Simultaneous Occurrence of Calcification and Amyloid Deposit in Pilomatricoma

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Amyloid deposition was encountered in 10 of 16 samples of pilomatricoma, indicating that the deposition of amyloid is nearly as common as calcification in pilomatricoma. In addition, a simultaneous occurrence of calcification and amyloid deposit in pilomatricoma was recognized in 9 of 16 samples. The calcification and the deposition of amyloid developed topographically in the same area within the shadow cell masses. Such an area was revealed as moderately basophilic, amorphous, or hyalinized by H&E staining. Electron microscopy revealed spotty calcium deposits in amyloid. No light chains of human immunoglobulin were detected in the amyloid-deposited area. Amyloid in this tumour may facilitate calcification or serve as a matrix for subsequent calcification.

Key words: Shadow cell; Histology; Electron microscopy.

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Pilomatricoma or calcifying epithelioma of Malherbe is a unique skin tumour in which calcification frequently occurs. Recently, deposition of amyloid has been demonstrated in pilomatricoma (1). This study was therefore undertaken to confirm the deposition of amyloid in 16 collected samples of pilo-

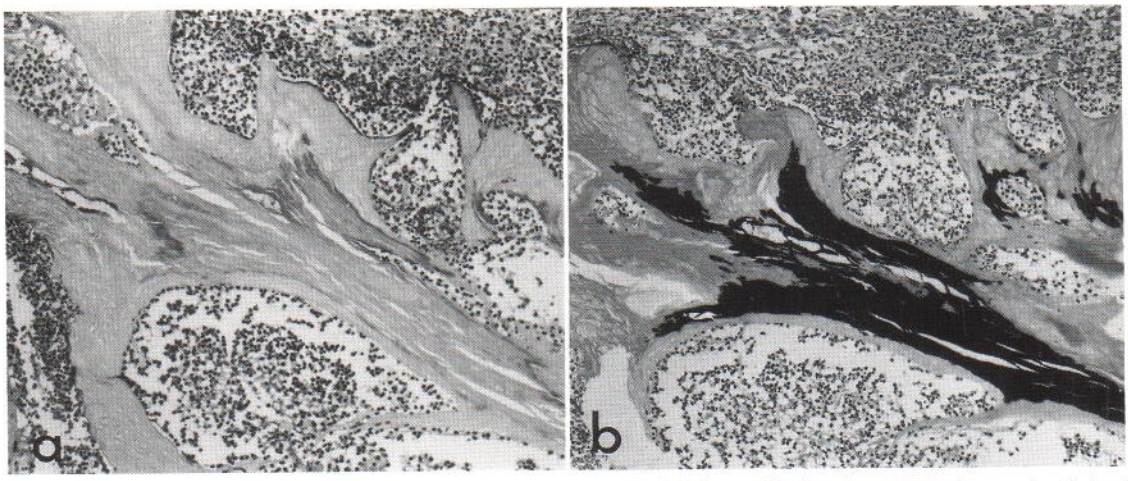

Fig. 1. A sample from a 13-year-old male patient. *a* (*left*): In the nest of shadow cells, there is an amorphous or hyalinized area in which the cellular boundaries are scarcely identifiable. One such area is stained basophilic and contained numerous irregularly shaped, artificial fissures. There is an infiltration of neutrophils in the stroma (H&E; ×57). *b* (*right*): Nests of basophilic cells, shadow cells and squamoid cells. Calcification is recognized in a hyalinized area within the nest of shadow cells (von Kossa stain; ×57).

matricoma and to elucidate the relationship between amyloid deposits and calcification, using histology and electron microscopy.

MATERIALS AND METHODS

Histology

Sixteen specimens of pilomatricoma were taken from 16 patients who visited Tottori University Hospital and Tsuyama Central Hospital. They were 4 males and 12 females ranging in age from 1 to 69 years. The specimens were fixed in a 3.5% buffered formalin solution and embedded in paraffin wax. Staining methods were as follows; hematoxylin and eosin (H&E); periodic acid-Schiff (PAS) for glycogen and neutral polysaccharides; von Kossa for calcium phosphate; Dylon (2) and thioflavine-T for amyloid.

Immunohistochemistry

Both light chains (κ and γ) of human immunoglobulin were investigated with the use of a PAP kit (DAKO Japan Co, Ltd., Kyoto, Japan). The primary antibodies were purchased from the same company.

Electron microscopy

Paraffin-embedded sections for histology were processed for conventional electron microscopy. They were deparaffinized in xylene, and rehydrated in a graded series of ethanol. After fixation with 1% osmium tetroxide solution, they were dehydrated in the same ethanol and embedded in Epon 812. Then thin sections of the hyalinized shadow cell areas (mentioned later) were stained with uranyl acetate and Reynolds' lead citrate, and observed in a Hitachi HU12A electron microscope.

RESULTS

Calcification and amyloid deposits were observed in 10 of 16 samples. Both calcification and amyloid deposits were observed simultaneously in 9 samples. A series of representative photographs obtained from the same sample are shown in Fig. 1. In H&E stain, there were nests of basophilic cells, shadow cells and squamoid cells. In the nest of shadow cells (Fig. 1*a*) there was an amorphous or hyalinized area in which the cellular boundaries were difficult to identify. One such area was stained moderately basophilic and had numerous irregularly shaped artificial fissures running parallel with the long axis of the lesion. This hyalinized area was stained weakly positive with PAS stain and black with von Kossa stain (Fig. 1*b*). Furthermore, its area was stained brown with Dylon and fluorescent with thioflavine-T under a fluorescence microscope. As a result, both amyloid deposits and calcification were demonstrated in the area coinciding with the hyalinized shadow cell nest. Immunohistochemically, neither of the light chains was demonstrated in the area of calcification and amyloid deposits.

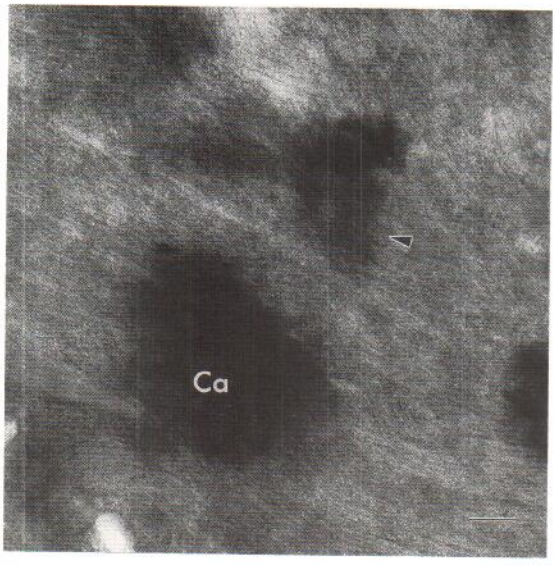

Fig. 2. Electron micrograph shows amyloid as rigid and unbranched fibrils with a diameter of about 10 nm, running in various directions. Calcium deposits (*Ca*) are also observed as irregularly shaped, electron-dense aggregations measuring approximately 0.3 to 0.6 μm. Amyloid fibrils (*arrowhead*) can be seen in calcium deposits which are less dense (×30,000, scale bar; 0.1 μm).

In electron microscopy, amyloid substances were demonstrated in Dylon-positive and thioflavine-T-positive area (Fig. 2). The findings were typical ultrastructural features for amyloid, in which many calcium deposits were observed. Amyloid fibrils could be recognized in peripheral portions where calcium deposits were less numerous.

DISCUSSION

In pilomatricoma, it is well known that calcification occurs only in the shadow cell nests, particularly hyalinized shadow cell areas, as shown in this study. In addition, this study revealed that the hyalinized shadow cell nests contained amyloid. Thus amyloid deposition as well as calcification may be unique characteristics of pilomatricoma.

The relationship between calcification and amyloid substances was earlier described in a study of multiple myeloma by Glans (3) and in the skin disease (1). Recently, Ladefoged & Rohr (4) reported a highly significant simultaneous occurrence of calcium and amyloid in sclero-calcific heart valves. However, to our knowledge, there have been no reports of epithelial tumours in which amyloid deposits and calcification were observed simultaneously, with the exception of calcifying epithelial odontogenic tumor (5,6). The causal relationship between amyloid deposit and calcification has not been clearly demonstrated. Our study revealed that calcium deposits were scattered in the amyloid substances, and also that amyloid fibrils could be recognized in the area where calcium was sparsely deposited. These features suggest that calcification is preceded by the deposition of amyloid. It seems probable that the amyloid substance in this tumour serves as a matrix for subsequent calcification.

Most commonly the deposition of amyloid masses of localized cutaneous amyloidosis takes place in the dermis, whereas, in pilomatricoma, amyloid as well as calcification develop within the tumour cell nests where there are no dermal components or inflammatory cells. A similar finding has been recognized in the calcifying epithelial odontogenic tumour (5,6). In general, many tumours – including epithelial tumours – have foci of dystrophic calcification which follow degeneration or necrosis (7). Amyloid in pilomatricoma is, as discussed later, also possibly related to the degeneration of tumour cells, and therefore, amyloid degeneration in the shadow cell nest may be liable to induce calcification due to a pathogenesis different from that of amyloid formed in the dermis.

Recently, degeneration of the epithelium with resultant transformation of the tonofilaments into amyloid deposits has been observed in skin (1); furthermore, immunohistochemistry has shown that antisera against keratin cross-react with amyloid fibrils (8). Amyloid substances were invariably found in a hyalinized area within the shadow cell nest, in which keratinization takes place. As a result, it is quite likely that the amyloid in pilomatricoma is derived from cytoskeleton proteins, especially keratin filaments of the tumour cells, as suggested by Hashimoto & Kobayashi (1).

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