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ORIGINAL ARTICLE

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PE-35-related antigen expression and CD1a-positive lymphocytes in thymoma subtypes based on Müller-Hermelink classification

An immunohistochemical study using catalyzed signal amplification

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Abstract PE-35 monoclonal antibody, detecting a cell-surface antigen of various types of carcinoma and normal epithelium, reacts exclusively with the medullary epithelium in the thymus; therefore, the antigen has been considered as a marker of medullary differentiation in thymomas. Using the catalyzed signal amplification method, which made it possible to apply PE-35 to routinely processed, archival tissues, we examined expression of this antigen, together with CD1a reactivity of lymphocytes, in 40 thymic epithelial tumors subclassified using the Mü1ler-Hermelink system. Medullary thymomas infiltrated with a small number of CD1a-negative lymphocytes were PE-35 positive, although many of the long spindle tumor cells were PE-35 negative. Mixed thymomas and predominantly cortical thymomas, both with prominent CD1a-positive lymphocytes, were also PE-35 positive, although some areas of the latter type were PE-35 negative. Cortical thymomas with decreased numbers of CD1a-positive lymphocytes were largely PE-35 negative. In well-differentiated thymic carcinomas with a few CD1a-positive lymphocytes, two cases were negative, but four cases were at least focally positive with PE-35. All high-grade thymic carcinomas infiltrated with some CD1a-negative lymphocytes were PE-35 positive. These results suggested that medullary thymoma generally possesses the medullary nature, although the latter tends to be lost in the long spindle tumor cells. Mixed and predominantly cortical thymomas may have mixed medullary phenotype and cortical function. Cortical thymoma and many well-differentiated thymic carcinomas may possess the cortical nature, while the large polygonal tumor cells tend to lose immature T-lymphocyte-retaining function.

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Introduction

It is generally accepted that thymic epithelial tumors are separated into thymoma and thymic carcinoma according to the degree of cellular atypia [28]. However, the classification of thymomas and designation of the subsets are still controversial because of their heterogeneity of histological features and clinical behaviors [11]. One prevailing classification is based on the lymphocyte to epithelial cell ratio and on epithelial cell types, i.e., polygonal cell, spindle cell, and mixed polygonal and spindle cell types [16, 17, 28]. Although this classification is relatively easy to apply histologically, it showed no significant correlation with their clinical behavior [14, 17, 19, 23]. In addition, there are variations in the polygonal cells, e.g., small and large cells [15] and also in the spindle cells, e.g., short and long cells [28].

Recently, Müller-Hermelink and colleagues [8, 9, 20] have proposed a new classification of thymic epithelial tumors based on histological resemblance of the neoplastic epithelial cells to various types of epithelial cell of the normal thymus. According to their classification, thymic epithelial tumors are divided into medullary thymoma, mixed medullary and cortical thymoma, predominantly cortical thymoma, cortical thymoma, well-differentiated thymic carcinoma (WDTC), and high-grade thymic carcinoma (HGTC). Several investigators supported the usefulness of this classification system in predicting the aggressive potential of thymomas [7, 14, 25, 26]. However, the issue still remains to be established and the histogenesis of thymic epithelial tumors has not been clarified [11, 12].

PE-35 is a monoclonal antibody defining a novel cell-surface antigen of small cell lung carcinoma with a molecular weight of 35,000 Da [30, 31]. It has been confirmed that various types of normal epithelium and carci-

noma as well as lung carcinoma show immunoreactivity with PE-35 [30]. Interestingly, in the normal thymus, PE-35 positivity is restricted to the medullary epithelial cells, and the other types of epithelial cells, i.e., subcortical, cortical, or Hassall's corpuscular cells, are PE-35 negative [5, 31]. Therefore, this antigen is considered to be a valuable marker for medullary epithelium in the thymus. Unfortunately, PE-35 was applicable to cryostat sections only [5, 30, 31]. However, we have found that catalyzed signal amplification (CSA) [1], amplifying the signals of streptavidin-biotin-peroxidase reaction by catalyzing the deposition of a biotinylated phenolic compound, made it possible to apply PE-35 to formalin-fixed and paraffin-embedded tissues. The staining with the commercially available anti-CD1a antibody [13] was also greatly enhanced by the CSA method.

In the present study, we have tried to phenotypically and functionally characterize the thymoma subtypes in the Müller-Hermelink (M-H) classification, reviewing the tumor cell cytology in each subset and performing the CSA immunohistochemistry for the PE-35-related antigen and CD1a-positive lymphocytes.

Materials and methods

Thirty-seven surgically resected thymomas were retrieved from the archives of the Department of Pathology, Nagoya City University Medical School, Nagoya, Japan. Three thymic carcinomas from the same source were added to the series for comparison. All of the tissues had been fixed in 10% neutral-buffered formalin and embedded in paraffin. The diagnosis was confirmed by retrieval of the pathology report and review of the hematoxylin and eosin sections. All the thymoma cases were reclassified according to the criteria of Müller-Hermelink and colleagues [8, 9, 20] as medullary, mixed, predominantly cortical, or cortical thymomas, or WDTC. Cases of thymic carcinoma were designated as HGTC. The stages of the tumors were determined according to the criteria of Masaoka and colleagues [21]. Specimens of normal thymus were obtained from four children at the time of thoracic surgery. They were used as non-neoplastic control.

Immunohistochemical stainings for PE-35-related antigen and CD1a were performed on paraffin sections using the CSA method and Dako CSA system peroxidase kits (Dako, Carpinteria, Calif.), essentially according to the manufacturer's instructions. The monoclonal antibody PE-35, which was provided by courtesy of Prof. Ryuzo Ueda, Department of Internal Medicine, Nagoya City University Medical School, was used at a 1:1000 dilution. Anti-CD1a antibody (clone O10, Immunotech, Marseille Cedex, France) was prediluted. Briefly, sections were deparaffinized, rehydrated in graded alcohols, and subjected to heat-induced epitope unmasking by immersing the slides in 0.05 M Tris-HCl containing 0.1% Tween 20 and then placing them in a microwave oven for 5 min twice. Endogenous peroxidase activity was blocked using 3% hydrogen peroxide for 5 min. Then, sections were incubated with a protein block to suppress nonspecific binding of subsequent reagents, followed by incubation with primary antibodies for 15 min at room temperature, after which sections were incubated with biotinylated rabbit anti-mouse immunoglobulins, streptavidin-biotin-peroxidase complex, biotinyl tyramide, and streptavidin-peroxidase for 15 min. Peroxidase activity was then visualized with diaminobenzidine, followed by counterstain with hematoxylin. Immunohistochemical staining with pancytokeratin antibody AE1/3 (Dako) and with anti-vimentin antibody (Dako) was also performed using the streptavidin-biotin-immunoperoxidase method [6] and Histofine immunostaining kits (Nichirei, Tokyo, Japan).

The immunoreactivity of PE-35 by the CSA method in paraffin sections was compared with that by the usual peroxidase method in frozen sections of normal thymic tissues from three children; both methods were similar with regard to topographic localization (data not shown). In addition, to confirm the PE-35 reactivity with the medullary epithelial cells of the thymus, sections of normal thymus were subjected to double immunostaining with PE-35 and AE1/3, as described previously [24], with some modification. On the day following CSA immunostaining for PE-35, the same sections were incubated with AE1/3 for 1 h at room temperature. Rabbit anti-mouse immunoglobulins (Z0259, Dako; 1:25) were applied for 30 min and then complexes of calf intestinal alkaline phosphatase and mouse monoclonal anti-alkaline phosphatase (D651, Dako; 1:25) were applied for 40 min. AP Kit I (SK-5100, Vector Laboratories, Inc. Burlingame, Calif.) was used for chromogenic substrate.

Results

Histological findings

The histological types of tumors reclassified by the M-H system together with the clinical informations are listed in Table 1. There were four cases of medullary thymoma, showing diffuse proliferation of short spindle or mixed short spindle and some small polygonal cells with scant lymphocyte infiltration. Two cases (cases 2 and 3) showed hemangiopericytoma-like patterns and the other cases (cases 1 and 4) showed pseudogland formations. The latter cases also showed bundles of long spindle cells and abundant collagen fibers intermingling with short spindle cells. Eighteen cases were mixed thymoma, which showed a mixture of short spindle and small polygonal cells associated with varying numbers of lymphocytes [25]. Noted in these tumors were foci of medullary thymoma in six cases, foci of predominantly cortical thymoma in two cases, and foci of both medullary and predominantly cortical thymomas in three cases. These thymomas were classified as mixed type because the features of mixed-type thymoma were predominant. Five cases were predominantly cortical thymomas, with features resembling the architecture of the thymic cortex. The tumor cells were relatively small and polygonal and were embedded in abundant lymphocytes. Some tumor cells showed elongated nuclei, resembling short spindle cells (Fig. 1). The scattered macrophages produced "starry sky" appearance. Foci of "medullary differentiation" were more or less prominent, in which some polygonal cells were present without well-formed Hassall's corpuscles. Medullary-like areas with Hassall's corpuscles could be found only in the periphery of these type thymomas. There were four cases of cortical thymoma, consisting predominantly of large polygonal cells. These cells generally possessed a small but distinct nucleolus, more conspicuous than that of small polygonal cells. The lymphocytic infiltration was less than that of predominantly cortical thymoma, with a tendency to be patchy. Some small polygonal cells indistinguishable from those of mixed thymoma or predominantly cortical thymoma were also seen, particularly in areas with more lymphocytes. One case (case 28) showed foci of predominantly

Table 1 Clinical, histological, and immunohistochemical features in thymic epithelial tumors. *Med* medullary thymoma; *Mix* mixed thymoma; *PCor* predominantly cortical thymoma; *Cor* cortical thymoma; *WDTC* well-differentiated thymic carcinoma; *HGTC* high-grade thymic carcinoma; *PRCA* pure red cell aplasia; *EM* erythema multiforme; *HGG* hypogammaglobulinemia; *MG* myasthenia gravis; *HT* Hashimoto's thyroiditis; *AND* alive with no evidence of disease; *AWD* alive with disease; *DOC* died of other cause or complication; *DOD* died of disease; *FU* follow-up

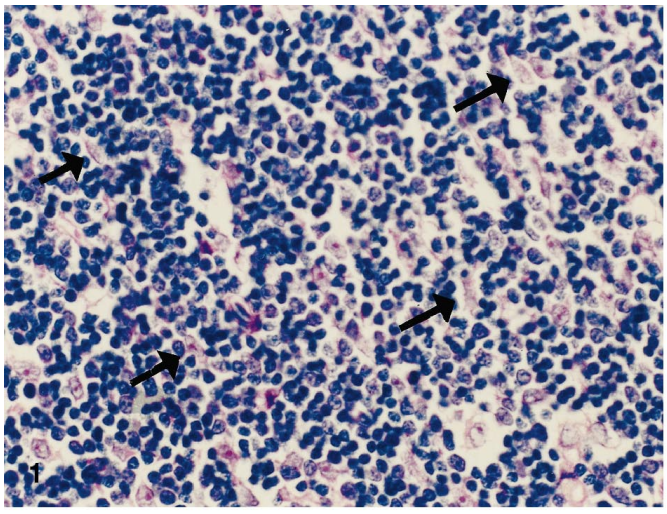
Case no.	Age/gender	Stage	Subtype[a]	PE-35[b]	CD1a[c]	Complication and follow-up
1	60/M	I	Med	+	–	PRCA; DOC, 4 years
2	72/M	I	Med	+	–	EM; AND, 10 years
3	56/F	II	Med	+	–	AND, 10 years
4	42/F	I	Med, Mix	+	+*	AND, 2 years
5	48/M	I	Mix	+	+	Lost FU
6	64/F	I	Mix	+	+	AND, 5 years
7	71/M	I	Mix	+	+	PRCA; DOC, 1 year
8	50/M	II	Mix	+	+	AND, 8 years
9	60/F	II	Mix	+	+	Lost FU
10	65/M	II	Mix	+	+	Lost FU
11	74/M	II	Mix	+	+	Lost FU
12	38/M	I	Mix, Med	+	+	PRCA; AND, 17 years
13	55/F	I	Mix, Med	+	+	PRCA; AND, 10 years
14	68/F	I	Mix, Med	+	+	AND, 1 year
15	49/F	II	Mix, Med	+	+	AND, 13 years
16	66/F	II	Mix, Med	+	+	Lost FU
17	79/M	II	Mix, Med	+	+	HGG; AND, 6 years
18	39/F	I	Mix, PCor	+	+	Lost FU
19	65/F	I	Mix, PCor	+	+	AND, 5 years
20	29/M	I	Mix, Med, PCor	+	+	AND, 12 years
21	56/M	I	Mix, Med, PCor	+	+	AND, 10 years
22	75/F	I	Mix, Med, PCor	+	+	AND, 3 years
23	60/M	1	PCor	+*	+	MG; AND, 10 years
24	40/F	II	PCor	+	+	HT; AND, 1 year
25	44/F	II	PCor	+	+	DOD, 8 years
26	62/F	II	PCor	+*	+	Lost FU
27	62/F	II	PCor	+*	+	AND, 13 years
28	54/F	III	Cor, PCor	+*	+	AND, 1 year
29	76/F	I	Cor	–	+	Lost FU
30	26/F	IVb	Cor	–	+	AND, 2 years
31	50/F	IVb	Cor	–	+	Lost FU
32	54/M	II	WDTC, Cor	+*	+	AND, 1 year
33	38/M	III	WDTC, Cor	–	+	AND, 4 years
34	68/M	II	WDTC	+	+	MG; Lost FU
35	38/M	III	WDTC	–	+	Lost FU
36	45/M	IVa	WDTC	+*	+	AND, 1 year
37	78/F	IVa	WDTC	+*	+	AWD, 2 years
38	61/M	II	HGTC	+	–	AND, 3 years
39	53/M	III	HGTC	+	–	AND, 5 years
40	50/F	IVa	HGTC	+	–	DOD, 4 years

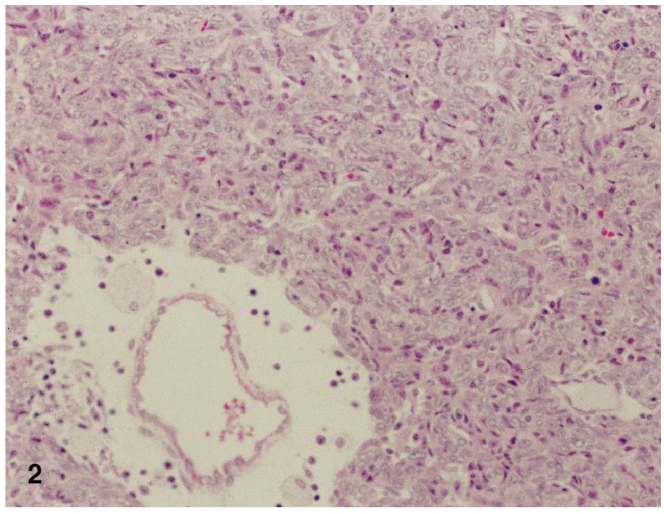
*Focally positive
[a] Subtype listed first is predominant
[b] Positivity of tumor cells
[c] Positivity of lymphocytes

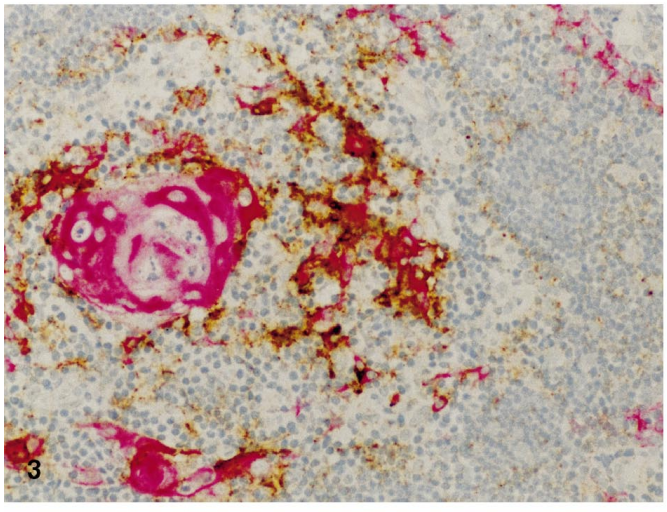
cortical thymoma in part. There were six cases of WDTC. All but one case (case 34) showed a solid growth of large polygonal cells, with mild nuclear pleomorphism and with scanty lymphocytes. Small areas of cortical thymoma were identified in two cases. Tumor cells of case 34 consisted of short spindle cells resembling those of medullary thymoma, but showed mild nuclear pleomorphism and some conspicuous nucleoli (Fig. 2). All cases showed perivascular spaces with tumor cell palisades, although the latter is not so distinct in the spindle cell WDTC. The three cases of HGTC showed features of squamous cell carcinoma with obvious cytologic atypia and mitoses.

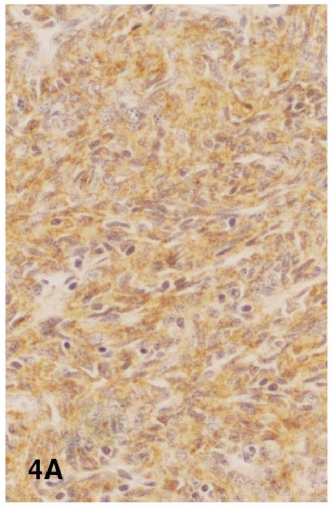
Immunohistochemical findings

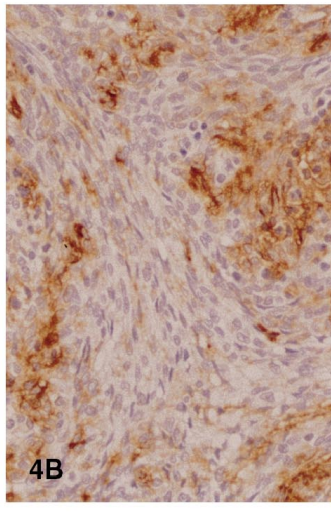
Immunohistochemical study using the CSA method showed a dispersed finely granular background staining; however, this was negligible and the staining was interpreted as positive when the epithelial cells or lymphoid cells showed identifiable staining on the cell membrane. In normal thymus, the double immunostaining revealed that both PE-35 and AE1/3 were positive in the medullary epithelial cells except those of Hassall's corpuscles, but only AE1/3 was positive in the cortical epithelial cells (Fig. 3). Almost all lymphocytes in the cortex and few lymphocytes in the medulla showed positive staining for CD1a. In medullary thymomas, PE-35 positively stained most of the short spindle and small polygonal

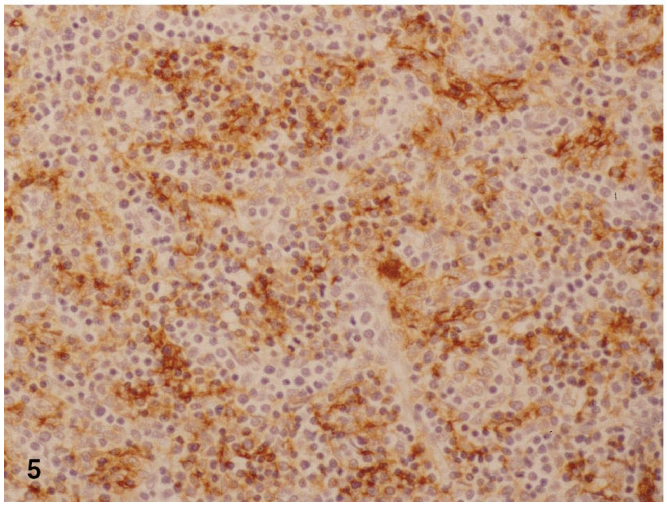












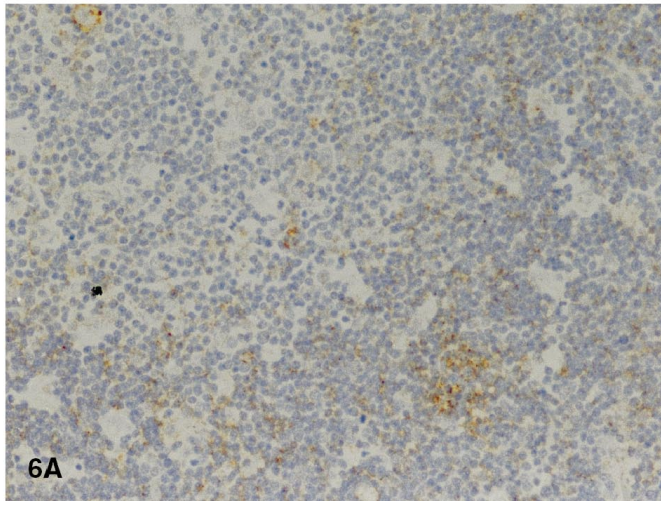


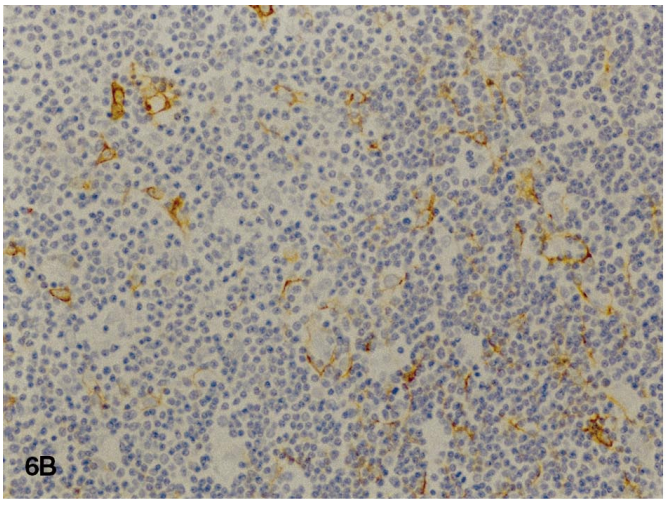
Fig. 1 Predominantly cortical thymoma. Small polygonal cells and some spindle cells (*arrows*) are present among abundant lymphocytes (hematoxylin and eosin, ×270)

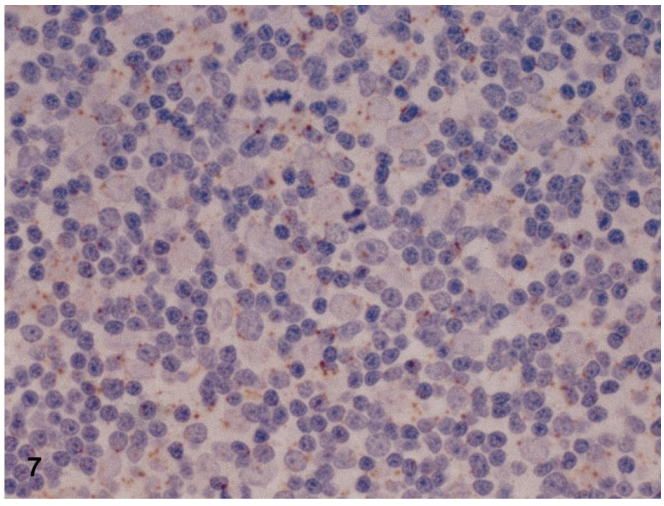
Fig. 2 Spindle cell type well-differentiated thymic carcinoma. Tumor is comprised of spindle cells with mild atypia, showing some palisades around the perivascular space (hematoxylin and eosin, ×220)

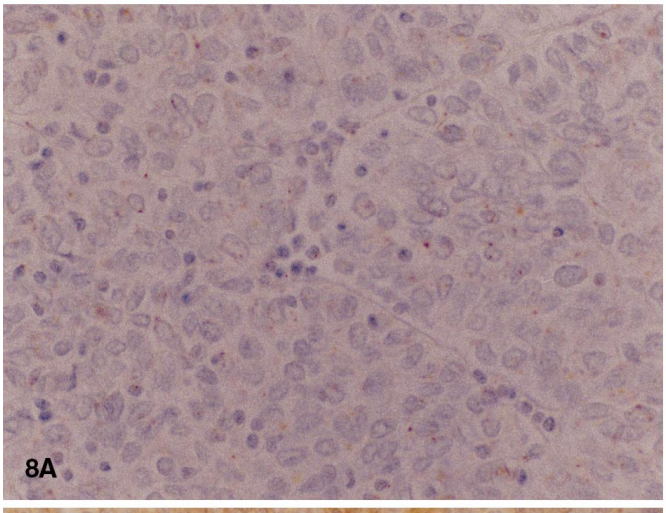
Fig. 3 Normal thymus. PE-35 reacts with epithelial cells of the medulla, which are positive for AE1/3, but not with those of the Hassall's corpuscles and cortex, which are positive only for AE1/3 (double immunostaining for PE-35 (*brown*) and AE1/3 (*red*) using catalyzed signal amplification method and streptavidin-biotin immunoperoxidase method with hematoxylin counterstain, ×220)

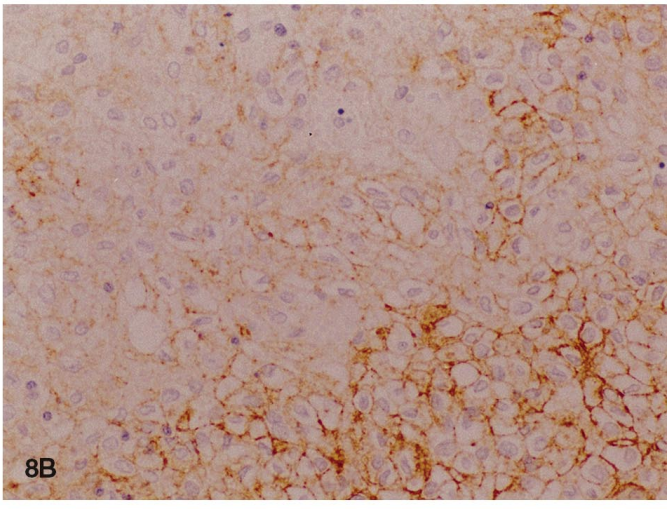
Fig. 4 Medullary thymoma (**A** case 2; **B** case 4). The short spindle tumor cells are PE-35 positive (**A**) and the long spindle tumor cells are PE-35 negative (**B**) (catalyzed signal amplification method with hematoxylin counterstain, ×220 for both figures)

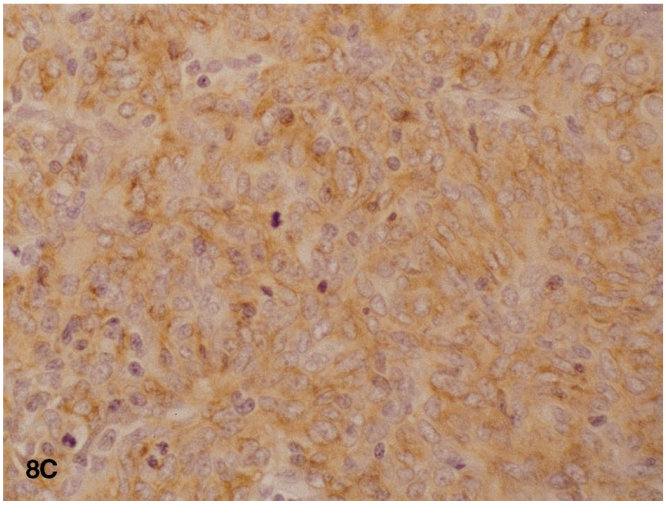
Fig. 5 Mixed thymoma (case 12). The short spindle and small polygonal tumor cells react with PE-35 (catalyzed signal amplification method with hematoxylin counterstain, ×220)











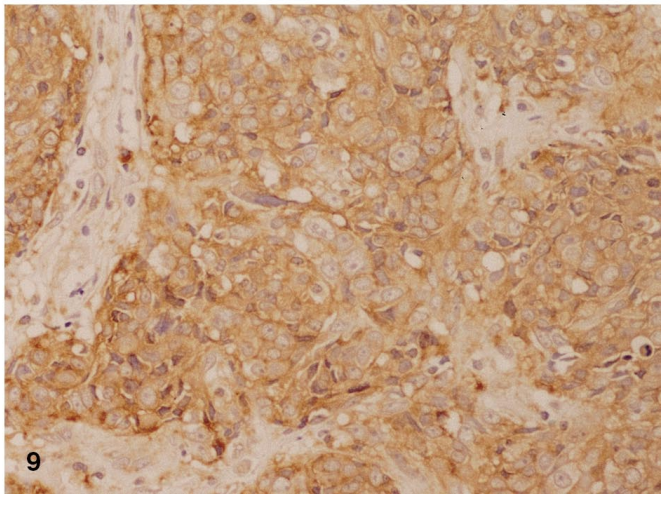


Fig. 6 Predominantly cortical thymoma with "medullary differentiation" (case 24). Many small polygonal tumor cells are positive with PE-35 (**A**). The area of "medullary differentiation" (*left*) shows only a few PE-35-positive epithelial cells (**A**) and some AE1/3 positive epithelial cells (**B**) [**A** and **B** are serial sections, catalyzed signal amplification method (**A**) and streptavidin-biotin-immunoperoxidase method (**B**) with hematoxylin counterstain, ×220 for both figures]

Fig. 7 Cortical thymoma (case 29). The large polygonal tumor cells are negative with PE-35 (catalyzed signal amplification method with hematoxylin counterstain, ×330)

Fig. 8 Well-differentiated thymic carcinoma (**A** case 35; **B** case 36; **C** case 34). The polygonal tumor cells are PE-35 negative (**A**). More atypical area (*right*) is positive with PE-35 (**B**). Spindle cell type tumor is diffusely positive with PE-35 (**C**) (catalyzed signal amplification method with hematoxylin counterstain, ×330 for **A** and **C**, ×220 for **B**)

Fig. 9 High-grade thymic carcinoma (case 38). The tumor cells are intensely positive with PE-35 (catalyzed signal amplification method with hematoxylin counterstain, ×220)

cells, but only some of the long spindle cells (Fig. 4A, B). This reactivity pattern of PE-35 was almost the same with that of AE1/3 staining the cytoplasm. Many of the long spindle cells reacted for vimentin more intensely than cytokeratin. Vimentin was positive only in the long spindle cells, but was negative in other types of tumor cells including the short spindle cells. Almost all the epithelial cells, both the short spindle and small polygonal cells, of mixed thymomas reacted with PE-35 in the staining pattern similarly to AE1/3 (Fig. 5). The majority of lymphocytes in medullary thymomas were negative for CD1a, whereas nearly all lymphocytes in mixed thymomas were positive for CD1a. Immunostaining results of foci of medullary thymoma in mixed thymoma were the same as those of pure medullary thymomas. In predominantly cortical thymomas, many small polygonal cells stained also positive with PE-35 as well as AE1/3. However, in places, the number of PE-35-positive small polygonal cells was diminished in comparison with that of AE1/3-positive cells. Although there was no apparent cytologic difference of tumor cells, the PE-35-negative areas had the tendency to be packed more densely with CD1a-positive lymphocytes.

Interestingly, in the foci of "medullary differentiation" with CD1a-negative lymphocytes, there were a few PE-35-positive cells (Fig. 6A) and some AE1/3-positive cells only (Fig. 6B). Medullary-like areas with Hassall's corpuscles in the periphery of the tumor disclosed many PE-35 positive cells. Cortical thymomas were largely PE-35 negative (Fig. 7); the small polygonal cells as well as large polygonal cells did not react with PE-35, although all these cells strongly reacted with AE1/3. Foci of predominantly cortical thymoma in cortical thymoma (case 28) were PE-35 positive. Almost all lymphocytes of predominantly cortical thymomas and cortical thymomas reacted for CD1a except for the lymphocytes in the foci of "medullary differentiation" in the former. WDTCs, by contrast, showed heterogeneous reactivities with PE-35, while they were evenly positive with AE1/3. Two cases were PE-35 negative (Fig. 8A), whereas four cases showed immunoreactivity with PE-35 at least in part (Fig. 8B). The spindle cell type WDTC was one of the latter tumors, and almost all the tumor cells in this case were positive with PE-35 (Fig. 8C). In the other three WDTCs, tumor cells with more atypical nuclei and clear cytoplasm showed positive staining with PE-35. Lymphocytes were more scanty in PE-35-positive areas, although they were positive for CD1a. In spindle cell type WDTC, some CD1a-negative lymphocytes were also present. The tumor cells of HGTCs were intensely positive with PE-35 and AE1/3, and the lymphocytes in them were negative for CD1a (Fig. 9).

Discussion

It is well recognized that epithelial cells of the thymus are heterogeneous and are divided into several subtypes according to their location, cytological appearance, and immunohistochemical phenotypes [2, 3, 5, 18, 25, 28]. The histological classification system of thymoma proposed by Müller-Hermelink and colleagues [8, 9, 20] is based on the premise that spindle and polygonal tumor cells of thymoma are derived from the medulla and cortex of the thymus, respectively. However, morphologically similar small polygonal epithelial cells are present in both of the latter regions. Actually, medullary thymoma in M-H classification may not consist of monomorphic proliferation of spindle cells; some small to medium-sized polygonal cells are also intermingled, sometimes forming pseudoglandular structures. However, the spindle cells that are not observed in the cortex of the normal thymus may also constitute a part of predominantly cortical thymoma. Furthermore, two or even three types of thymoma, such as medullary, mixed, and predominantly cortical type foci, frequently coexist in the same tumor. Thus, it may be impossible to decide the origin of the tumor cells of thymoma by the histological appearance alone.

To date, immunohistochemical studies using various epithelial markers could not detect any definite differences between medullary and cortical type thymomas [4, 10, 22, 25, 27, 32]. There were only two studies using PE-35 in cryostat sections of thymomas to characterize the tumor cells. The PE-35-related antigen is positive in a wide range of epithelial cells including those of the thymic medulla, but is negative in the other epithelial cells of thymus including those of the cortex [30, 31]. Takahashi and colleagues [31] reported that this antigen in thymomas showed a tendency to be expressed in the epithelial type and mixed lymphoepithelial type thymomas with the medullary lymphoid components. Fukai and colleagues [5], using PE-35 and other markers, reported that although there was heterogeneity of epithelial cell antigen expression in thymomas, 28 of 46 polygonal cell thymomas were of the cortical type, whereas 10 of 18 spindle cell thymomas and 7 of 17 mixed cell thymomas were of the medullary type. While the results of these studies and ours show some overlap, the detailed histological features were not described in their studies and the classification systems of thymoma they adopted do not distinguish some important subtypes in M-H classification.

In the present study, we examined PE-35-related antigen expression in thymic epithelial tumors based on M-H classification and using the CSA immunohistochemistry. The latter method made it possible to apply the antibody to formalin-fixed and paraffin-embedded tissues, and a precise morphology of the tumor cells was easier to observe. Short spindle and small polygonal cells of the present medullary thymomas reacted with PE-35, which may confirm that this type of thymoma originates from or differentiates to medullary epithelium as proposed by Müller-Hermelink and colleagues [20]. Scarcity of CD1a-positive cells in the lymphocytic infiltrate if present is also compatible with this hypothesis. The long spindle cells, only some of them reacting with PE-35 and AE1/3, may be losing the medullary nature and acquir-

ing more mesenchymal phenotype as these cells were positive of vimentin. Short spindle and small polygonal cells of mixed thymomas also showed positive staining with PE-35, but most of the lymphocytes showed CD1a-immunoreactivity. While Müller-Hermelink and colleagues characterized mixed thymoma as tumors consisting of both cortical and medullary epithelial cells [20], this type may be considered as a tumor with the tumor cells expressing medullary phenotype and cortical function retaining the immature T lymphocytes.

Some small polygonal cells of predominantly cortical thymoma, small and large polygonal cells of cortical thymoma, and many large polygonal cells of WDTC did not react with PE-35. These types of thymoma were associated with CD1a-positive lymphocytes as also described previously [9, 25, 28]. Furthermore, PE-35-negative areas of predominantly cortical thymoma were associated with more densely packed immature T-lymphocytes in comparison with PE-35-positive areas. Therefore, these PE-35-negative tumor cells may have cortical function as well as cortical phenotype. However, since the number of immature T-lymphocytes of cortical thymomas was less than that of predominantly cortical or mixed thymomas, the cortical thymomas may have a decreased cortical function in comparison with the latter two types of thymoma. This tendency becomes more obvious in WDTCs, where infiltration of lymphocytes, although positive of CD1a, is scanty. The foci of "medullary differentiation" may require a different interpretation. Although they resemble the medulla of the thymus, with mature lymphocytes in the present as well as previous studies [28], these foci were largely PE-35 negative, with only some keratin-positive epithelial cells in the present study. Hassall's corpuscles could be found only in the medullary-like areas with abundant PE-35-positive cells located in the periphery of the tumors; these areas may be pre-existing non-neoplastic thymus involved in thymoma. Further investigation is needed to see whether all these foci represent an intact tumor parenchyma.

Whereas two WDTCs were PE-35 negative, which is compatible with cortical phenotype, the other four WDTCs were at least focally positive for this antigen. All the HGTCs in the present study were also PE-35 positive. The possibilities are that either HGTC showed differentiation to medullary epithelial cells or HGTC acquired the antigen on the step of tumor progression. The latter hypothesis may be reasonable because three of the four WDTCs showed PE-35-positivity, especially in the histologically atypical foci; according to the recent suggestion by Suster and Moran [29], atypical thymomas (including WDTC in M-H classification) may progress to HGTC. The remaining one WDTC, consisting of spindle cells and conforming to atypical spindle cell thymoma [25], was diffusely positive for the PE-35-related antigen. This tumor may have possessed this antigen from the inception, in relation to the medullary epithelium.

In conclusion, the present study, with PE-35-related antigen as a medullary marker and CD1a-positive lymphocytes as a cortical functional marker, supported the medullary nature of medullary thymoma and the cortical nature of cortical thymoma and most WDTCs in M-H classification. However, the long spindle tumor cells in medullary thymoma tend to lose an epithelial character. In addition, the presence of mixed medullary phenotype and cortical function was suspected in mixed and predominantly cortical thymomas. The large polygonal tumor cells of cortical thymoma and WDTC tend to lose cortical function. Rarely, medullary thymoma may progress to spindle cell WDTC directly.

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