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Ultrastructural Study of Varied Calcified Materials in the Pleomorphic Adenoma Occurring in the Soft Palate

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Summary

A case of pleomorphic adenoma derived from the palatine gland was studied with light microscope, transmission electron microscope and X-ray microprobe analysor. Some irregular basophlic masses were found under the light microscope in the stromal tissue close to tumor cells, which were ultrastructurally composed of needle-shaped crystals and bordered by a lamina limitans-like structure. Furthermore, transmission electron microscopic findings showed that numerous membranous vesicles with appatite deposited in various degrees were scattered between collagen fibers. These vesicles seemed to be matrix vesicles which may be related to a stromal change of pleomorphic adenoma.

Introduction

Pleomorphic adenoma is the most common neoplastic lesion of the salivary gland, characterized by the complex combination of epithelial and mesenchymal features (Thackray and Lucas, 1974[14], Ishikawa, 1980[8]). The histogenesis of mesenchymal regions has been studied frequently, however a theoretical consensus has not yet been reached. Although the myxomatous change was recognized as the result of epithelial or myoepitherial function (Yate and Paget, 1952[17], Fukushima, 1968[6], Kraevsky, et al.,1982[10], Dardick, et al., 1982[4]), there was no consensus whether the nature of the chondroid tissue in this tumor was epithelial or mesenchymal in origin (Azzopard and Smith, 1959[2], Welsh and Meyer, 1968[16], Doyle, 1968[5], Huber, et al., 1971[7]). The investigation of stromal change is considered to be important to clarify the nature of this neoplasm. It has already been reported that calcification in ductal content and keratin pearls may occur in old lesions, and has been studied by light microscopy (Thackray and Lucus, 1974[14]). Calcification in a pleomorphic adenoma was also been observed by transmission electron microscopy.

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Case Report

A 58-year old famale was admitted to the second Department of Oral and Maxillofacial Surgery of Matsumoto Dental College Hospital with a complaint of a left palatal swelling which was painless and slow-growing in May, 1983. Clinical examination revealed a localized, elastic soft mass. No other remarkable symptoms were found. Following clinical diagnosis as adenoma, surgical resection was performed. The tumor mass was well circumscrived and measured about 20 mm in diameter. After the operation the prognosis was good, and no evidence of recurrence was disclosed.

Methods

The chief material resected was fixed in 10% formalin solution and partially demineralized in 10% formic acid with formalin for light microscopy. After embedded in paraffin, specimens were sectioned and stained with hematoxylin and eosin (H-E). For electron microscopy and X-ray microprobe analysis, the remnant material was fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4), and post fixed in 1% osmium tetroxide in 0.1 M phosphate buffer (pH 7.4). Minced tissues, untreated or demineralized with EDTA, were embedded in Epon 812. Ultrathin sections, stained with uranyl acetate and lead citrate (U-Pb) or unstained, were observed by a JEOL JEM-100-B electron microscope. Furthermore, undemineralized and unstained sections were analyzed by means of a JEOL JEM-1200 EX and a Tractor Northern TN-2000 energy dispersive X-ray spectroscope (EDS).

Results

Light microscopic findings:

The tumor mass was well defined and encapsulated with thin fibrous connective tissues. The gross view of the lesion showed many tubular structures, sheets or strands, solid or diffuse proliferation of epithelial and myoepithelial cells, and hyalinized or myxomatous stroma. Squamous metaplasia or keratin pearl formation was not frequent (Fig. 1). In some areas, masses strongly stained with hematoxyline, being irregular in size and shape, were present on the undemineralized sections and were approximately 25μm to 150μm in diameter. Some cells were observed around these, and hyalinization was not so strong (Fig. 2). Fig. 3 shows an area of poor cellular zone, composed of plump and round cells. Vacuolation change occurred in the cytoplasms of some cells which resembled cartilage cells. Although calcified materials were seen, cartilagenous formation did not occur. In addition, stromal tissue was seldom hyalnized. The material corresponding to the region of Fig.3 was examined using an electron microscope, the findings will be refered to later on.

Electron microscopic findings:

In the demineralized specimens stained with U-Pb, the calcified mass showed a moderate electron dense, and had numerous needle-shaped spaces. The mass was circumscribed by an irregular electron dense line resembling lamina limitans. Many small globules attached to and/or fused with this border. Some epithelial cells containing tonofilaments were found adjacent to the calcified masses. Elongated cytoplasmic process or cell clusters laid either in contact with or close to lamina limitans. Collagen fibers were tightly packed among intercellular spaces (Fig.4). In the untreated specimens, neither demineralized nor stained, needle-shaped crystals deposited in the masses. These crystals were distributed randomly in most areas (Fig.5), and arranged in radial fashion in some

areas (Fig.6). Regarding ultrastructural features of the region corresponding to Fig.3, numerous membranous vesicles, from 0.1μm to 2μm in diameter, intermingled with collagen fibers. In the demineralized and stained specimens the vesicles consisted of unit membrane (Fig.7). On the other hand, in the untreated specimens the electron dense crystals were deposited peripherally, centrally or generally in the vesicles, but some of them had no crystals (Fig.8). Ultimate analysis revealed that the composition of these crystals was both calcium and phosphorus (Fig.8, inset). The intimate relationship between these mineralized vesicles and calcified masses with lamina limitans-like structure was not shown.

Discussion

In pleomorphic adenomas, cartilage or pseudcartilage, and bone or osteoid sometimes appear in the stroma, the latter is not so common (Ishikawa, 1982[8]). In our case, although, there was neither typical cartilage nor bone formation, light microscopical study revealed small calcified masses. These calcifications may occur based on intraductal content or keratinized substance (Thackray and Lucas, 1974[14]). Because the squamous metaplasia was extremely rare in this case, many of calcified bodies were thought to originate from concerning ducts. The initial site of mineral deposition might not be biological, but distrophic. Developed masses caused the duct lining cells to degenerate, and become free in the stromal tissue. Ultrastructural findings of the developed mass showed the electron dense border similar to lamina limitans (Scherft, 1972[11]), globular structures, and also tumor cells and cell clusters found around the mass. These appearances could suggest that calcified masses might continue to grow gradually in biological course.

Another interesting feature was the existance of numerous mineralized membranous vesicles intermingled with collagen fibers observed under a transmission electron microscope. These vesicles were reported previously in other cases such as mucoepidermoid carcinoma (Kawakami, et al., 1986[9]), cardiac myoma (Tanimura, et al., 1985[13]), and aging caltilage (Bonucci and Deardon, 1976[3]). It was concluded that they acted as matrix vesicles. Anderson (1969[1]) and others emphasized that the function of the vesicles was initiation of calcification. Thyberg and Friberg (1970[15]) reported that matrix vesicles were classified into type I and II in the epipheseal caltilage. They thought that type I intimately related with calcification, and type II had lysosomal enzyme, which was important to resolution of proteoglycans. We regard the role of membranous structures as type II from the result of ultimate analysis. But their function as type I is uncertain so that their lysosomal activity was not examined.

It was thought that chondrogenesis in this neoplasm is depended upon epithelial cell, myoepithelial cell or stromal mesenchymal cell. There are problems such as whether true chondrocyte exists or not, and what the origin of chondrocyte is. Doyle (1968[5]) refered that neoplastic myoepthelial cells underwent partial metaplasia enabling them to produce a typical chondroid matrix. And Stacey, et al. (1981[12]) thougt that neoplastic cells which were not myoepithelial cells had multidirectional potential differentiating to chondrocyte. But Welsh and Meyer (1968[16]) stated that this tumor was not purely of epithelial origin because of the existence of two kinds of cartilage cells; one was epithelial in origin, and the other was mesenchymal in origin. That is, the latter were true cartilage cells. In our case, the area with calcified membranous structure closely resembled aging cartilage, and also the vacuolated cell found in this area was similar to chondrocyte. Assuming that such an area could change into pseudcartilage, the functions of matrix vesicle and degeneration of cell may related to this change. It is thought that cartilagenous tissue could appear as a result not

only of metaplasia or differentiation, but also due to other courses, as mentioned above.

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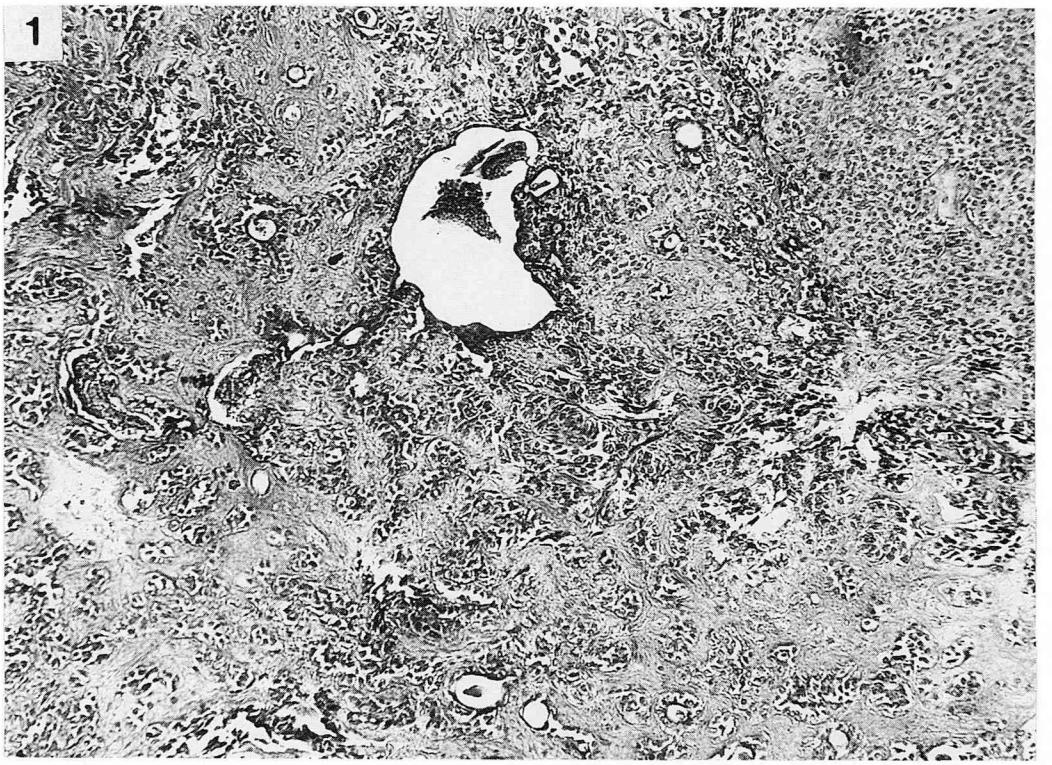
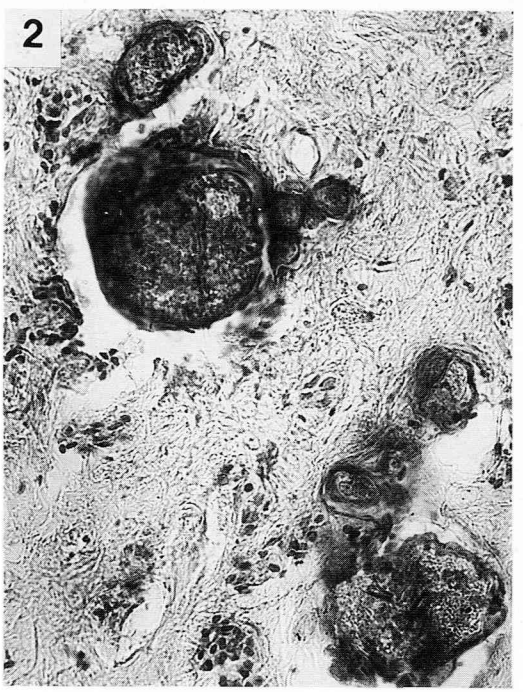




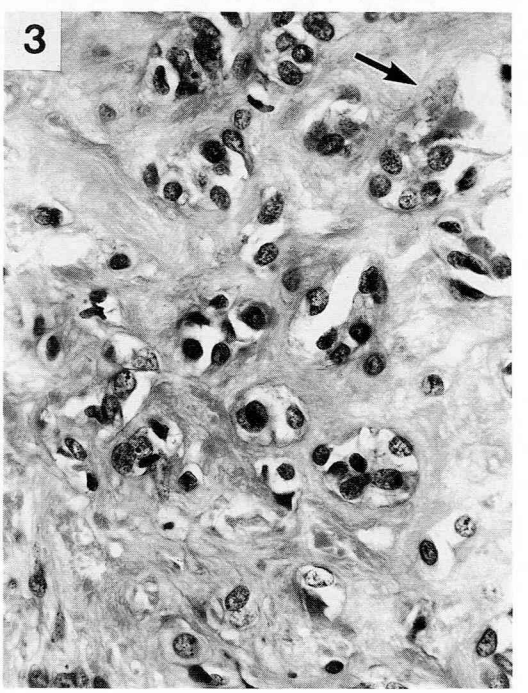
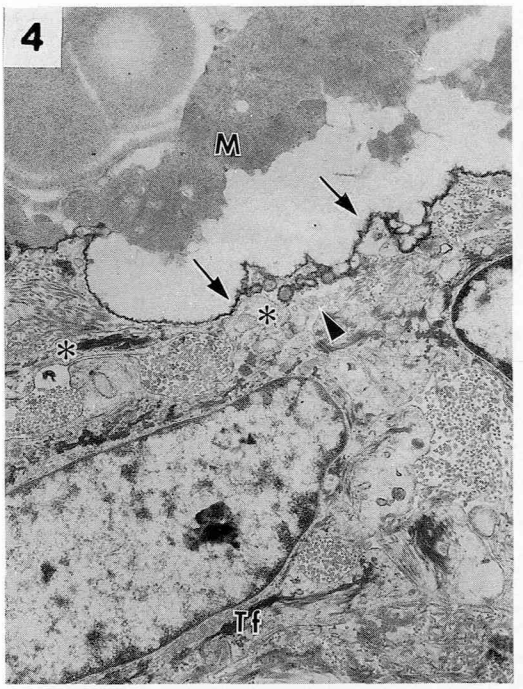
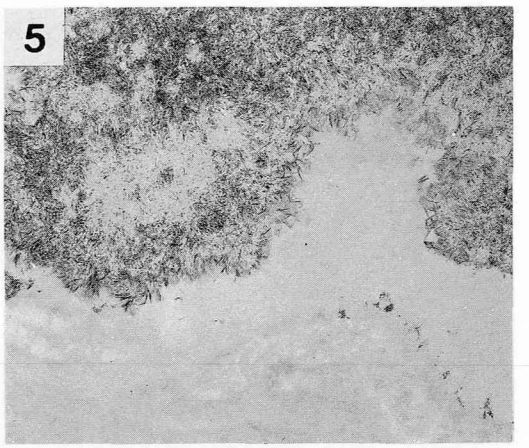
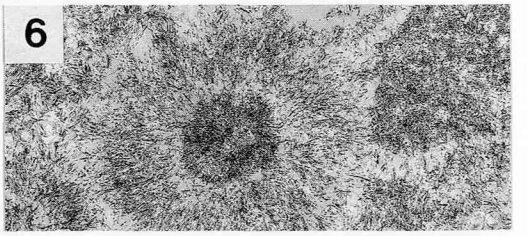


Fig. 1. A general histological view showed variable proliferating pattern of epithelial cells: duct formation, sheets or strands and solid nests, and hyalinized or myxoid changes in the stroma. H-E stain. ×125.
Fig. 2. Hematoxylin positive masses were evident in undemineralized specimen. H-E stain. ×190
Fig. 3. Calcified material became unclear (arrow) in demineralized specimens, but vacuolated changes in cytoplasms were seen, H-E stain. ×400







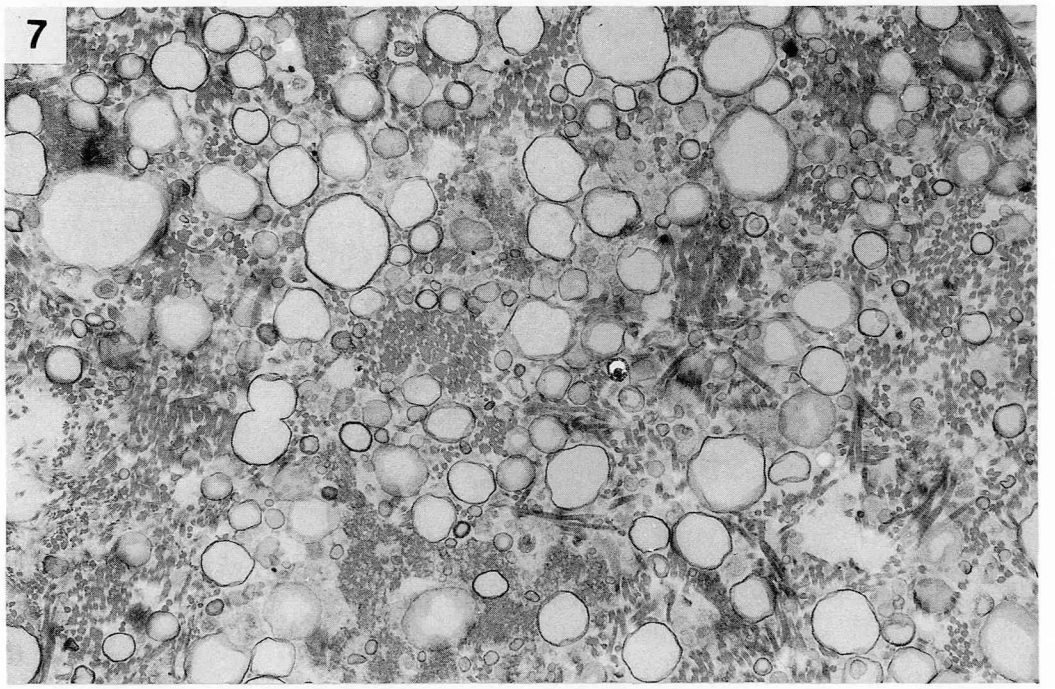


Fig. 4 . Ultrastructural feature disclosed the mass bordered by electron dense line (arrows) adjacent to the cytoplasms (asterisks) of tumor cells with tonofilaments (Tf). Arrowhead showed cell clusters. Demineralized specimens. U-Pb stain. ×5,700

Fig. 5 . Undemineralized and unstained specimen showing depositon of needle-like crystals in disorder. ×9,000

Fig. 6 . Crystals arranged in radial fashion. Undemineralized and unstained specimen. ×19,000

Fig. 7 . Membranous and vacuolated vesicles dispersed between collagen bundles in demineralized specimen stained with U-Pb. ×12,000

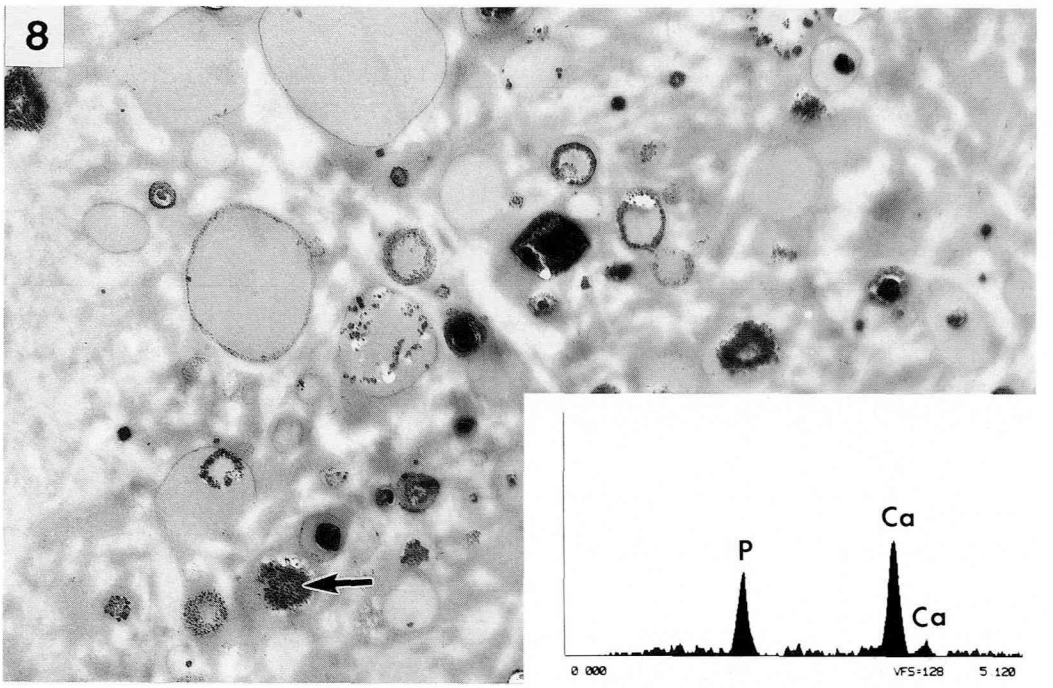


Fig. 8. Crystals deposited in variable degrees. Undemineralized and unstained. ×14,000
Inset : ultimate analysis with EDS of the body situated in the figure (Arrow).