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Malignant papillary cystadenoma lymphomatosum

Light and electron microscopic study

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Summary. A case of malignant papillary cystadenoma lymphomatosum was investigated by light and electron microscopy. Histologically, the tumor consisted mainly of a characteristic papillary cystadenoma lymphomatosum, but the feature of epithelial components varied greatly in separate portions of the tissue; papillary adenoma-, tubular adenocarcinoma-, acinic cell tumor- or oncocytoma-like features were observed, the tumor cells showing "atypia" in major components. Numerous intracytoplasmic inclusions were found in many oncocytic cells arranged in acinar structure, and stained with PAS, AZAN or Alcian blue. Electron microscopically, large numbers of very large, swollen and closely packed mitochondria were observed in almost all the tumor cells. The intraplasmic inclusions were composed mainly of a long dense body enclosed by a single unit membrane, and the contents constituted bundles of fine filament running parallel to the longer axis. The inclusions were often observed to unite with lysosomes and to co-exist with secretory granules in the cytoplasm.

Key words: Malignant papillary cystadenoma lymphomatosum – Crystalloids

Papillary cystadenoma lymphomatosum (PCL) is an uncommon benign tumor, which had a characteristic histologic pattern, showing papillary proliferation of duct epithelium with abundant lymphoid stroma. The epithelial element is composed of two characteristic rows of eosinophilic granular cells. It arises mostly in the lower portion of the parotid gland, and the incidence has been assessed as 5–12.5% of all parotid gland tumors (Thackray and Lucas 1974; Seifert et al. 1980). Malignant PCL, on the other hand, appears to be extremely rare. Foote and Frazell (1954) did not accept a reported case of primary malignant change from benign PCL. Although

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a few malignant variants have been described subsequently (Little and Rickles 1965; Döbrössi et al. 1972; Kessler et al. 1977; Seifert et al. 1977), the characteristics of the histological pattern of malignant PCL have not yet been elucidated.

The present study describes a supposed case of malignant PCL with variegated features. Light and electron microscopic observations were performed, and both peculiar and unique manifestations were observed. A striking feature was the inclusion in the cytoplasm of numerous oncocytic cells.

Case report

A 73-year-old Japanese male complained that a painless tumor mass, which was noticed about 3 years earlier in his left parotid region, had been increasing in size. Physical examination revealed a hemispheric movable growth, 5 cm in diameter, in the parotid. Any lymph nodes were impalpable. Incisional biopsy was performed. Histological examination revealed oncocytic cells forming an acinic structure surrounded by lymphocyte aggregates. The specimen was too small to diagnose, so it was tentatively diagnosed as PCL or oncocytoma of the parotid gland. After the first incisional biopsy, the tumor mass increased rapidly in size, so the tumorous tissue was extirpated. The tumor was located immediately outside the confines of the parotid gland. At this writing, 13 months after surgical extirpation, there is no evidence of recurrence or metastasis.

The extirpated tumor, measuring $8 \times 10 \times 5$ cm, was well encapsulated. In the cross section, a large irregular cyst, filled with a mucous brownish fluid, was found. Many fine papillary projections were observed to protrude into the lumen of the cyst. In a portion of the cyst wall, a solid mass including many microcysts was observed.

Materials and methods

The tumor tissues obtained from the surgical excision were fixed in buffered formalin, embedded in paraffin and sectioned. The tissue sections were stained by the following dyes: H-E, periodic acid Schiff's reagent, orcein, Alcian blue, AZAN, silver impregnation, and Congo red. Some frozen sections were stained by Sudan III. In order to detect the glycosaminoglycan components, a digestion test was performed with chondroitinase ABC (Yamagata et al. 1968) (pH 8.0, 10 units/ml, 37° C, 1 h), Streptomyces hyaluronate lyase (Ohya and Kaneko 1970) (pH 5.0, 100 turbidity reducing units/ml, 37° C, 1 h) or heparitinase (Cifonelli and Dorfman 1970) (pH 8.0, 0.3 units/ml, 37° C, 1 h). Some tissues were fixed in cold 2% glutaraldehyde in 0.05 M phosphate buffer solution (pH 7.4) and post fixed in 2% Millonig's phosphate buffered osmium tetraoxide, and embedded in Epon 812. Thin sections were observed under a Hitachi electron microscope operating at 75 kV.

For comparative purposes, two cases of oxyphilic adenoma, and two cases of benign PCL were examined light-microscopically.

Results

Histological findings

The major parts of tumor tissue have a cystadenomatous pattern, as shown in Fig. 1. The tumor cells are arranged in duct- or cyst-like structures, and the spaces among them are filled with mucinous substance. The interstitial stromal element consists of lymphoid aggregation with germinal follicle for-

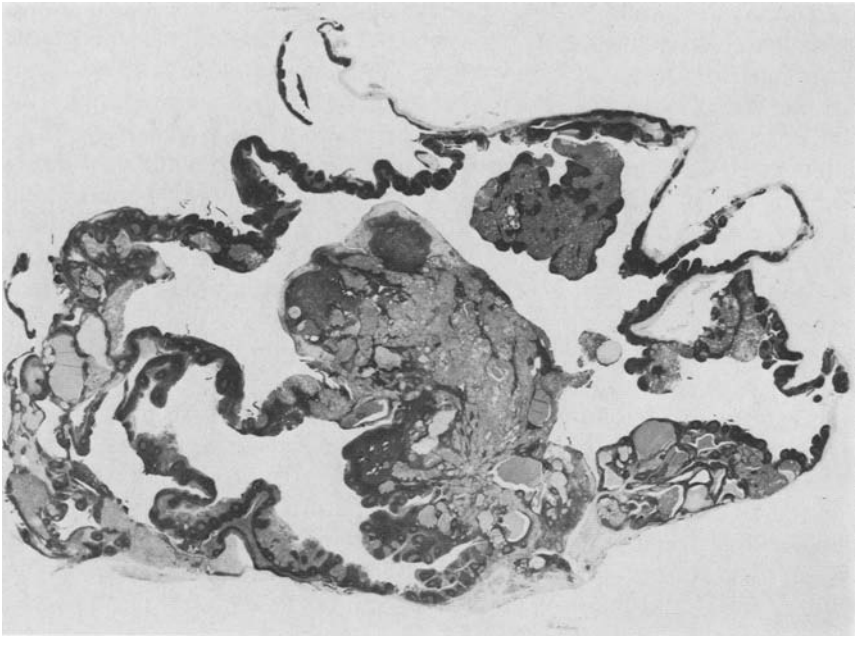

Fig. 1. Microscopic section of the tumor tissue. Lymphoid stroma with many germinal follicles is only focally pronounced in the cyst wall (HE, 2.2:1)

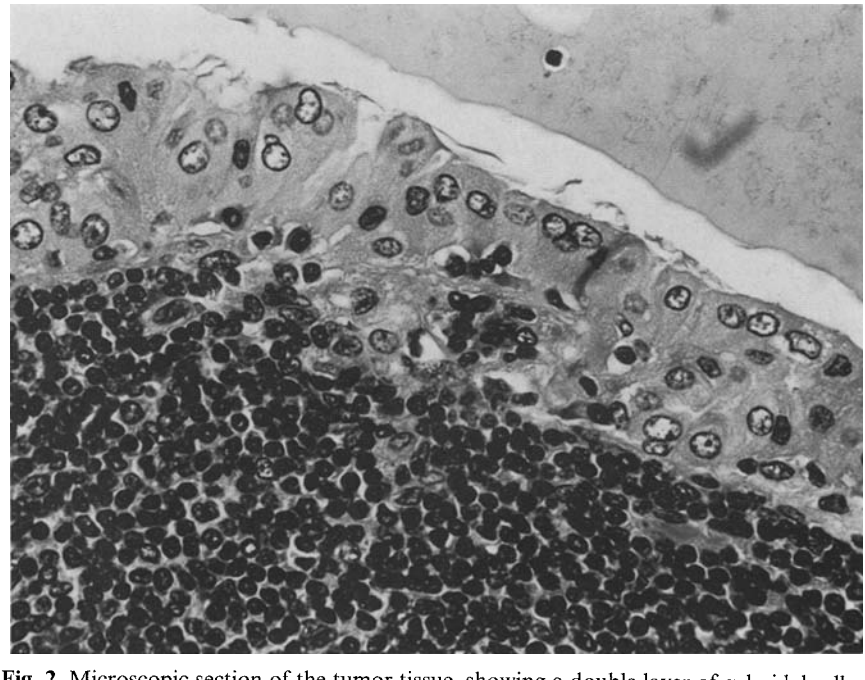

Fig. 2. Microscopic section of the tumor tissue, showing a double layer of cuboidal cells covering the inner surface of the cyst wall which is composed of lymphoid aggregation (HE, 240:1)

mation (Fig. 1). In minor parts of the tumor tissue, solid masses of tumor cells are noted. The great variation of the epithelial components in separate portions of the tissue is a striking feature, though the majority of the tumor cells contain more or less oxyphilic fine granules in their large cytoplasms. In some portions, the inner surface of the cyst wall is covered by a single or double layer of cuboidal cells which contain a few oxyphilic granules (Fig. 2). The nuclei of the cells are relatively small, hyperchromatic and irregular in arrangement. Mitosis is rare. In other portions, a papillary structure, consisting of multilayered cells, is observed (Fig. 3). Cribriform cell nests resembling an acinic cell tumor are also visible (Fig. 4). In these areas, the tumor cells are small and cuboidal, and their nuclei are small, round and hyperchromatic. Mitoses are numerous, and in some areas one mitosis per 3 high power fields is seen. The tumor cells immediately adjacent to mucous fluid have a foamy cytoplasm, resembling a sebaceous cell. Some elongated columnar cells with faintly eosinophilic cytoplasm, producing a large amount of mucous fluid, form a tubular structure not unlike that encountered in adenocarcinoma (Fig. 5). Their nuclei are large, vesicular, pleomorphic and irregular in arrangement. In some places, patchy squamous epithelial cell nests are observed in solid mosaic sheets. In other areas, oncocytoma-like, solid mass of large eosinophil cells containing numerous eosinophilic granules are found (Fig. 6). The cells have a single, large irregular nucleus with one or two prominent nucleoli. They are arranged in an acinar pattern, and the mucous material is observed in the acinar lumen: many secretory granules are visible in their cytoplasm. In some peripheral portions, the small acini tend to penerate into the fibrous connective tissue.

The most noticeable finding in the histologic section of the present tumor is the many tumor cells surrounding the acinar lumen or the cystic spaces containing inclusion bodies in their cytoplasm (Fig. 7). The inclusions show bundles of long bacilliform structure, the longest of which measured 14 μm. They are positively stained with either PAS, AZAN or Alcian blue, and reddish brown with silver impregnation. With H-E, orcein or Congo red, they are not stained. Some of the cells with these inclusions often reveal round hyalin bodies together in their cytoplasms (Fig. 7), suggesting that the round secretory granules are transformed into the long bacilliform inclusion-bodies. The same bacilliform bodies as the intraplasmic inclusions can be also observed in some cavities of the small cyst lined by the oncocytic cells (Fig. 8). In polarized light the inclusions show form birefringence, indicating the presence of elongated submicroscopic units oriented in the direction of the inclusion axis.

The mucinous substances observed in the cystic spaces and the acinar lumen are heavily stained with Alcian blue. In the major parts, the mucinous material remains virtually intact by the treatment with any mucopolysaccharidases, but in some areas where the tumor consists of ductal and acinar structure, resembling tubulo-acinar adenoma, the Alcian blue-positive material in the space among the ductal or tubular structure has a high sensitivity to the treatment with chondroitinase ABC. Stainability is not diminished with hyaluronidase and heparitinase.

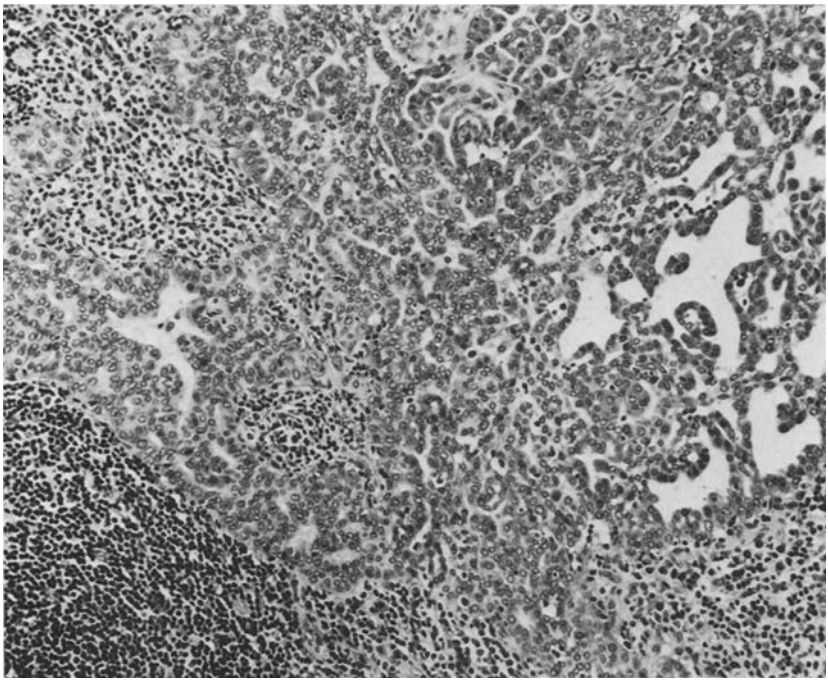

Fig. 3. Microscopic section of the tumor tissue, showing a papillary structure consisting of multilayered cells. Lymphoid aggregation with germinal follicle formation is observed in the interstitial stroma (HE, 160:1)

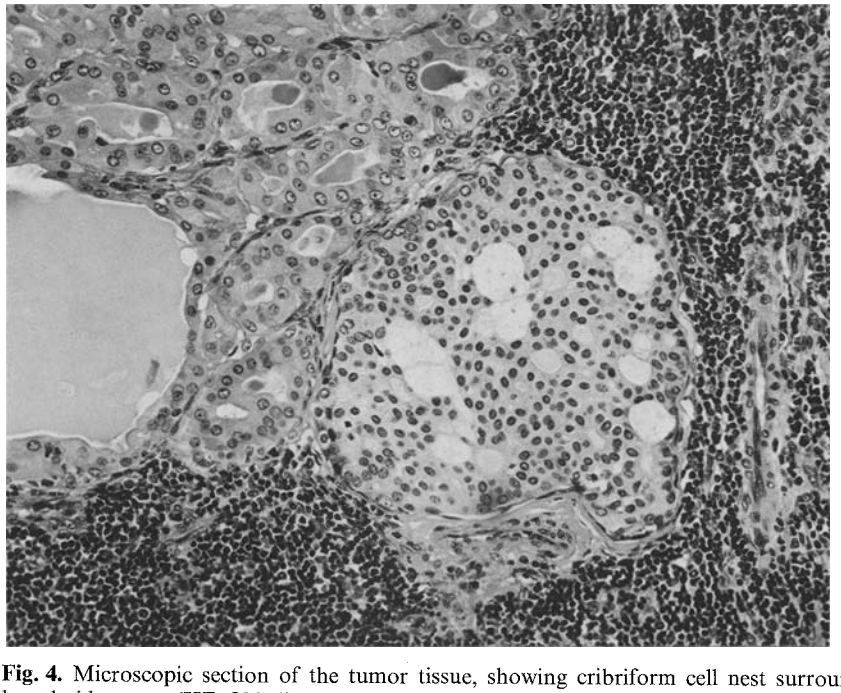

Fig. 4. Microscopic section of the tumor tissue, showing cribriform cell nest surrounded by lymphoid stroma (HE, 200:1)

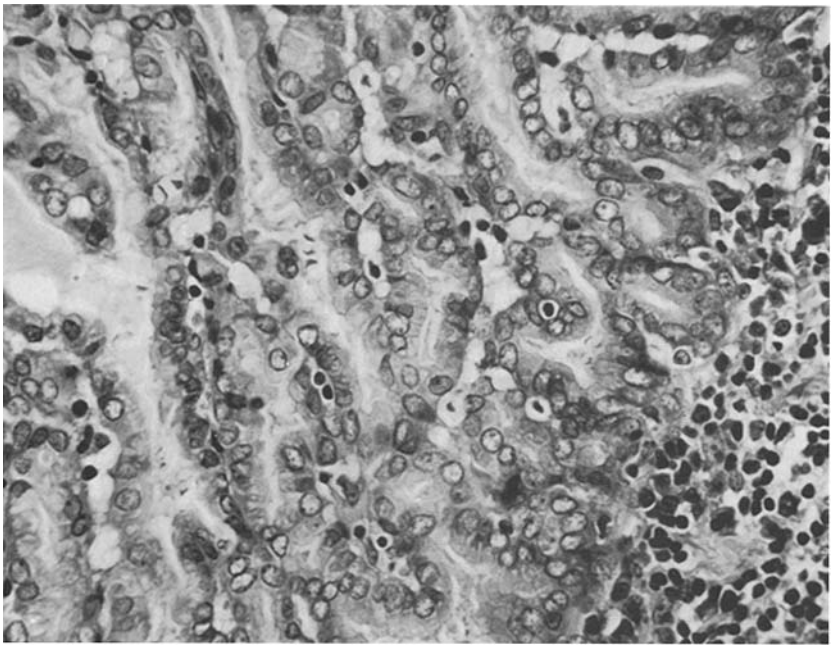

Fig. 5. Microscopic section of the tumor tissue, showing a tubular structure not unlike that encounters in adenocarcinoma (HE, 400:1)

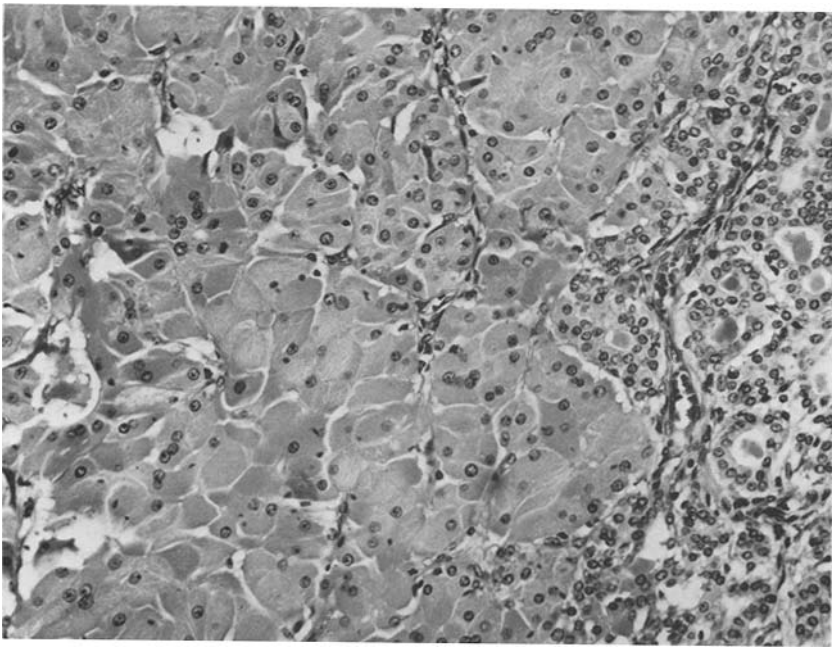

Fig. 6. Microscopic section of the tumor tissue, showing an oncocytoma-like, solid mass of large eosinophilic cells (HE, 200:1)

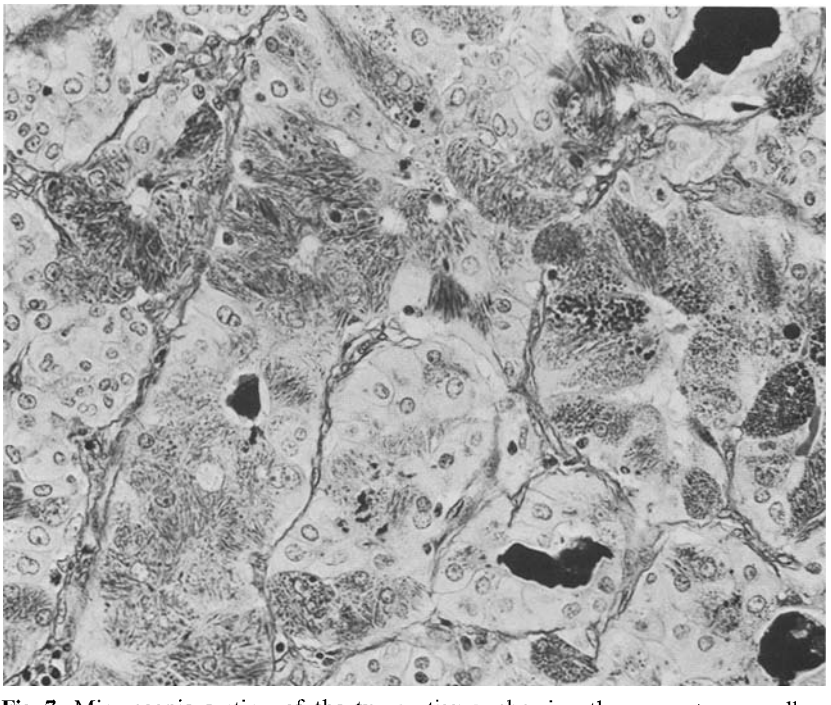

Fig. 7. Microscopic section of the tumor tissue, showing the many tumor cells containing inclusion bodies in their cytoplasm. The inclusions are composed of PAS-positive bundles of long bacilliform (PAS, 320:1)

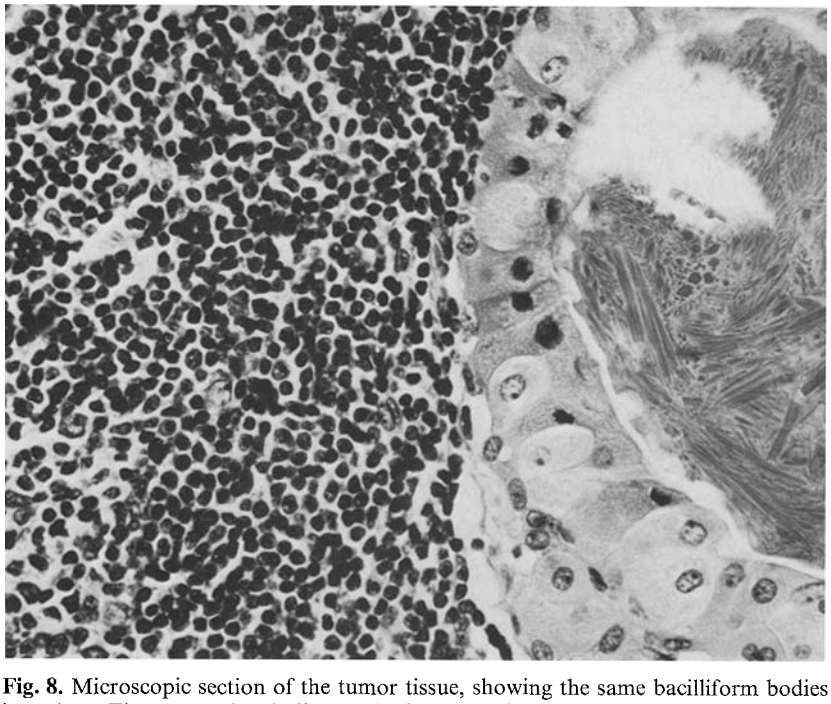

Fig. 8. Microscopic section of the tumor tissue, showing the same bacilliform bodies observed in a duct. They are quite similar to the intracytoplasmic inclusions as shown in Fig. 7 (HE, 400:1)

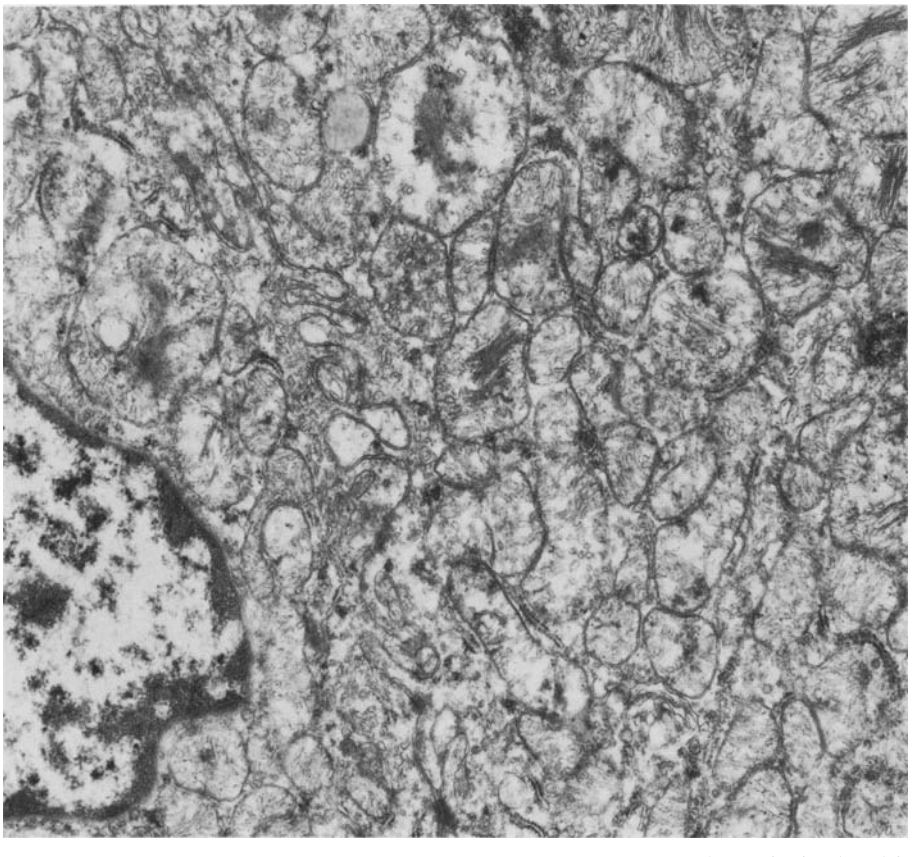

Fig. 9. Electron micrograph showing a tumor cell containing large numbers of mitochondria, which are very large and closely packed together (10,000:1)

Electron microscopic observations

Almost all the tumor cells contain great numbers of mitochondria. In the large cells, the mitochondria are very large, swollen, and closely packed together (Fig. 9), and only a small number of rough endoplasmic reticulum and secretory granules can be seen between them. In the large cells arranged in acinar structure, numerous intracytoplasmic inclusions are found (Fig. 10). The inclusions are composed mainly of long dense bodies enclosed by a single unit membrane, and the contents constitute bundles of fine filament running parallel to the longer axis (Fig. 11). Each filament is 8–10 nm in diameter, with about a 6–8 nm interval in between. The dense bodies occasionally conglutinate each other, forming a complex inclusion in structure. The inclusions often unite with lysosome, and contain electron-dense granules or a lipid-like material arising probably from lysosomes. There are several instances in which these inclusions co-exist with secretory granules in the cytoplasm. In the areas of papillary growth, relatively fewer mitochondria and considerably more numerous free ribosomes are observed.

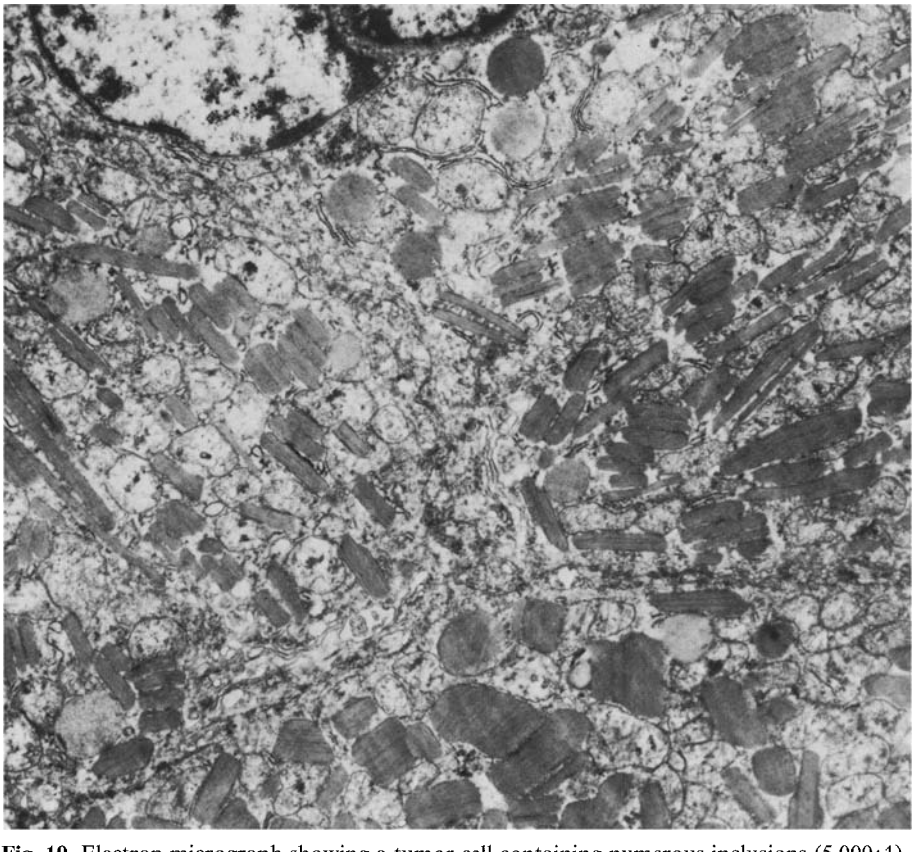

Fig. 10. Electron micrograph showing a tumor cell containing numerous inclusions (5,000:1)

Discussion

The present study described the histologically rare features of a supposed case of malignant PCL. The first task must be to determine whether the present tumor, in fact, belongs to the PCL category. PCL has been reported to occur mostly in the region of the parotid gland of elderly males (Seifert et al. 1980). Ashley (1978) described a case of acinic-cell carcinoma of parotid gland consisting of eosinophilic cells simulating oncocytes. In the present tumor tissue, the acinic cell tumor-like feature could be observed in some areas, but in others monomorphic adenoma-, adenocarcinoma- or oncocytoma-like features were visible. However, electron microscopically almost all of the cells were found to have large numbers of mitochondria, packed together in their cytoplasm, and both the gross appearance and the stromal component with abundant lymphocytes with lymph-follicles were characteristic as PCL. Seifert et al. (1980) subclassified the cystadenoma of the parotid gland into three subtypes, depending on to the ratio of epithelial tumor component to lymphoid stroma. The lymphoid stromal component is only focally pronounced in this tumor. Therefore according

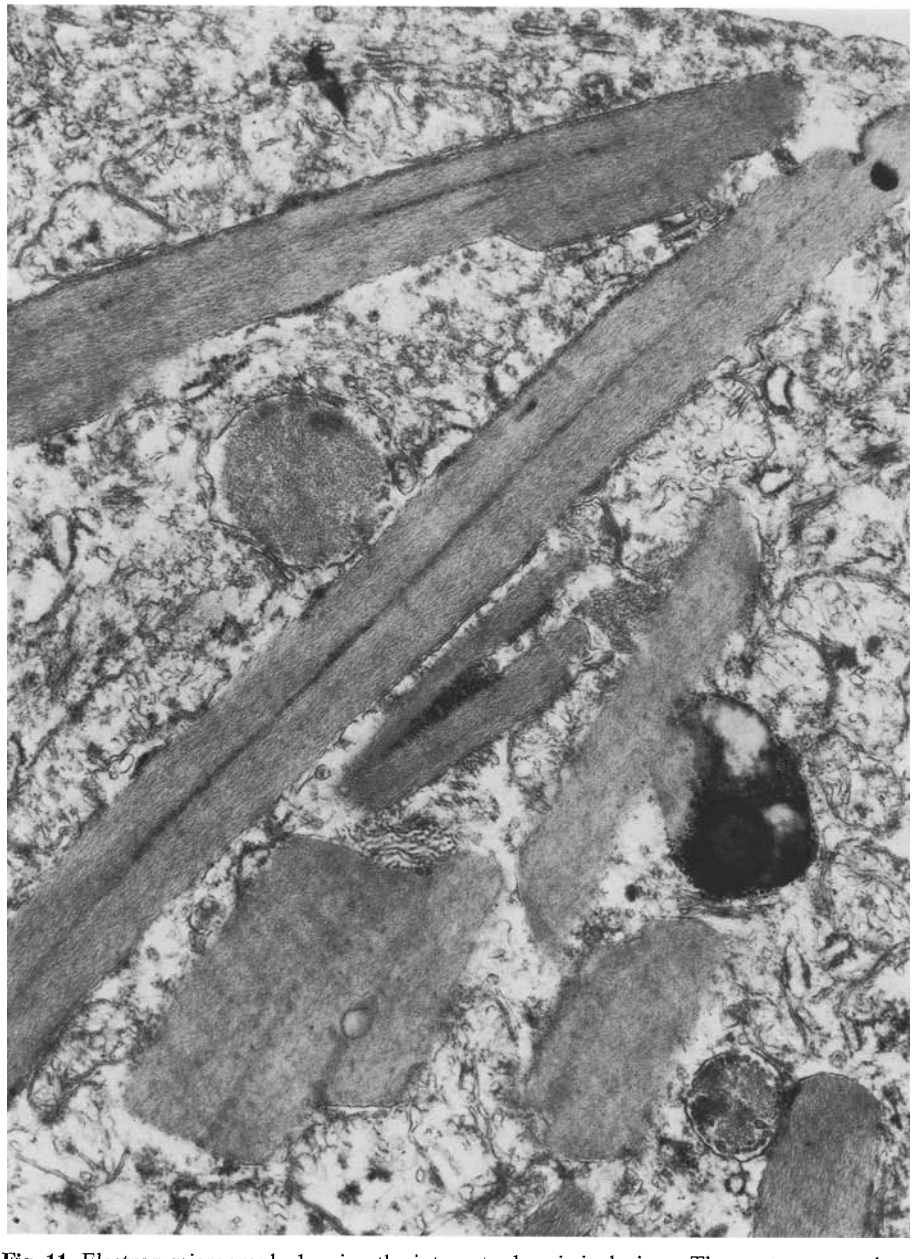

Fig. 11. Electron micrograph showing the intracytoplasmic inclusions. The contents constitute bundles of fine filament running parallel to the longer axis (16,000:1)

to them it is probably classified to "stroma-poor adenolymphoma". It was quite conceivable that the present tumor belongs to the category of PCL, though the typical double row of oxyphil epithelial cells could be observed in only a few areas.

Secondly, one must ascertain the malignant nature of the present tumor.

There has been no evidence to date of metastasis or recurrence (about 13 months after the excisional biopsy) in the case under study, but in the region of papillary growth with few stromal components, mitotic figures were numerous, and generally pleomorphism and atypism were marked. Nuclei were irregularly arranged. In the solid mass of tumor cells, oncocytic cells also had irregularly arranged atypical nuclei. In some peripheral portions, although not pathognomonic for malignancy the small acini tended to penetrate into the fibrous connective tissue. The histologic features of the present tumor rather closely resemble those reported by Ruebner and Bramhall (1960) and Seifert et al. (1977). From all of the above mentioned findings, it was considered that the present tumor should be diagnosed as a malignant PCL in situ. It is also quite conceivable that this tumor did not develop by malignant change from a benign PCL, but rather was malignant in nature from its inception.

Several cases of malignant changes from benign PCL have been described: mucoepidermoid carcinoma (Gadient and Kalfayan 1975), sebaceous carcinoma (Rawson and Horn 1950; McGavran et al. 1960; Wasan 1971; Cameron and Stenram 1979), Squamous cell carcinoma (De la Pava et al. 1965; Assor 1974; Uchibori et al. 1983) adenocarcinoma (Little and Rickles 1965; Kessler et al. 1977; Seifert et al. 1977) cystadenocarcinoma (Fleischer et al. 1980) and undifferentiated carcinoma (Döbrössi et al. 1972; Moosavi 1980) were reported to be derived from PCL. In the present case, various malignant features were observed in separate areas of the tumor tissues. It was reported that a few groups of cells were eosinophilic and displayed the characteristics of oncocytes in an occasional adenocarcinoma of salivary gland (Thackray and Lucas 1974). However, the present tumor tissue seemed to show that the oncocytic cells may well have been transformed into acinic cell carcinoma, papillary carcinoma and mucous secreting adenocarcinoma, instead of oncocytic change from a carcinoma cell. Seifert et al. (1980) suggested that the oncocytic cell proliferation, being observed mostly at greater ages, was due to a disturbance of the immune system.

Another interesting finding was the numerous inclusions occasionally observed in the oncocytic cells surrounding acini. Tandlar and Shipkey (1964) reported crystalloids in the pyramidal cells of PCL and speculated that the inclusions may be accumulations of a secretory product which cannot be liberated from the cells. Sholley et al. (1981) found cytoplasmic crystalloids in parotid acinar cells of rats given a large (6,400 R) single exposure of X-rays to the head and neck. In the cytoplasm of a cell arranged in the outer layer of PCL, Thackray and Lucas (1974) noted a dense body which is somewhat similar to the inclusions found in the present tumor. In the case under study, the inclusions were often found together with secretory granules, and the amorphous parts of the inclusions were similar to the secretory granules both in structure and in electron density. The inclusions had an unit-membrane similar to that of the secretory granules, suggesting that the inclusions had been derived from the secretory granules.

Over the past decade, the authors have examined 122 cases of parotid gland tumor. Fifteen of them (12.3%), including the present case, were PCL and only two cases were females. Two oxyphilic adenomas were ob-

served. The youngest of 15 patients was 48 years old and the oldest was 76 years old; The average age was 65.6 years old. The tumors are common in the seventh and eighth decades.

In the previous study (Takeuchi et al. 1978), an analysis of glycosaminoglycan and glycoprotein in neoplastic and nonneoplastic cells of the salivary gland revealed that the regenerating duct cells as well as the pleomorphic adenoma cells synthesized mainly glycosaminoglycan, whereas acinar mucous cells (nonneoplastic) produced mostly glycoprotein (about 80–90%). In the present investigation, chondroitinase-sensitive material could be found only in minor parts which showed a tubular adenoma-like pattern. Most of the Alcian blue-positive substance observed in the space of cyst or tubular lumen was not diminished by the treatment with any enzymes. The results indicated that mucinous material consists mainly of glycoprotein. The oncocytic cells thus would appear to have the ability to synthesize the same kind of mucinous substance as acinar cells.

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