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CALCIFYING EPITHELIAL ODONTOGENIC TUMOR: HISTOCHEMICAL AND ELECTRON MICROSCOPIC OBSERVATIONS ON A CASE

BY

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ABSTRACT

A case of calcifying epithelial odontogenic tumor was studied histochemically and electron microscopically. This case was characterized by an abundant amount of homogeneous substance and the presence of numerous clear cells. Although the homogeneous substance was shown to be an amyloid material in nature, it was supposed to be an extracellular product of the epithelial tumor cells. The clear cells contained abundant glycogen granules in their cytoplasm.

INTRODUCTION

Although many papers have been written about the calcifying epithelial odontogenic tumor since Pindborg's first description [1], the nature and origin of the homogeneous substance seen in this tumor remain an unsolved problem. It is known that this tumor infrequently contains an unusual number of clear cells (Franklin and Pindborg [2]). The nature of the clear cells is another controversy. We examined a case of calcifying epithelial odontogenic tumor histochemically and electron microscopically and discussed these problems.

CASE REPORT

A 36-year-old man noted a painless swelling in the anterior portion of the right mandible 3 months before the operation. At the time of admission to our University hospital, a firm and ill-defined swelling was seen on the lingual side of the right mandible ($\overline{5\text{–}7}$). A shallow ulcer of the oral mucosa, 8×4 cm in size, was seen on the lingual side of the interdental papilla between the canine and the first premolar. The roentogenography revealed a radiolucent lesion without an evident radiopacity in the upper region of the mandible ($\overline{6\text{–}1}$). Pathologic examination of the tissue removed from the lesion revealed a calcifying epithelial odontogenic tumor. After that, a partial resection of the mandible was performed in June 1978. To date, there have been no signs of recurrence.

MATERIALS AND METHODS

For the histologic study, the materials were fixed in 10% neutral buffered formalin. Serial sections were prepared for histologic reconstruction of the tumor nests.

For the histochemical study, the following stains were employed: Congo red,

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thioflavine T, crystal violet, standardized toluidine blue (STB) (Wolman [3]), van Gieson, p-dimethylaminobenzaldehyde-nitrite (DMAB) reaction (Adams [4]) and periodic acid Schiff (PAS) reaction with or without diastase digestion. Congo red- and STB-stained sections were examined with polarized light. In order to compare the staining results of the homogeneous substance, the following materials were studied: tongue affected with generalized amyloidosis, myeloma kidney, medullary carcinoma of the thyroid gland and hyalinized Langerhans' islets of the pancreas in diabetes mellitus.

For the electron microscopic study, the specimens were taken from the original formalin-fixed materials and refixed in 2.5% glutaraldehyde (phosphate buffer), and the other specimens were originally fixed in 2.5% glutaraldehyde (phosphate buffer). After post-fixation in 2% osmium tetroxide (phosphate buffer) and dehydration, they were embedded in Epon. The ultrathin sections were stained with uranyl acetate and lead citrate and examined with the Hitachi HU-12 electron microscope. Electron microscopic observations were also made on the tongue affected with generalized amyloidosis.

Pathologic Findings

Grossly, the tumor measured 2.5×2.5×1.0 cm. It was gray-white, solid and relatively well demarcated. The tumor was not associated with the embedded tooth.

Microscopically, the tumor consisted of sheets or nests of polyhedral epithelial tumor cells in an abundant amount of stroma. The cytoplasm of the tumor cells

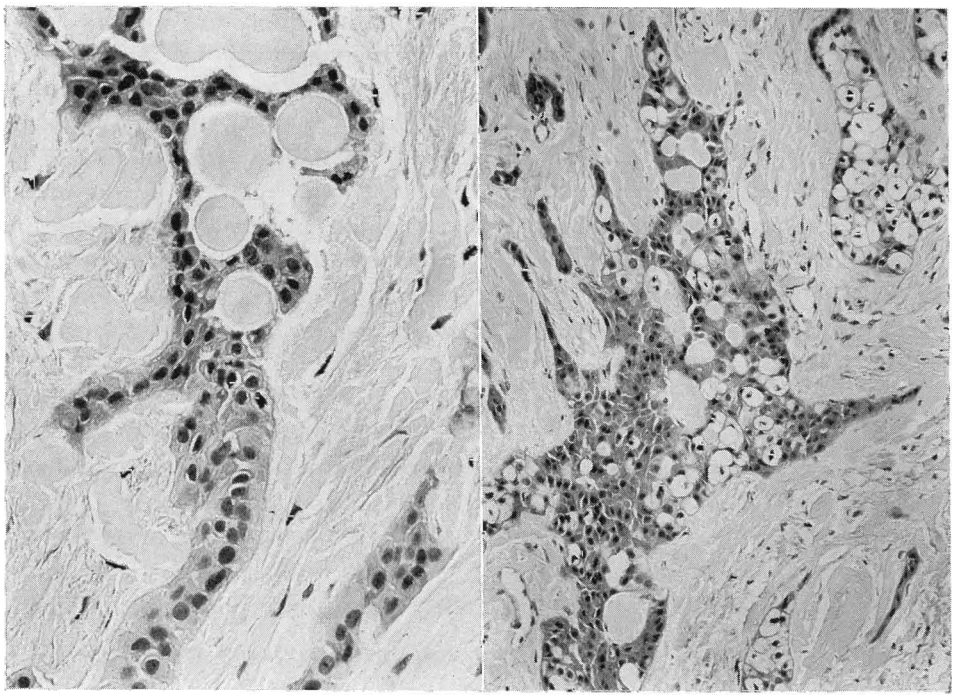

Fig. 1. A homogeneous substance is seen among the tumor cells and in the stroma. H & E. ×300.

Fig. 2. Clear cells scattered in the tumor nests. H & E. ×120.

was eosinophilic and their nuclei were round to oval in shape. Atypism and mitoses were not evident. There was an abundant amount of eosinophilic homogeneous substance among the tumor cells and in the fibrous connective tissue stroma (Fig. 1). The homogeneous substance was occasionally associated with irregular calcification. Histologic reconstruction of the serially sectioned preparations revealed a continuation between the homogeneous substance within the tumor nests and those in the stroma and the isolated presence of the homogeneous substance within the tumor nests. PAS reaction and silver impregnation suggested the lack of the basement membrane between the tumor cells and the homogeneous substance within the tumor nests.

Moreover, the present case was characterized by the appearance of numerous clear cells in the tumor nests (Fig. 2). They showed a voluminous clear cytoplasm with a well-defined cell border and round to oval nuclei.

Histochemically, the homogeneous substance was stained positively with the following reactions: Congo red, crystal violet, STB, van Gieson and PAS. Congo red-stained specimens showed a green birefringency and the STB-stained ones showed an orange-red birefringency with polarized light. The amyloid materials of various sources used in this study showed the same stainability in the above-mentioned reactions. The homogeneous substance was negative by the DMAB reaction. The amyloid materials of the medullary carcinoma of the thyroid gland and hyalinized Langerhans' islets of the pancreas were also negative by the DMAB reaction. On the other hand, the amyloid materials of the tongue

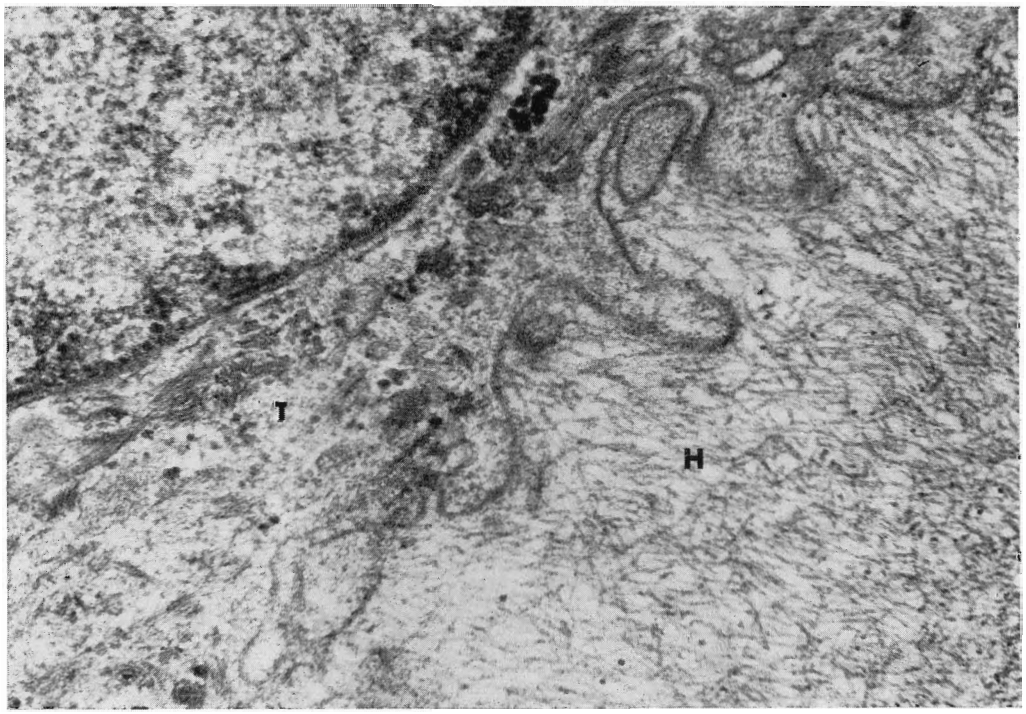

Fig. 3. The homogeneous substance seen histologically within the tumor nest shows a fibrillar structure. Basal lamina is absent between the homogeneous substance (H) and the tumor cell (T). ×6000.

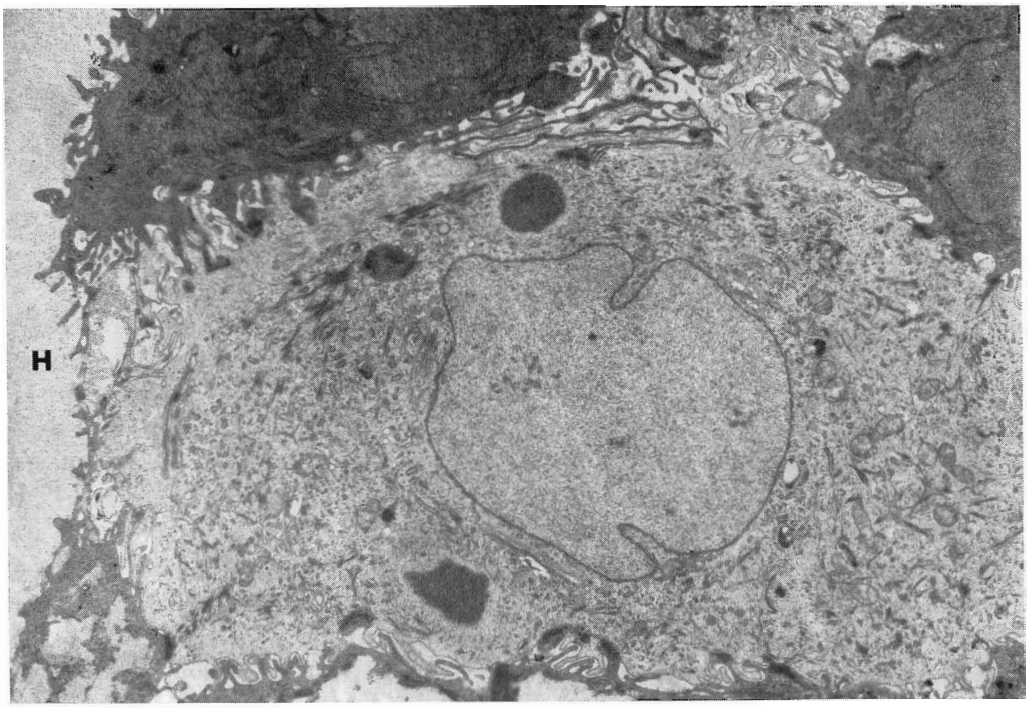

Fig. 4. The tumor cell contains electron-dense bodies in the cytoplasm. H: Homogeneous substance. ×7800.

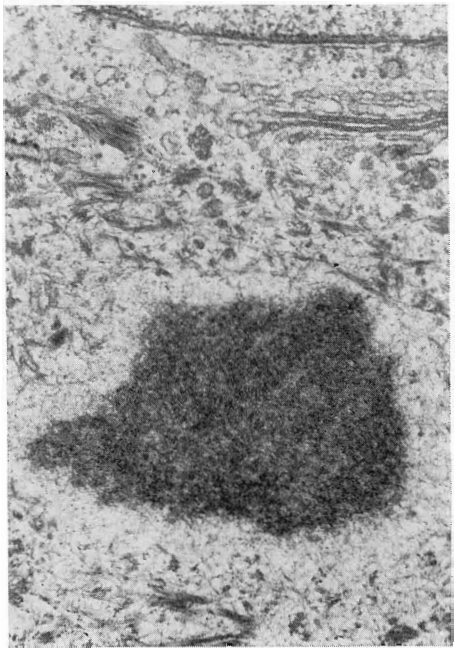

Fig. 5. High-power view of the electron-dense body. ×33700.

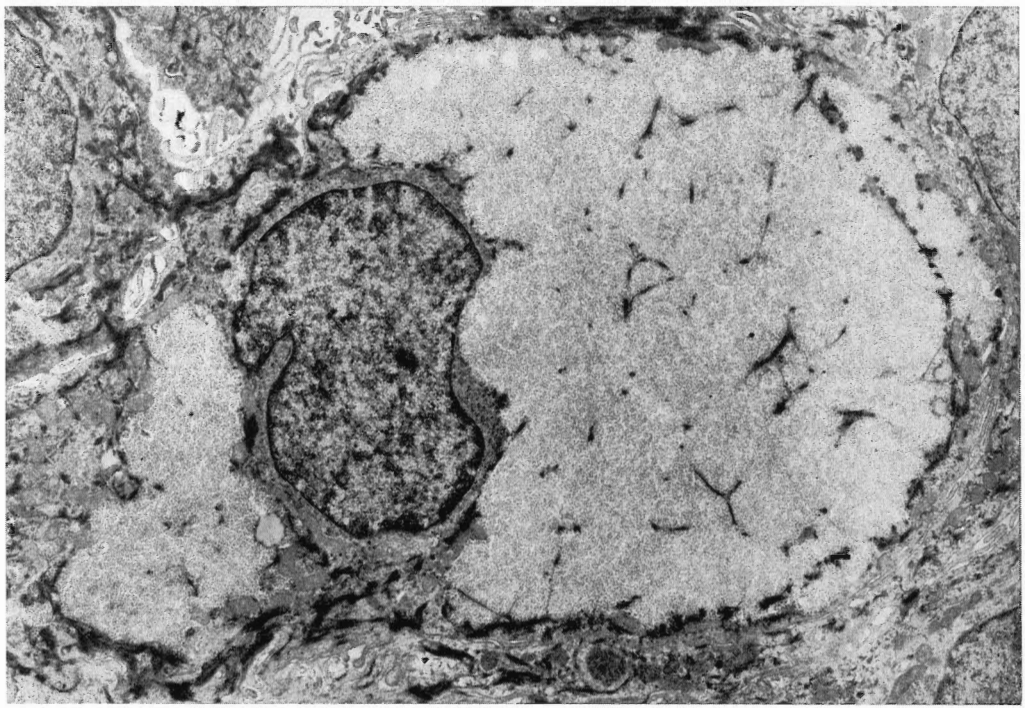

Fig. 6. The clear cell contains numerous glycogen granules in the cytoplasm. ×9000.

with generalized amyloidosis and myeloma kidney were positive by the DMAB reaction. The clear cells contained numerous glycogen granules, which were stained with PAS reaction and digested by diastase.

Electron microscopically, the cell membrane of the adjacent tumor cells showed frequent intercellular digitation of the cytoplasmic processes with associated desmosomes. The cytoplasm was abundant and contained mitochondria and tonofilaments. Sometimes rough-surfaced endoplasmic reticulum, free ribosomes and Golgi apparatus were seen. Small aggregates of glycogen granules were occasionally found in the cytoplasm.

The homogeneous substance seen with a light microscope appeared as either a fibrillar or granulo-fibrillar material. The fibrillar material was rigid and non-branching and measured 10–15 nm in width. It showed the same ultrastructural features as the amyloid material examined in the control material. The fibrillar material within the tumor nests was found directly adjacent to the cell membrane of the tumor cells. A basal lamina was not found between the tumor cells and the fibrillar material (Fig. 3). The cell membranes frequently formed microvillous structures (Fig. 3, 4). Discontinuity of the cell membrane facing the fibrillar material was scarcely seen. The fibrillar material could not be found in the cytoplasm. Sometimes a few electron-dense bodies were seen in the cytoplasm (Fig. 4). They were round to polyhedral, 1–3 μm in diameter, and had a fibrillar structure in the periphery without a limiting membrane (Fig. 5). However, the transition between these bodies and the extracellular fibrillar material was not seen.

The clear cells were filled with numerous glycogen granules in the cytoplasm, and a small amount of tonofilaments and mito-

chondria was seen among them (Fig. 6). These tumor cells were joined to the adjacent cells by the desmosomes.

Discussion

Although histochemical and electron microscopic studies of the homogeneous substance seen in the calcifying epithelial odontogenic tumor have been reported by several authors, its nature and origin remain as disputable problems. Most authors consider the homogeneous substance to be either amyloid (Ranløv and Pindborg [5]; Meenaghan *et al.* [6]; Page *et al.* [7]) or enamel matrix (Chaudhry *et al.* [8]; Mori *et al.* [9]). A few thought keratin (Chaudhry *et al* [8]), basal lamina (Anderson *et al.* [10]) or glycoprotein (Mainwaring [11]) to be the nature of the substance. On the basis of our histochemical and electron microscopic studies, we would support the amyloid concept. The negative stain result of the DMAB reaction, which was considered important in the recognition of the amyloid material (Meenaghan *et al.* [6]; Cooper [12]), may be ascribed to some differences in the chemical components from the classical amyloid (Pearse *et al.* [13]). Other authors (Meenaghan *et al.* [6]; Mori *et al.* [9]) reported a positive stain result of the DMAB reaction in the calcifying epithelial odontogenic tumor. This suggests some diversity in the protein components of the homogeneous substance in each case. On the other hand, Mori *et al.* [9] showed that there are similarities in the histochemical reactions between the homogeneous substance and the enamel matrix of the human developing teeth. However, it is emphasized that the common nature of the amyloid material from the various sources is the beta-protein conformation (Glenner and Page [14]). Proteins of the enamel matrix also have a beta-protein conformation (Glemcher *et al.* [15]). Therefore, it may be possible that the amyloid material in this tumor has some similar structure to the enamel matrix with a beta-protein conformation (Page *et al.* [7]; Glenner and Page [14]).

The origin of the homogeneous substance is another point of discussion. Ranløv and Pindborg [5] and Gon [16] reported on the intracytoplasmic homogeneous substance seen with a light microscope, and Mainwaring *et al.* [11] and Chaudhry *et al.* [8] noted a fibrillar material in the cytoplasm with electron microscopy. In this study the electron-dense bodies having a fibrillar structure in their periphery were seen in the cytoplasm. However, the transition between these bodies and the homogeneous substance could not be found. Though some authors regarded cellular degeneration as important in the formation process of the homogeneous substance, cellular degeneration associated with the formation of homogeneous substance was scarcely seen in our case. The histological reconstruction of the serial sections revealed the isolated presence of the homogeneous substance within the tumor nests and the continuation between the homogeneous substance within the tumor nests and that of the stroma. Electron microscopically the basal lamina was absent between the tumor cells and the homogeneous substance within the tumor nests, and a microvillous structure was found associated with the homogeneous substance. These findings suggest that the homogeneous substance is originally formed by the tumor cells and subsequently transported to the stroma.

Our case was characterized by the appearance of numerous clear cells. Anderson *et al.* [10] interpreted these cells from the electron microscopic observation as a representation of the degenerative process. Our

study revealed that the clear cells contained a large amount of glycogen granules in their cytoplasm. Wallace and MacDonald [17] also noted that they were rich in glycogen. The morphological features of the glycogen granules seen in the clear cells have a resemblance to that of the epithelial cells in the human enamel organ [18]. It might be also considered that the clear tumor cells represent a feature of cytodifferentiation rather than that of the simple degenerative process.

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