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Light and Electron Microscopic Studies of Microcalcifications Appearing in Monomorphic Adenomas

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Summary

Microcalcifications appearing in two cases of monomorphic adenomas were studied histopathologically, electron microscopically, and electron-microanalytically. One case was basal cell adenoma that occurred in a 56-year-old man and the other was canalicular adenoma in a 71-year-old woman. The calcified granules were observed both in the lumina formed by the tumor cells and in the stromal tissues. The surroundings of the granules were stained by alcian blue and showed a sulfur peak by EPMA. These facts suggest that the surroundings contain sulfated glycosaminoglycans and that sulfur has a significant role in the mechanisms of pathological calcification as well as physiological calcification.

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Introduction

Microcalcifications are found in both non-neoplastic and neoplastic tissues. The former includes meninges choroid plexus, pineal boby and thyroid, and the latter is most common in the meningiomas, papillary tumors of the thyroid and ovary[11]. In addition, they can be seen in tumors derived from the other organs such as the breast, lung, kidney, endometrium, stomach, pituitary gland[11], and salivary gland[6]. Although microcalcifications are most common in pleomorphic adenoma, the calcification is extremely rare in other salivary gland tumors[7].

The purpose of the present article is to report the light and electron microscopic findings of the microcalcifications appearing in monomorphic adenomas and to discuss the role of sulfur in the mechanisms of pathological calcifications.

Materials and Methods

The specimens used in the present study were obtained from two patients clinically diagnosed as salivary gland tumors.

Case 1:

A tumor, located in the right sublingual region and in 2.3×1.7×1.2 cm in size, was surgically removed from a 56-year-old man. It was examined histopathologically and diagnosed as basal cell adenoma. The patient was discharged from an eventful postoperative course.[17]

Case 2:

A globular tumor mass, 1.5 cm in diameter, was enucleated from the right floor of the mouth of a 71-year-old woman and was submitted for histopathological examination. The diagnosis of canalicular adenoma was rendered. The postoperative course was not eventful.

Both specimens, fixed in 10% formalin, were processed for light microscopy by the following stainings: hematoxylin-eosin (H-E), van Gieson's, alcian blue, toluidine blue (pH 2.5), and von Kossa's.

For transmission electron microscopy, after removing the paraffin, the remnant materials were washed with 0.1 M cacodylate buffer, fixed in Karnovsky's solution, and embedded in Epon 812. The specimens were not treated with any demineralization. To obtain good orientation and suitable specimens, 1μm thick sections were stained with toluidine blue and checked by light microscopy. Ultrathin sections were cut with a LKB ultratome equipped with a diamond knife (Diatome: Switzerland) and observed under a JEOL JEM 100-B electron microscope operating at 80KV. For scanning electron microscopy and electron probe microanalysis (EPMA), the specimens were processed by critical-point drying and coated with carbon by cathodic sputtering to be examined with a JCXA 733 Super Probe scanning electron microscope.

Results

Light Microscopy:

Both tumor masses, covered by a thin fibrous tissue, were composed of cuboidal and/or columnar cells with rather large, deep bosophilic, round to ovoid nuclei. Scant cytoplasm was occasionally found. No mitotic figures were observed in any fields. In case 1, the tumor cells generally formed solid nests and partly luminal or cystic structures at the center of the nests (Fig. 1). On the other hand, the tumor cells of case 2 were closely packed and formed wide-spread cyst-like lumina. In both cases, many lumina contained a bosophilic spherical shaped materials (Figs. 1, 2

arrows). They were positive to von Kossa's stain (Figs. 3, 4 arrows), but their periphery was partially negative to the stain. Intercellular stroma was scant and inconspicuous. In a part of the intercellular stroma, von Kossa's positive small granules (Figs. 3, 4), which could not be confirmed in the H-E stained sections, were also found (Figs. 3, 4). These areas were stained uniformly red by H-E, blue by Mallory's azan stain and by alcian blue stain (Fig. 5), red by van Gieson's. Furthermore, the areas showed metachromasia by toluidine blue (pH 2.5).

Transmission Electron Microscopy:

Ultrastructurally, the spherical shaped materials in the lumina of the both cases seemed to be formed by the fusion of adjacent smaller globules and were sharply delimited, surrounded by aggregates of electron-moderate fine globular and/or granular materials (Fig. 6). The calcifications exhibited a relatively electron-lucent center and an electron-dense peripheral end was composed of fine fibriller or needle-like crystallites. No calcified cores could be observed in these structures.

In case 1, the light microscopical von Kossa's positive granules in the intercellular stroma were 0.2-3μm in size and distributed both in fibrous connective tissues (Figs. 7, 8) and in multilayered basal laminae (Fig. 9). Many parts of these fibrous connective tissues were electron dense and partially hyalinized. In case 2, the granules were also located in the relatively electron-dense intercellular stroma, but the distinction between the basal laminae and fibrous connective tissues were not clear.

The small granules were round or ovoid in shape, but a small number were cuboid (Fig. 8), and made of various forms of calcium deposits: amorphous form or crystalline forms of polyheadral or needle shapes, and some deposits formed concentric laminae. The relationship between the calcium depositions and the membrane of vesicles could not be found.

Scanning Electron Microscopy and Electron Probe Microanalysis:

Under the scanning electron microscope (composition image), relatively large spherules in the lumina and many small granules in the intercellular connective tissues of both cases were recognized as light structures (Figs. 10, 11). EPMA revealed that they mainly consisted of both calcium and phosphorus and that the sulfur peak existed in the contiguous tissues (Figs. 12A-D).

Discussion

Both basal cell adenoma and canalicular adenoma are relatively rare and belongs in a classification of monomorphic adenoma. Although variety changes, including pathological calcification, appear in pleomorphic adenoma (Fukushima 1968[4], Hasegawa et al. 1987[5], Kameyama et al. 1987[6]), these two tumors show very little changes in the stroma.

To the best of our knowledge, no research has been done concerning the microcalcifications in monomorphic adenoma. Therefore, both of our cases having calcification are thought to be extremely rare.

Recently, many facters regarding calcification have been taken up[9]: collagen fiber, proteoglycan (glycosaminoglycans), lipid, osteocalcin, and matrix vesicle. As the calcification proceeds, some of them are thought to remain as the structural component in the calcifying matrix while others disappear. Chondroitin sulfate, a major constituent of proteoglycans and the most highly acidic substance, has been thought to play a role as calcified nuclei, because of their strong calcium-binding capacity[9]. Now, it seems more likely that these compounds act as inhibitors of

calcification and have to be removed before mineralization can occur[1~3,10,12,13,15,16].

Regarding the quantitative and qualitative changes of glycosaminoglycans in the calcifying process, many reports have shown the decrease of glycosaminoglycans (particulary sulfated glycosaminoglycans) with the development of calcification in cartilage[1~3,10,12,13,15,16]. The fact that the surroundings of the calcified masses of the intercellular stroma were stained by alcian-blue and metachromatic by toluidine-blue (pH2.5) in our study strongly suggests the existance of sulfated glycosaminoglycans within these areas. The presence of a sulfur peak in the contiguous tissues to the calcified masses may support this suggestion. These may also indicate a significant role of sulfur (chondroitin sulfate, that is sulfated glycosaminoglycans) in the mechanisms of pathological calcification as well as a physiological one.

Fukushima (1968)[4] has reported the abnormal epithelial secretions in pleomorphic adenoma and adenoid cystic carcinoma. Recentry, Yamazaki, et al. (1987)[17] reported a case of basal cell adenoma, which is the same as one of our cases (casel), and described the relationship between the abnormal epithelial secretions and the stromal calcifications. Judging from the fact that the cellular basement membrane was positive to PAS reaction, the origin of sulfur responsible for calcification is thought to be the tumor cell.

On the other hand, a membrane of vesicles has been repeatedly demonstrated at the initial calcification[7,8]. Intracytoplasmic organellae, especially mitochondria, have been considered to be a focus of intracytoplasmic calcification in cartilage, bone, or aortic valve[8], with consequent discharge from the cytoplasm and conversion to a matrix vesicle. We were unable to find an obvious membrane of vesicle despite careful observation. Similar findings, namely the calcification having no relationship to the vesicles, were observed in duodenal carcinoid tumor by Murayama et al. (1979)[11]. Although there is no denying the matrix vesicle type in our cases because of poor fixation, we postulate that some of the calcifications will be occurred without concerning membrane of vesicles.

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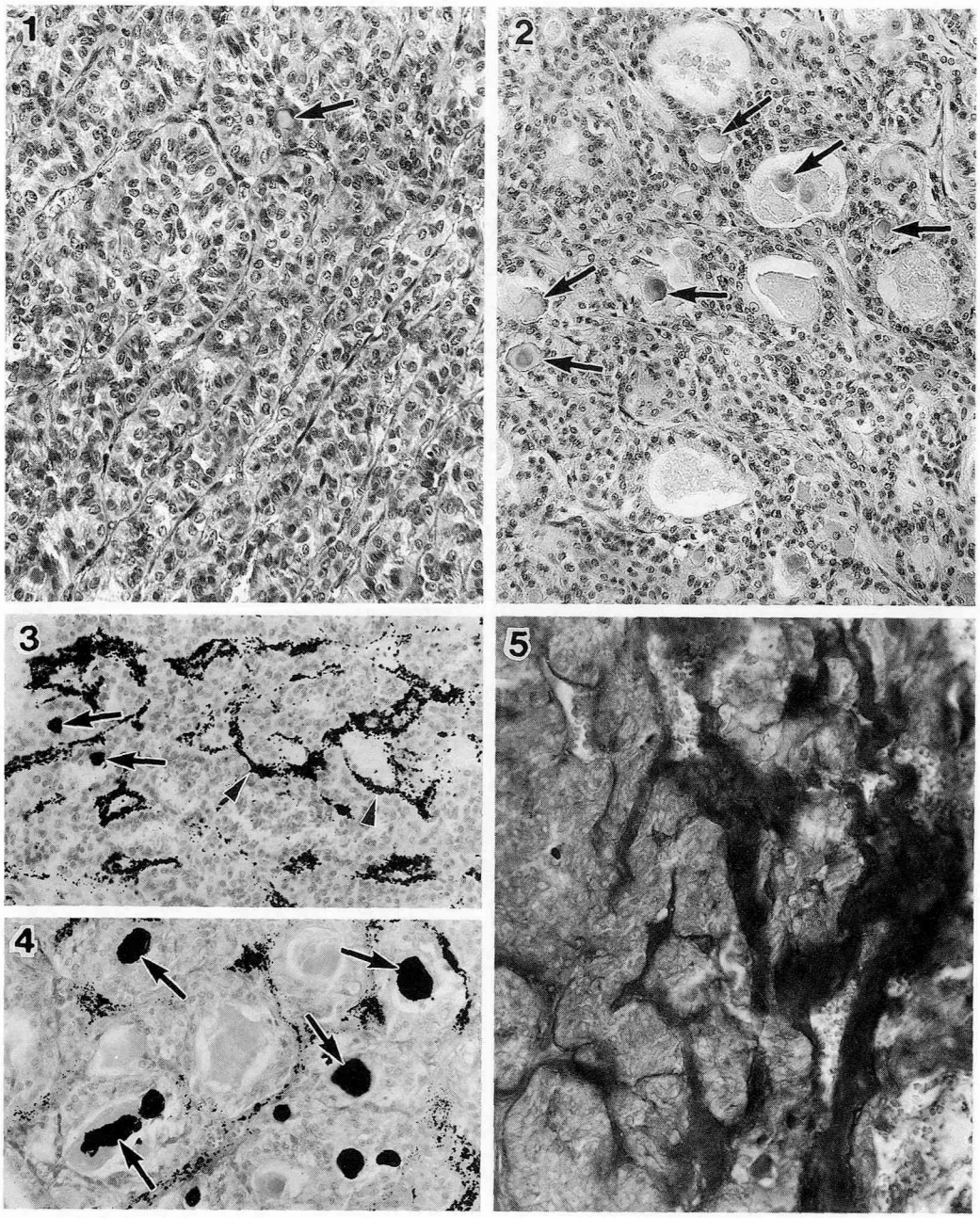


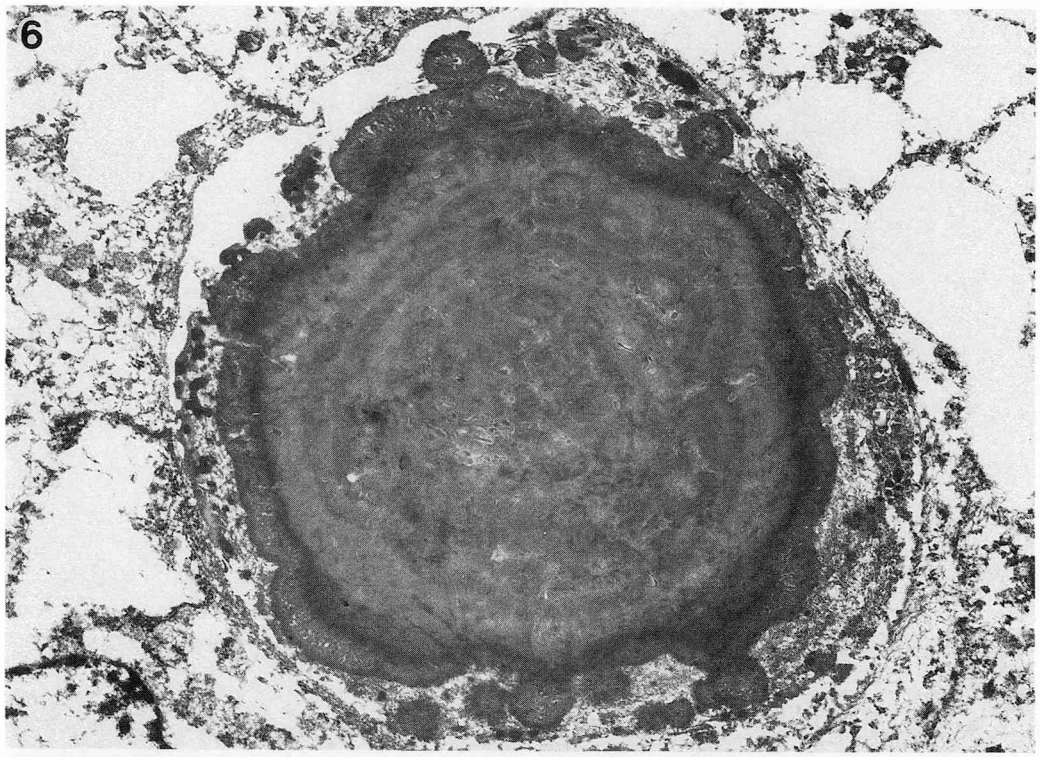
Fig. 1 : Tumor cells are arranged in a trabecular or solid pattern. Basophilic spherical shaped materials in the luminal structure (arrow). (case 1, H-E, ×230)

Fig. 2 : Spherical shaped materials (arrows) in some of the luminal spaces.(case 2, H-E, ×150)

Fig. 3 : Von Kossa's positive granules (arrowheads) in the intercellular stroma and spherical shaped materials in the lumina (arrows). (case 1, ×150)

Fig. 4 : Spherical shaped materials in the lumina (arrows) are positive to von Kossa's stain.(case 2, ×150)

Fig. 5 : Intercellular stroma was stained blue by alcian blue stain.(case 1, ×230)



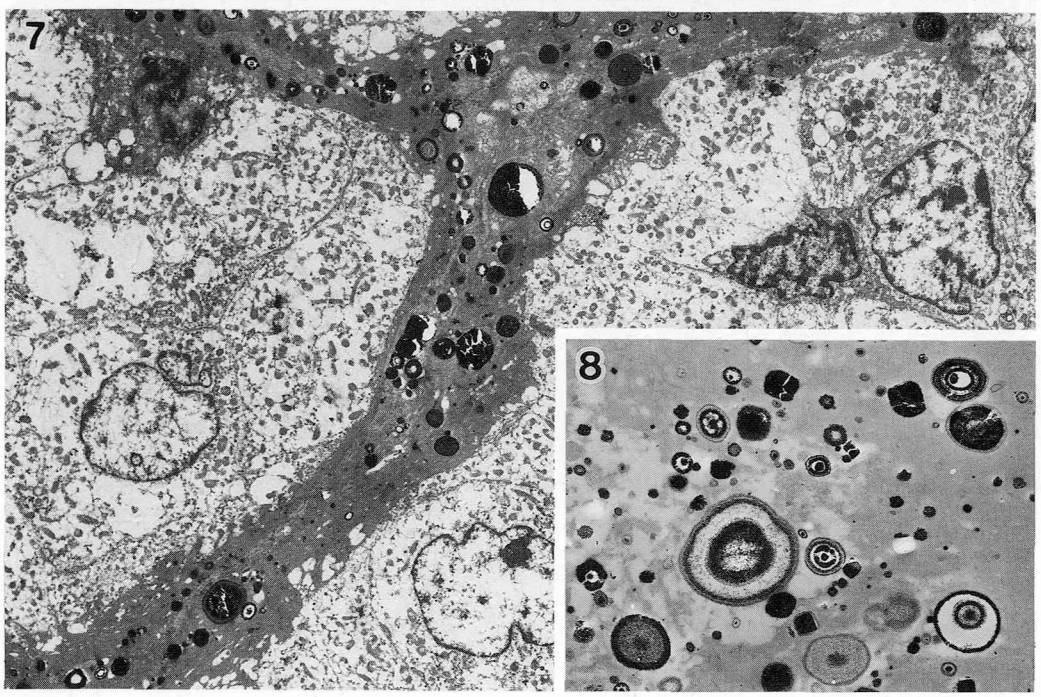
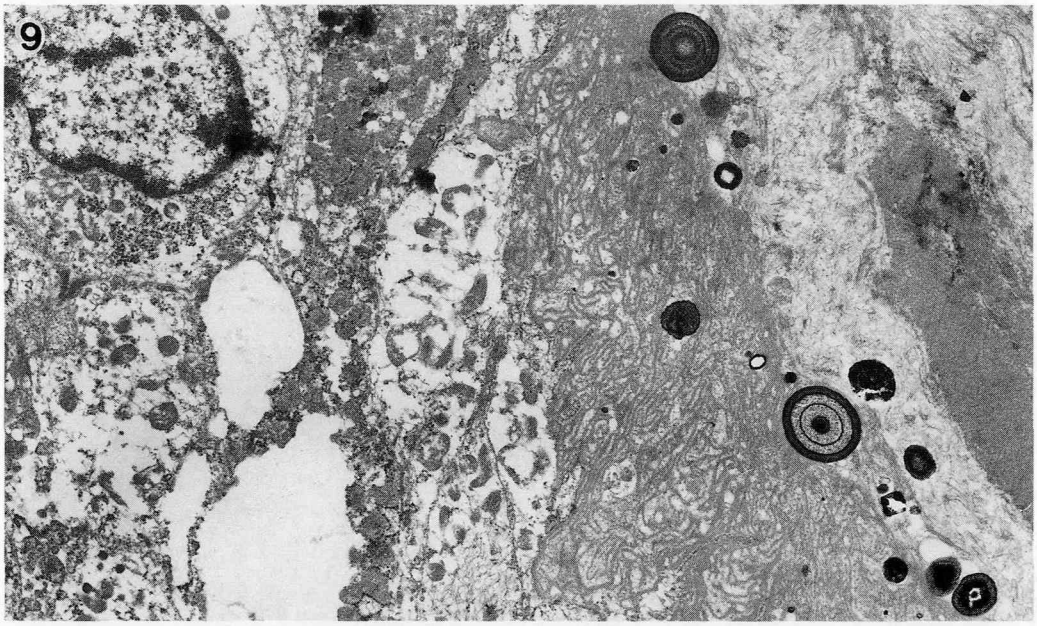
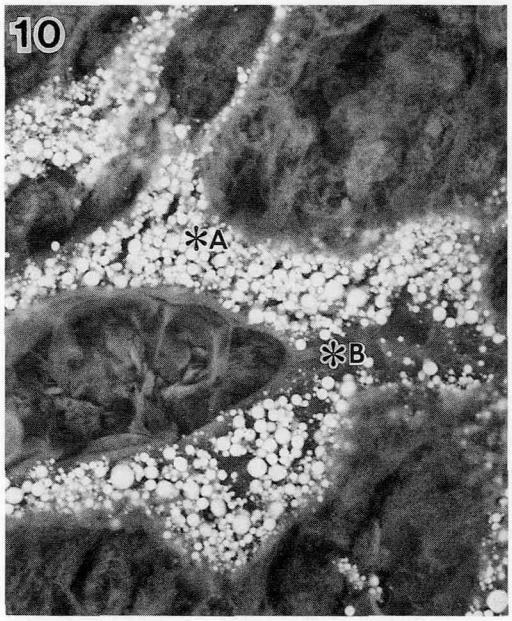


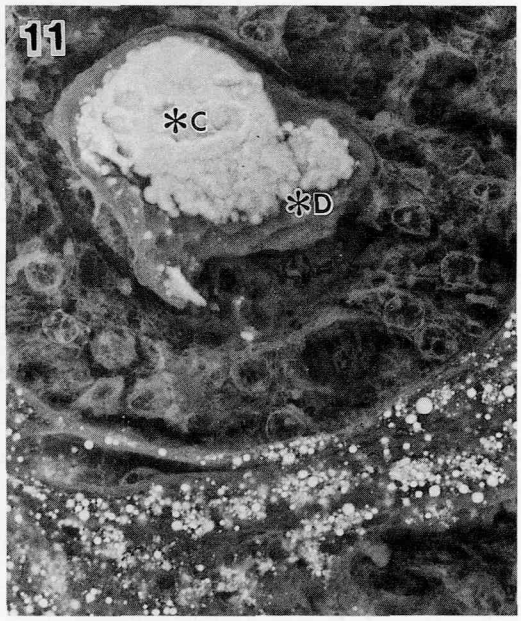
Fig. 6 : Spherical shaped material in the lumen formed by the fusion of adjacent smaller globules. (case 2, TEM×6,500)

Fig. 7 : Electron-dense granules scattered in fibrous connective tissues. (case 1, TEM×3,100)

Fig. 8 : The granules are round or ovoid in shape, and shoving lamelated appearance or solid. (case 1, TEM×5,500)







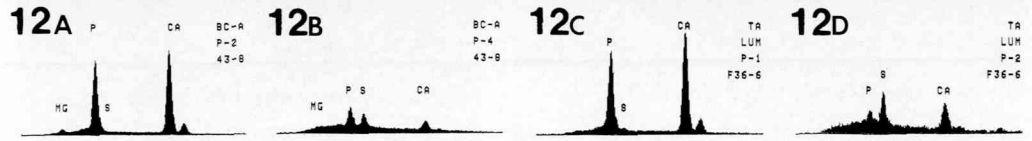


Fig. 9 : Electron-dense granules within the multilayered basal lamina. (case 1, TEM×9.000)

Fig. 10 : There are many calcifying granules (*A) in the intercellular stroma (*B). (case 1, SEM Composition image ×1,200)

Fig. 11 : Spherical shaped materials (*C) in the lumen and granules (*D) in the intercellular stroma are showing calcification. (case 2, SEM Composition image ×950)

Fig. 12 : Results of EPMA of points A, B (Fig. 10), C and D (Fig. 11). The surroundings of both the granule and the spherical shaped material show a composition sulfur in addition to Calcium and Phosphorus.