Virchows Archiv A Pathol Anat (1992) 420:185–189

Virchows Archiv A
Pathological Anatomy and Histopathology

Case report

Microcytic variant of thymoma: histological and immunohistochemical findings in two cases

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Received July 11, 1991 / Received after revision October 7, 1991 / Accepted October 8, 1991

Summary. Two cases of microcytic variant of thymoma are presented. Both tumours were well encapsulated with a yellow-whitish colour and soft consistency. Microscopically, they consisted of round cells, having ample vacuolated cytoplasm. Fat droplets were not detected in one case where fat staining was performed. Immunohistochemically, the tumour cells were strongly positive for AE1/AE3, MB1, MB2, and LN1 and faintly positive for epithelial membrane antigen. They lacked any other leucocyte antigens. Leu 7 showed a positive immunoreaction in a ring-like or homogeneous pattern, compatible with the cytoplasmic vacuoles or cytoplasm. Ultrastructurally, the vacuoles resembled cystically dilated rough-surfaced endoplasmic reticulum. Desmosome-like structures (case 1) and intermediate junctions (case 2) were identified between adjacent cells. These findings indicate that the present tumours belong to a category of microcystic thymoma. The vacuoles were attributed to excess accumulation of Leu-7-positive material, probably in the cystically dilated endoplasmic reticulum.

Key words: Thymoma – Microcystic variant – Leu 7 – Excess accumulation

Introduction

Thymomas are classically divided into subtypes: predominantly lymphocytic, mixed and predominantly epithelial (Lattes 1962). However, Rosai and Levine (1976) advocated that the term thymoma should be restricted to epithelial tumours of thymic origin, regardless of the presence or absence of lymphocytes. They found microcystic change on about 16% of thymomas and regarded it as a degeneration of the neoplastic cells. However, we have found only 2 cases of thymomas with microcystic change when reviewing 123 cases in our institutes. In these 2 cases, the tumour cells were striking and showed a lipoblastic or signet-ring cell-like appearance. In addition, recent immunohistochemistry showed a positive reaction for Leu 7 as well as epithelial markers on thymomas (Chan et al. 1984; Kodama et al. 1986). This indicates that Leu 7 may be a useful marker of thymomas. We have examined these 2 cases using ultrastructural and immunohistochemical methods and will discuss the origin of the cytoplasmic vacuoles and the differential diagnosis from other types of tumours.

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Case reports

Case 1

A 51-year-old Japanese man was admitted to Niigata University Hospital because of myasthenia gravis. Chest radiographs revealed a questionable thymic mass and the lateral view showed a mass lesion in the upper portion of the anterior mediastinum. The patient underwent a thymectomy with a diagnosis of thymoma. He was well 15 years after operation, without recurrence or metastasis.

Case 2

A 48-year-old Japanese man was admitted to Tachikawa General Hospital because of a questionable mediastinal mass. Chest radiography, CT scan, and magnetic resonance imaging showed a $3 \times 3 \times 4$ cm solid lesion in the upper anterior portion of the mediastinum. A thymectomy was carried out with a diagnosis of thymoma. The patient was well 3 years after operation, without recurrence or metastasis.

Materials and methods

We reviewed 123 cases of thymomas resected surgically from 1960 to 1990 at Niigata University Hospital and Tachikawa General Hospital. We found only 2 cases of thymoma composed entirely of vacuolated tumour cells. The specimens were immediately fixed in 10% neutral formalin and multiple blocks had been made and treated routinely, followed by embedding in paraffin. Thin sections were stained with haematoxylin and eosin. Special stains included

periodic acid-Schiff with or without diastase digestion, elastica-Weigert, alcian blue (pH 2.5), and silver impregnation. Sudan III stain was also carried out in formalin-fixed material of one case (case 2), but not in another (case 1) because all specimens were embedded in paraffin.

For ultrastructural observation, a few small specimens were taken from the paraffin-embedded specimen of case 1 and formalin-fixed material of case 2. The specimens were treated as described previously (Fukuda and Ohnishi 1991) and observed with a Hitachi H-800 electron microscope (Hitachi, Tokyo).

For immunohistological examination, thin sections were stained using Vecstain ABC kit (Vector, Burlingame, Calif.). The antibodies used were against the following antigens; keratin (polyclonal), epithelial membrane antigen (EMA), carcinoembryonic antigen (CEA), S-100 protein, vimentin, actin, and leucocyte common antigen (Biogenex, San Ramon, USA). Monoclonal anti-keratins, AE1/AE3 and CAM 5.2, were obtained from Hybritec (San Diego, Calif. USA) and Becton-Dickinson (Mountain View, Calif.) respectively. Leu 7 and Leu M1 (Becton-Dickinson) and MB1, MB2, MB3, MT1 (Bioscience, Tokyo, Japan), LN1, LN2, LN3, UCHL-1 (Nichirei, Tokyo, Japan) and L26 (Kyowa, Tokyo, Japan) were also used.

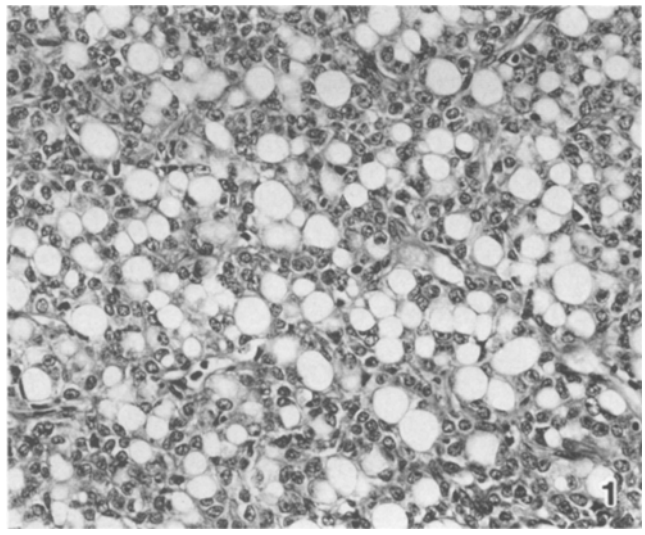

Fig. 1. Case 1. Vacuolated and non-vacuolated cells are seen. Vacuolated cells have eccentric nuclei and show signet-ring cell like configuration. H&E, ×250

Pathological findings

Both tumours were well encapsulated with yellow-whitish colour and soft consistency. The tumour in case 1 was 4×2×2 cm and lobulated; that in case 2 was 3×3×4 cm and non-lobulated with a homogeneous appearance. Necrosis, haemorrhage, and calcification were not observed.

In case 1, the tumour consisted of a sheet of both vacuolated and non-vacuolated cells, with predominance of vacuolated cells throughout the tumour. The nuclei were uniform and round or oval, vesicular, and frequently eccentric with or without a small nucleolus (Fig. 1). Mitotic figures were not present. The one or two vacuoles showing a slight variation in size presented in individual cells. There was a prominent vascularity in the stroma. A small number of small lymphocytes and neutrophils were scattered throughout the tumour, predominantly in perivascular areas. Mucin was not present anywhere. Reticulin fibres encircled tumour cell nests. Several tumour cells were trapped in the dense fibrous capsule. However, capsular penetration or vascular infiltration were not observed.

In case 2, the tumour was entirely composed of vacuolated cells with slight variation in size (Fig. 2). The cells had eosinophilic abundant cytoplasm with one or two vacuoles; the nuclei showed a slight variety in size and some of them exhibited a bizarre appearance. Mitotic figures were rare. The stroma was rich in vasculature and cholesterol clefts were occasionally observed. However, necrosis and degeneration of tumour cells were not seen. Thin reticulin fibres encircled tumour cell nests. Mucin was not detected anywhere. Sudan III failed to show fat droplets in vacuoles (Fig. 2, inset). Neither capsular permeation nor vascular infiltration was found.

Tumour cells of both cases reacted with AE1/AE3 and polyclonal anti-keratin (Fig. 3a) but not CAM 5.2 in ring manner and faintly positive for EMA. Leu 7 was also strongly positive in a ring-like or homogeneous pattern, corresponding to the vacuoles (Fig. 4), in several

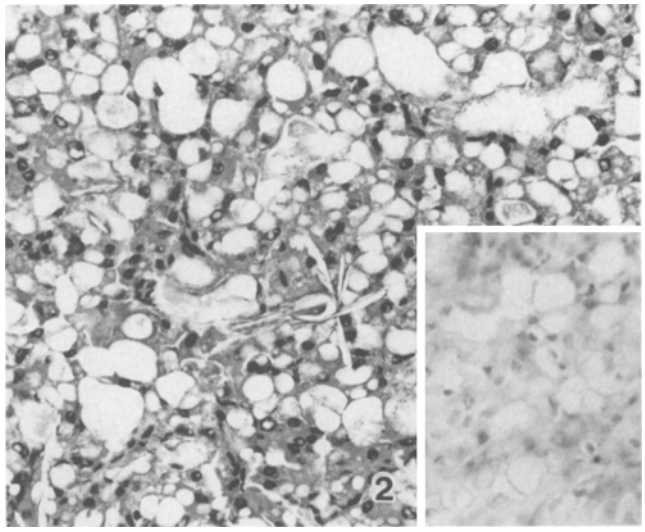

Fig. 2. In case 2 signet-ring like cells are seen having abundant eosinophilic cytoplasm. Cholesterol clefts are also present focally. H&E, ×250. Sudan III stain failed to show fat droplets in the vacuolated cells. *Inset:* Sudan III stain, ×250

tumour cells but not all. Tumour cells in the subcapsular region showed a stronger reaction for Leu 7. A small number of S-100-positive cells, some of which showed dendritic cell appearance, and actin positive cells were scattered throughout the tumours. Vacuolated tumour cells were negative for S-100 protein. MB1, MB2, and LN1 showed a positive immunoreaction in tumour cells (Fig. 3b). However, MB3, MT1, LN2, LN3, L26 and UCHL-1 failed to react. Vimentin, Leu M1 and CEA were not detected.

Although ultrastructural fine structures were not always preserved because of the poor preservation of the tumour cells, some were identified in several cells. The cells had one or two large vacuoles, which looked empty

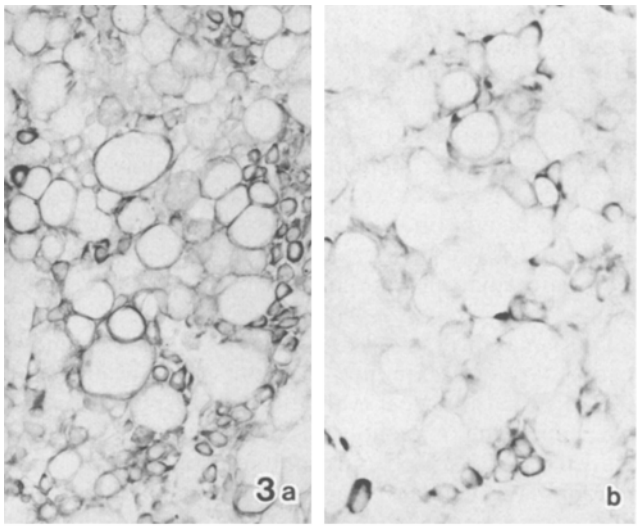

Fig. 3. Tumour cells show a positive immunoreaction for AE1/AE3 (**a**) and MB2 (**b**). ABC method; **a** ×220, **b** ×250

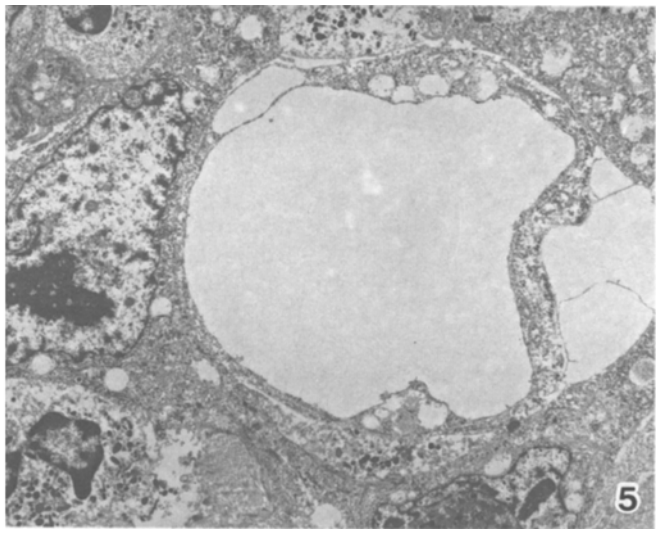

Fig. 5. Tumour cell possesses large empty vacuoles. ×3000

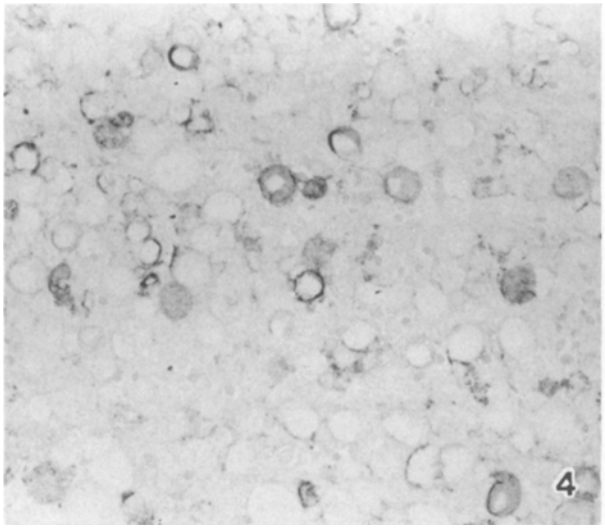

Fig. 4. Numerous tumour cells in both cases show a positive immunoreaction for Leu 7 in a ring-like or homogeneous pattern compatible with vacuoles or cytoplasm. ABC method, ×200

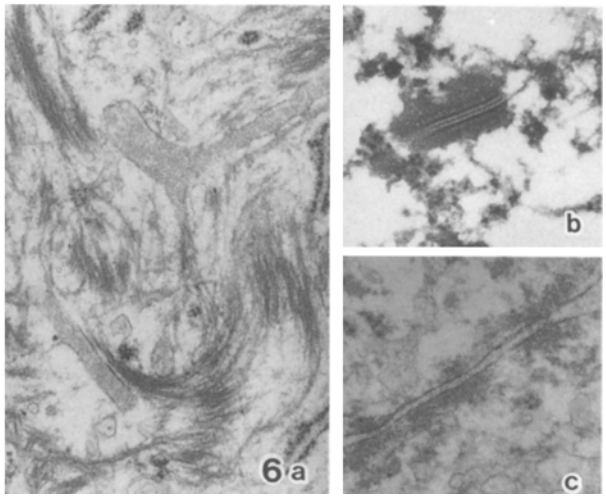

Fig. 6. a Tumour cells possess variable amounts of microfilaments resembling keratin filaments. ×22500. **b, c** Adjacent cells are connected by desmosome and intermediate junction-like structures. ×33000

and were separated by cytoplasmic septa (Fig. 5). The nuclei were eccentric and had medium sized nucleoli. There was a small number of mitochondria, rough surfaced endoplasmic reticulum (RER), and lysosomal granules. Most vacuoles showed an empty appearance with no specific structure but a few resembled RER because of the presence of ribosome-like structures on the surface. Tumour cells contained variable amounts of microfilaments, simulating tonofilaments (Fig. 6a). Adjacent tumour cells were connected by desmosome and intermediate junction-like structures (Fig. 6b, c). A few of the non-vacuolated cells possessed medium-sized, electron-dense granules, suggesting lysosomes. Neurosecretory granules were not identified in any tumour cells in either case.

Discussion

The term of signet-ring cell is applied to cancer cells showing vacuolated cytoplasm and an eccentric nucleus, attributable to accumulation of mucin (Ming 1971). Signet-ring cells or a vacuolated appearance of tumour cells have been reported in lymphoma, melanoma (Sheibani and Battifora 1988), squamous cell carcinoma (Cramer and Heggeness 1989), thyroid neoplasms (Schroeder and Boecker 1985) and meningioma (Lattes and Bigotti 1991). Regardless of the type of tumours, the signet-ring cell or vacuolated appearance is attributed to excess accumulation of some substances within the cytoplasm, produced by the tumour cells under usual conditions. For example, signet-ring cell melanomas contain a large

amount of filaments positive for vimentin and melanoma antigen (Sheibani and Battifora 1988). Vacuoles in signet-ring cell lymphoma are more heterogeneous; some are due to accumulation of immunoglobulin (Kim et al. 1978; van den Tweel et al. 1978), membrane-bound vacuoles in some others (Kim et al. 1978; Grogan et al. 1985; Weiss et al. 1985; Cross et al. 1989), and lamellar/reticular material without a limiting membrane in others (Navas-Palacios et al. 1983). Additionally, tumour cells undergoing fatty degeneration also have a vacuolated appearance.

Rosai and Levine (1976) found microcystic change in 16% of thymomas in their description and regarded it as a degenerative change. We reviewed 123 thymomas and found only 2 cases with an entirely vacuolated appearance. In these 2 cases, the tumour cells were all conspicuous and not degenerative. In addition, the vacuolated cytoplasm and eccentric nucleus reminded us of signet-ring cell or fat accumulation. However, the tumour cells in case 2 had no fat droplets. We considered that the vacuolated change must be attributed to causes other than degeneration. In this context, we considered Leu 7.

Leu 7 is an antibody reacting with natural killer cells and identified as a surface glycoprotein (Abo and Balch 1981). It reacts with neoplastic or non-neoplastic endocrine cells of the alimentary tract, neuroectodermal tumours, small-cell carcinoma of the lung and myelin fibre-associated protein (Abo and Balch 1981; Schuller-Petrovic et al. 1983; Caillaud et al. 1984; Tsutsumi 1984). Recent investigations have shown that thymoma is also positive for Leu 7, as well as epithelial markers (Chan et al. 1984; Chilosi et al. 1984). Kodama et al. (1986) also noted that a positive reaction of Leu 7 was confined to the outer cortex in the normal thymus and was found in the peripheral portion of some thymomas. The basic immunohistochemical findings in the present cases were similar to those described by Kodama et al. (1986). Interestingly, several tumour cells showed a positive reaction for Leu 7 in a ring or diffuse manner corresponding to cytoplasmic vacuoles, but the other did not. This finding indicates that the vacuoles contain the substance reactive with Leu 7 but that this dissolved easily.

Although we have no information whether the Leu-7-reactive substance is present on the cell surface or in the cytoplasm of thymomas, abnormal accumulation of some proteins may occur in some tumour cells, as described above. In the present study, although tumour cells were poorly preserved, the vacuoles resembled cystically dilated endoplasmic reticulum and not fat droplets or lysosomes. Indeed, a fat stain failed to show fat droplets in the cells. These findings also suggest the abnormal production and accumulation of the Leu-7-positive substance in tumour cells.

From the light microscopic observation, the present cases can be distinguished from those types of tumour which show signet-ring cell or vacuolated appearance. The lack of mucin, fat droplets, and S-100 protein readily excludes carcinomas and lipomatous tumours. Although MB1, MB2 and LN1 react with haematological tumours, such as signet-ring cell lymphoma, the cells in our tumours lacked any other haematological markers. Additionally, MB1, MB2 and LN1 react with normal epithelia and epithelial tumours, suggesting thymoma (Poppema et al. 1986; Fukuda et al., in press). Keratin positivity (Battifora et al. 1980) and the presence of tonofilament-like filaments and desmosome and intermediate-like intercellular junctions also exclude the possibility of haematopoietic tumours in the present cases. The lack of neurosecretory granules denies the possibility of endocrine cell tumours, such as the thymic carcinoid.

Leu 7 may thus be a helpful marker for the diagnosis of some types of thymoma. Caution should be exercised in the histopathological evaluation of the tumours showing a signet-ring or vacuolated cell appearance and more detailed ultrastructural and immunohistochemical examination may elucidate specific histological findings in various lesions.

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