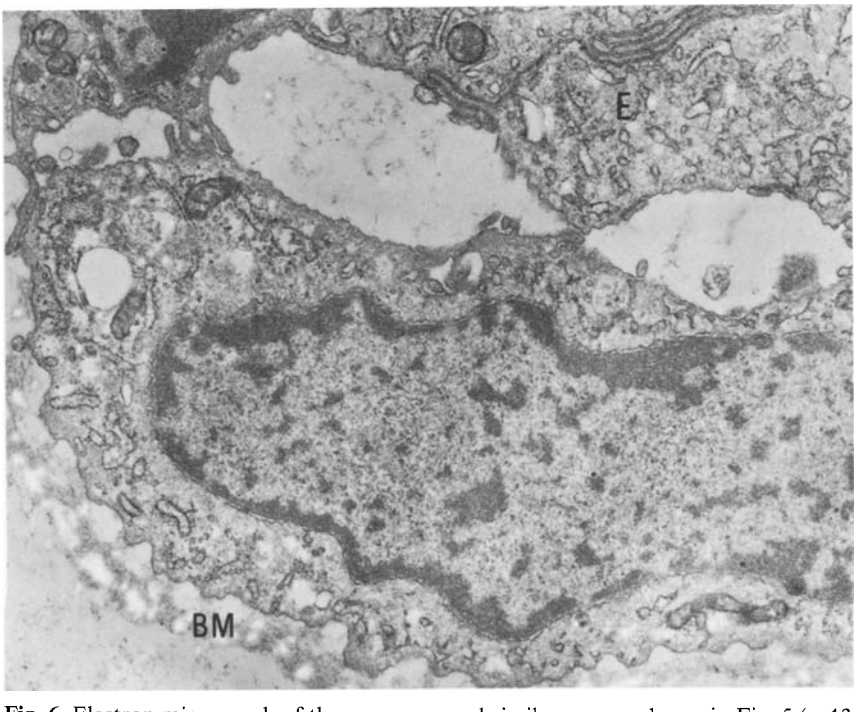

Fig. 6. Electron micrograph of the same case and similar area as shown in Fig. 5 (× 13,000)

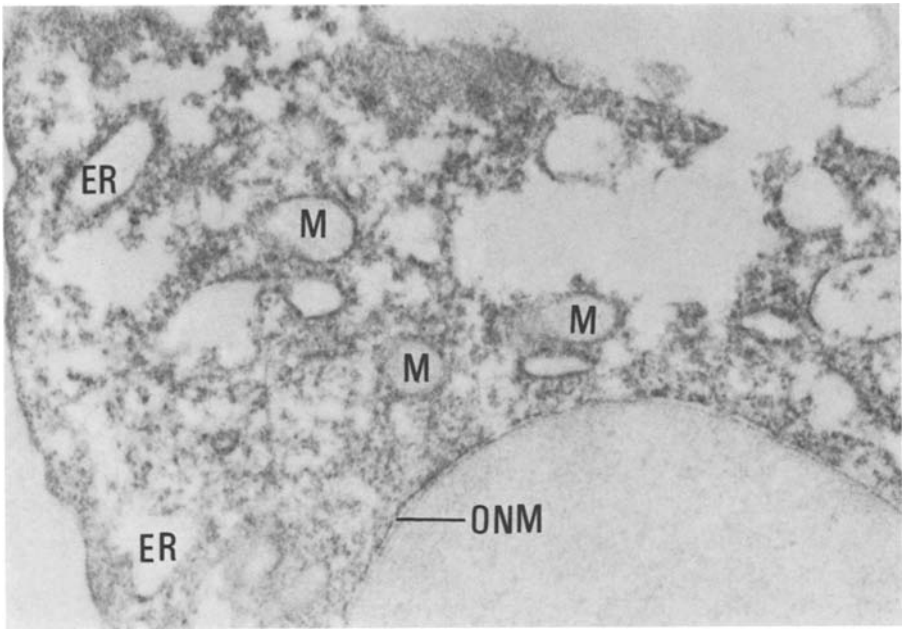

Fig. 7. Higher magnification of a part of Fig. 5. *ER*, endoplasmic reticulum; *M*, mitochondria; *ONM*, outer nuclear membrane (× 27,000)

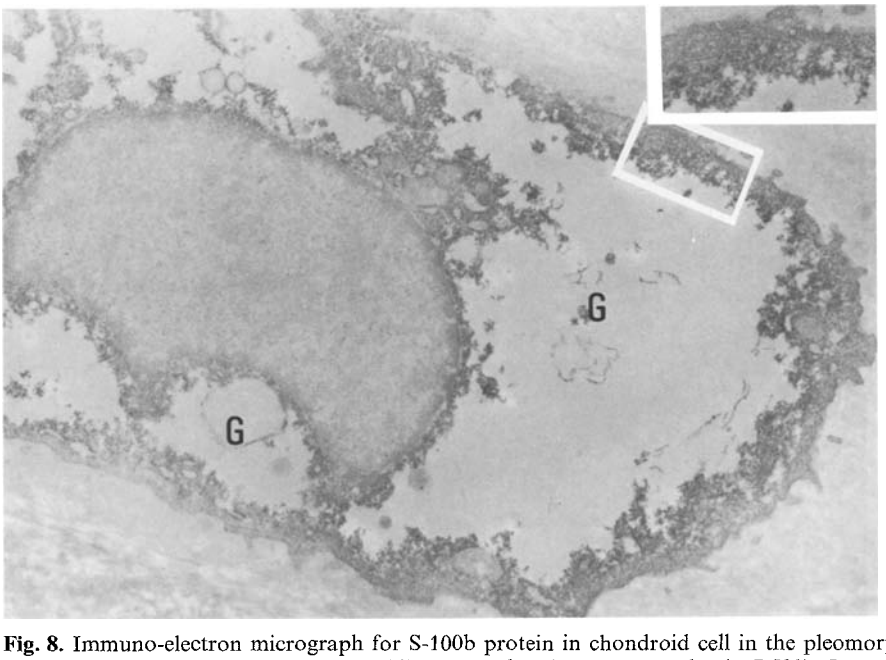

Fig. 8. Immuno-electron micrograph for S-100b protein in chondroid cell in the pleomorphic adenoma. Large electron lucent areas (*G*) seem to be glycogen granules (× 7,500). *Inset:* Enlargement of rectangle, pinocytotic vesicles are S-100b protein negative (× 13,000)

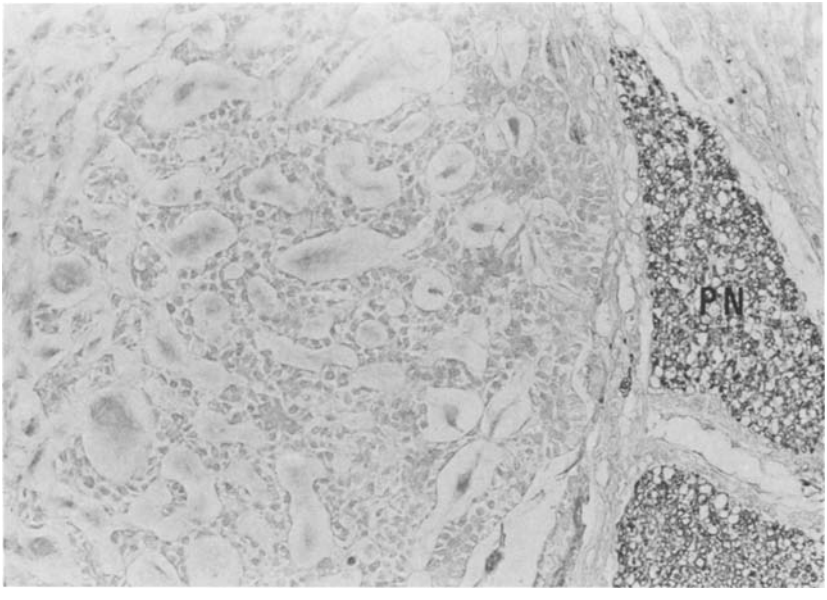

Fig. 9. Immunoperoxidase staining for S-100b protein in adenoid cystic carcinoma. No S-100b protein positive cells are found in the cribriform area. Peripheral nerve (*PN*) is intensely stained (× 200)

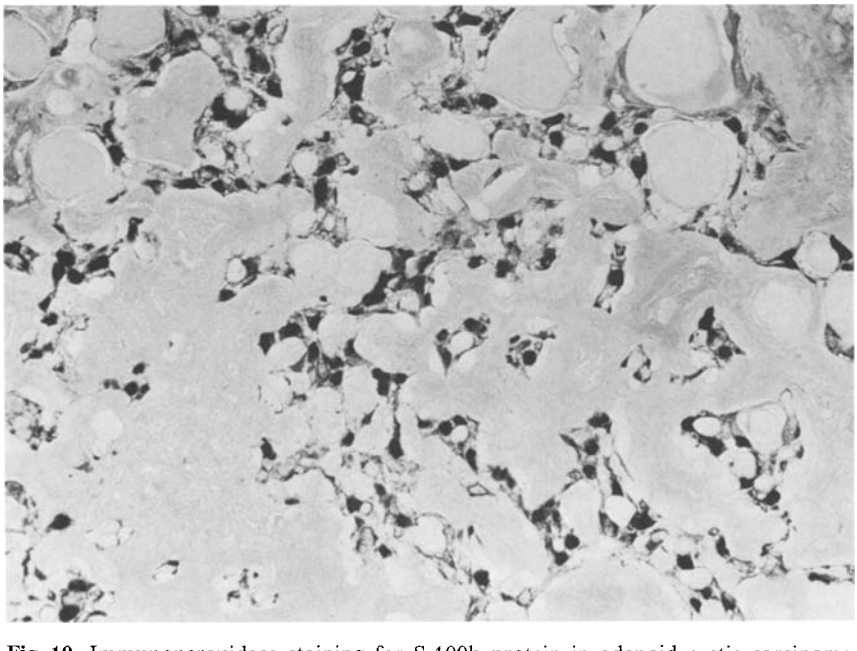

Fig. 10. Immunoperoxidase staining for S-100b protein in adenoid cystic carcinoma. In the trabecular area, S-100b protein positive cells are randomly scattered (× 250)

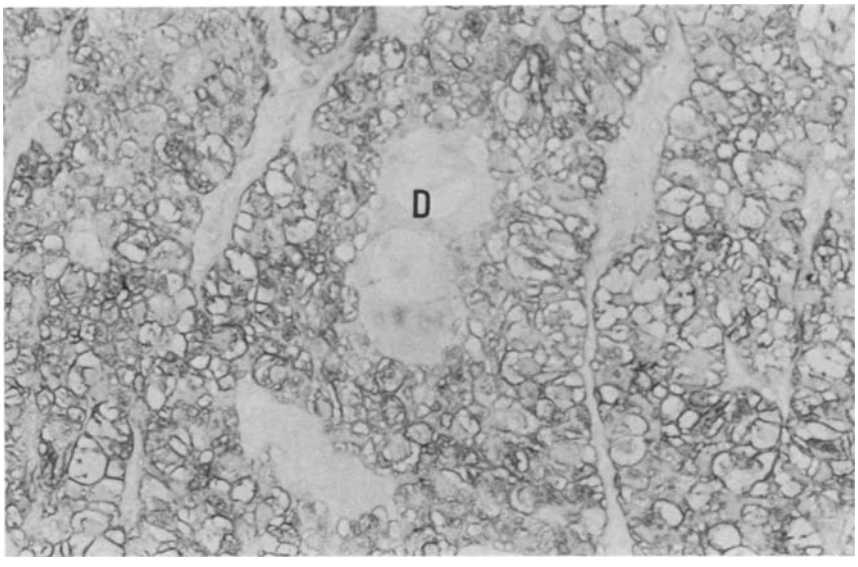

Fig. 11. Immunoperoxidase staining for S-100b protein in clear cell adenoma. Duct lining epithelial cells (*D*) are S-100b protein negative, but outer layered clear cells are S-100b protein positive (× 300)

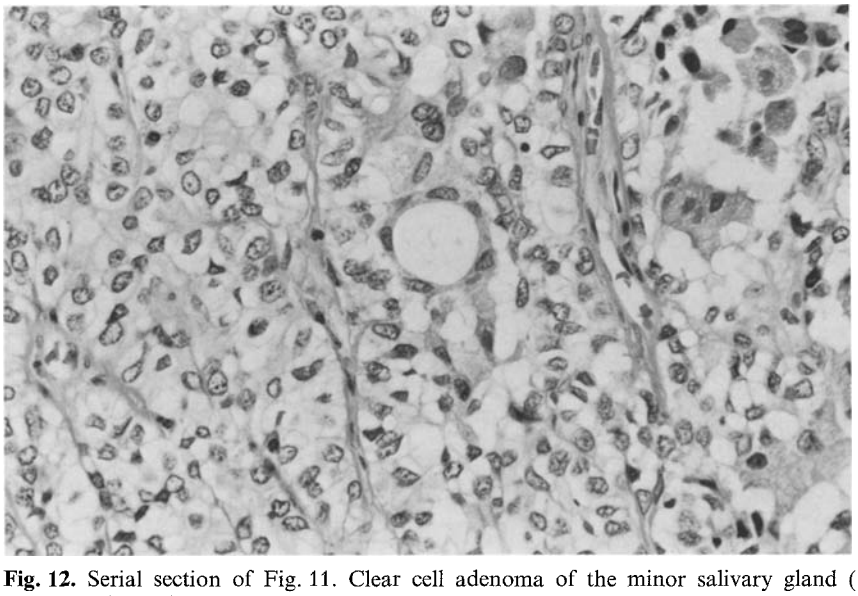

Fig. 12. Serial section of Fig. 11. Clear cell adenoma of the minor salivary gland (× 300). Hematoxylin and Eosin.

Clear cell adenoma

One case of clear cell adenoma was examined light microscopically. As shown in Figs. 11 and 12, duct-lining epithelial cells were negative for S-100b protein, but S-100b protein was readily detected in outer layered spindle-shaped or polygonal clear cells.

Other tumors

Warthin's tumor, oxyphilic adenoma, basal cell adenoma, mucoepidermoid tumor and acinar cell carcinoma were also examined. In these tumor tissues S-100b protein-positive cells were rarely found, though interdigitating reticular cells were S-100b protein positive in lymphoid tissue in Warthin's tumor.

Discussion

In the present study S-100b protein was specifically detected in myoepithelial cells of normal salivary glands.

In pleomorphic adenomas, with the exception of duct lining epithelial cells, almost all tumor cells were also S-100b protein positive. Since many researchers have reported that pleomorphic adenomas contained myoepithelial cells, producing a large amount of mucinous and chondroid materials (Mylius 1960; Doyles et al. 1968; Hübner et al. 1971), S-100b protein positive cells in pleomorphic adenomas seem to represent cells with myoepithelial differentiation. These findings indicate that S-100b protein is a marker protein of myoepithelial cells in non-neoplastic and neoplastic salivary glands. Our results pertaining to the pleomorphic adenoma disagree with those reported by Nakajima et al. (1982) in which S-100 protein was observed not only in chondroid cells and myxoid cells, but also in duct-lining epithelial cells. The disagreement seems to be due to the fact that our antiserum used in this study was produced with highly purified bovine S-100b protein (Hidaka et al. 1983).

In adenoid cystic carcinoma, S-100b protein positive cells were rarely found in cribriform and solid areas, but were distributed in trabecular areas. The absence of S-100b protein positive cells in cribriform areas does not support previous electron microscopic studies reported by others who claimed the presence of myoepithelial cells in adenoid cystic carcinoma (Hoshino and Yamamoto 1970; Hübner et al. 1969). Chomette et al. (1982) reported that few myoepithelial cells were found in adenoid cystic carcinomas and that the trabecular type had a better prognosis than the solid or cribriform types. Combining the results of Chomette et al. with ours, we assume that the adenoid cystic carcinoma with S-100b protein positive cells has a comparatively good prognosis.

Several markers were reported to be specific to myoepithelial cells, such as adenosine triphosphatase, alkaline phosphatase (Shear 1966), actin and myosin (Archer and Kao 1968; Drenckhahn et al. 1977; Nilsen and Donath 1981). But Garrett and Harrison (1970) reported that alkaline phosphatase was not observed in myoepithelial cells of human salivary gland. Caselitz et al. (1980) reported that actin was observed in myoepithelial as well as in excretory duct cells. Electron microscopically, myoepithelial cells are defined by the presence of myofilaments (Tandler 1965; Ellis 1965; Tamarin 1966). However, Saksela et al. (1972) suggested that clear cells in clear cell adenoma were of myoepithelial cell origin, although they failed to find myofilaments in the cytoplasm of clear cells. Our results, in which S-100b protein was distributed in clear cells, supported their view.

The present study, using immuno-electron microscopy showed that S-100b protein was distributed on the membrane of endoplasmic reticulum and the outer nuclear membrane, but absent from the cisternae of endoplasmic reticulum. This seems to indicate that the protein is produced by myoepithelial cells themselves.

Immuno-electron microscopically, Takahashi et al. (1981) reported that S-100 protein was distributed diffusely in the cytoplasm and the nucleus of the interdigitating reticular cells. In the present study, however, S-100b protein was absent from the cisternae of endoplasmic reticulum, mitochondria and the nucleus of myoepithelial cells, though light microscopically the nucleus was sometimes weakly positive for S-100b protein in paraffin-embedded materials by PAP method. This disagreement in immuno-electron microscopical findings may be due to the fact that our antiserum was produced with highly purified bovine S-100b protein (Hidaka et al. 1983).

As shown in Fig. 2, S-100b protein was observed to be associated with filamentous structures similar to certain cytoskeletal components of myoepithelial cells. This finding has not been reported previously, although further study is needed to clarify the true existence of S-100b protein and its functional significance in myoepithelial cells.

In conclusion, S-100b protein is a useful marker protein of myoepithelial cells not only for routine surgical pathology, but also for clarifying the histogenesis of salivary gland tumors.

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