J Clin Exp Hematop
Vol. 53, No. 1, June 2013

Original Article

The Evaluation of Immunohistochemical Markers and Thymic Cortical Microenvironmental Cells in Distinguishing Thymic Carcinoma from Type B3 Thymoma or Lung Squamous Cell Carcinoma

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Thymic carcinoma (TC) is often very difficult to distinguish from type B3 thymoma and lung squamous cell carcinoma (L-SCC) involving the anterior mediastinum. The present study evaluated the usefulness of immunohistochemical markers including c-Kit, CD5, glucose transporter-1 (GLUT-1), claudin-1 (CLDN-1), thymoproteasome β5t, p53 and Ki-67 (MIB-1) and thymic cortical environmental marker cells, cortical thymocytes (c-Thy) and thymic cortical dendritic macrophages (TCDMs) in distinguishing thymic carcinoma (TC) from type B3 thymoma or lung squamous cell carcinoma (L-SCC) using 17 cases of type B3 thymoma, 18 cases of TC and 12 cases of L-SCC. The results indicated that c-Kit and CD5 are very useful markers for TC, while GLUT-1, CLDN-1, p53 and Ki-67 are not. Thymic cortical microenvironmental marker cells, especially TCDMs, and thymic cortical epithelial cell-marker β5t are also useful for distinguishing TC from type B3 thymoma. Although none of these markers are adequate for making a distinction when used alone, the plural use of c-Kit, CD5, β5t thymic cortical environmental marker cells, c-Thys and TCDMs may therefore lead to a correct distinction between TC and type B3 thymoma or L-SCC. 〔*J Clin Exp Hematop 53(1) : 9-19, 2013*〕

Keywords: thymic carcinoma, type B3 thymoma, lung squamous cell carcinoma, immunohistochemical distinction

INTRODUCTION

Thymic carcinomas (TC) are rare malignant neoplasms of thymic epithelial cells (TECs). Squamous cell carcinoma (SCC) is the most common type of TC and it is generally accepted that the frequency of TC is higher in Asia than in the West. TC is exclusively present as an anterior mediastinal tumor and frequently invades adjacent organs or tissues such as the lungs and pericardium. On the other hand, lung squamous cell carcinoma (L-SCC) is one of the most common types of lung cancer and can invade the pleura and the anterior mediastinum. It is very important to correctly distinguish between TC and L-SCC, because the biological behaviors and therapies for TC are quite different from those for L-SCC.

It is often very difficult to correctly distinguish TC from type B3 thymoma, which is the most aggressive type of thymoma but is much less malignant than TC. Thymomas are uncommon neoplasms of TECs that can present with a wide variety of histological features. The great morphological variability and heterogeneity of thymomas has rendered the histological classification of thymomas a difficult and highly controversial field in pathology.[1] The World Health Organization (WHO) classifies thymomas into five types : A, AB, B1, B2 and B3.[2] Type A thymoma is characterized by the proliferation of spindle- or oval-shaped epithelial cells with scant cytoplasm. Type B thymoma is characterized by round, plump epithelioid tumor cells with abundant cytoplasm. Type AB thymoma includes tumors containing both type A and type B cells within the same tumor mass. Type B thymomas are further subdivided into types B1, B2 and B3 on the basis of proportional increases in the numbers of tumor

Received : October 20, 2012
Revised : November 22, 2012
Accepted : November 25, 2012
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cells in relation to lymphocytes and the emergence of atypia in tumor cells. Type B1 thymoma is characterized by a large majority of lymphocytes and few scattered round or polygonal epithelial tumor cells without cytological atypia. Type B2 thymoma is characterized by an approximately equal admixture of proliferating round epithelial tumor cells with inconspicuous cytological atypia and lymphocytes. Type B3 thymoma is characterized by an overwhelming majority of round epithelial tumor cells with conspicuous cytological atypia and small numbers of lymphocytes.

Prognostically, type B3 thymoma falls between thymic carcinoma and other types of thymoma. On the morphological level, while thymic carcinoma usually exhibits obvious cytological atypia, type B3 thymoma exhibits no or mild atypia with a minor component of immature T cells. Although this distinction is simple and straightforward, it is often very difficult to distinguish between these two entities. Some immunohistochemical markers, such as CD5, c-Kit (CD117) and glucose transporter-1 (GLUT-1), have been reported to be specifically expressed in TC, and not in typeB3 thymoma or L-SCC.[3-5]

In this study, we evaluated the usefulness of thymic carcinoma markers, including c-Kit, CD5, GLUT-1, p53 and MIB-1 (Ki-67), and thymic cortical epithelial cell (c-TEC)-differentiation marker thymoproteasome subunit β5t and thymic medullary epithelial cell (m-TEC)-differentiation marker claudin-1 (CLDN-1) in distinguishing TC from type B3 thymoma and L-SCC.

We also used thymic cortical microenvironmental marker cells, including cortical immature T-cells (cortical thymocytes ; c-Thys) and thymic cortical dendritic macrophages (TCDMs). It is well documented that c-Thys are specifically present in the thymic cortex and are accompanied by various types of thymoma, especially type B thymomas. TCDMs have recently been identified as thymic cortex specific macrophages that eliminate apoptotic cortical thymocytes.[6] These cells are thought to be attracted directly or indirectly by certain thymic cortical microenvironmental factors, such as chemokines, adhesion molecules and cytokines released or expressed by thymic cortical epithelial cells (c-TEC).[7,8]

We recently found that both c-Thys and TCDMs can be used as markers to identifying thymomas.[9] Therefore, we evaluated the usefulness of these cells as markers for type B3 thymoma in distinguishing this disease from TC.

MATERIALS AND METHODS

Specimens

A total of 35 surgical specimens of thymic epithelial tumors, including 18 cases of thymic carcinoma (TC) diagnosed as thymic squamous cell carcinoma, and 17 cases of type B3 thymoma, and 12 cases of lung squamous cell carcinoma (L-SCC) were used. These cases were diagnosed between 1998 and 2011 were retrieved from the pathology files of Okayama University Hospital (Okayama, Japan) and Okayama Red Cross Hospital (Okayama, Japan). These cases are summarized in Table 2. All surgical specimens were obtained with informed consent. The tissues were fixed in 10% formalin and embedded in paraffin.

Antibodies

The primary antibodies used in this study are listed in Table 1. The rabbit polyclonal antibody to human thymoproteasome subunit β5t was raised against the peptide encompassing resides 285 to 300 of human β5t, as described by Tomaru *et al*.[10] The antibody against the peptides was affinity purified and examined immunohistochemically. This antibody specifically reacted to c-TEC of the human thymus.

Immunohistochemical staining

Deparaffinized sections were immersed in citrate buffer (0.01 mol/L citrate, pH6.0), microwaved for 15 min at 600 W, and then cooled at room temperature for 20 min. The sections were rinsed with phosphate buffered saline (PBS) and examined immunohistochemically. The sections were treated

Table 1. Antibodies used for immunohistochemistry

Antibody	Clone	Property	Source	Concentration
c-Kit (CD117)	YR145	m-mAb	DAKO Cytomation	1:100
Ki-67	MIB-1	m-mAb	DAKO Cytomation	1:100
Claudin-1	IC5-D9	m-mAb	Abnova Corporation	1:100
CD5	4C7	m-mAb	DAKO Cytomation	1:100
p53	DO-7	m-mAb	DAKO Cytomation	1:50
GLUT-1	Polyclonal	r-pAb	ThermoFisher Scientific	1:200
Fascin	55K-2	m-mAb	DAKO Cytomation	1:100
HLA-DR	LN-3	m-mAb	MBL	1:100
CD99	12E7	m-mAb	DAKO Cytomation	1:100
β5t	Polyclonal	r-pAb	original	1:500

m-mAb, mouse monoclonal antibody ; r-pAb, rabbit polyclonal antibody

with methanol containing 3% H_2O_2 for 30 minutes to block endogenous peroxidase activity. The sections were washed with PBS, and incubated with PBS containing 10% normal goat sera for one hour at room temperature followed by overnight incubation at 4℃ with appropriately diluted primary antibodies as listed in Table 1. The sections were washed with PBS and stained following immunoperoxidase methods using the DAKO Envision System Peroxidase (Carpinteria, USA) according to the manufacturer's instructions. The sections were washed and counterstained with hematoxylin. The Ki-67$^+$ nuclei of the tumor cells were counted to calculate the MIB-1 index on a PC screen using Lumina Vision software made by Olympus.

Thymic cortical microenvironmental cells

c-Thy and TCDMs were applied as thymic cortical microenvironmental markers. CD99 was used as a specific immunohistochemical marker for c-Thy. Fascin was used as an immunohistochemical marker for TCDMs. Although mature dendritic cells (DCs) also express fascin, TCDMs are easily distinguished from mature DCs on HLA-DR staining because TCDMs are negative for HLA-DR, while DCs are intensely positive for HLA-DR.

RESULTS

Histological findings of tumors

1) Type B3 thymoma

The tumor cells tended to form lobules separated by thick fibrous hyalinized septa. The tumor cells were uniformly arranged forming tumor sheets with a vaguely solid or trabecular appearance (Fig. 1a). The tumor cells were relatively small polygonal cells with round vesicular nuclei. Neither keratinization nor necrotic foci were found. Palisades around the perivascular spaces and septa were often conspicuous.

2) Thymic carcinoma (TC)

All cases of TC were diagnosed as thymic squamous cell carcinoma. The tumor cells tended to form cell-sheets separated by thick fibrous or hyalinized septa. The tumor cells were uniformly arranged, medium-sized cells with round, vesicular nuclei (Fig. 1b). Necrotic foci were frequently detected while keratinization was rarely found.

3) Lung squamous cell carcinoma (L-SCC)

The tumor cells tended to form varied-sized cell-sheets separated by thick, fibrous and inflammatory septa. The tumor cells tended to exhibit marked nuclear atypia. Necrotic foci and keratinization were frequently found (Fig. 1c).

Thymic cortical microenvironmental marker cells

Thymic cortex-specific cells including CD99$^+$ c-Thys and fascin$^+$ TCDMs were used as thymic cortical microenvironmental marker cells. CD99$^+$ c-Thys were found to be specifically distributed in the thymic cortex of the normal human thymus (data not shown). Fascin$^+$ TCDMs were uniformly scattered exclusively in the thymic cortex, as described elsewhere.[6]

A varied number of CD99$^+$ c-Thys were found to be intermingled with tumor cells in 11 of 17 cases of type B3 thymoma (Fig. 1d). CD99$^+$ c-Thys were detected in considerable numbers (approximately 1/4～1/2 of tumor cells) in 5 cases and in small numbers (approximately 1/100～1/20 of tumor cells) in 6 cases. CD99$^+$ c-Thys were rarely found in remaining six cases of type B3 thymoma. CD99$^+$ Thys were not found in any case of TC or L-SCC at all (Fig. 1e & 1f).

In contrast, fascin$^+$ TCDMs were scattered regularly in considerable numbers (approximately 1/10 of tumor cells) in 11 of 17 cases of type B3 thymoma (Fig. 1g). These cells were rarely found in the remaining six cases of type B3 thymoma, from which CD99$^+$ Thys were also absent. Fascin$^+$ TCDMs were not found in any case of TC (Fig. 1h). These thymic cortical microenvironmental marker cells were not detected in any case of L-SCC (Fig. 1i).

c-Kit

The vast majority of cases of type B3 thymoma (14 of 17 cases) were negative for c-Kit (Fig. 2a). Intense membrane immunostaining for c-Kit was detected in one case, and a weakly diffuse cytoplasmic immunostaining for c-Kit was detected in the other two cases.

In contrast, the vast majority of cases of TC (16 of 18 cases) were positive for c-Kit. Among these positive cases, 12 cases were strongly positive, 3 cases were moderately positive, and one case was weakly positive for c-Kit (Fig. 2b). In all c-Kit positive cases of thymic carcinoma, immunoreaction products for c-Kit were detected at cell membrane and cytoplasm. The difference between type B3 thymoma and TC was statistically significant (chi-square test, $p < 0.01$).

In contrast to TC, almost all cases of L-SCC (10 of 12 cases) were negative for c-Kit (Fig. 2c). In two c-Kit-positive cases weakly diffuse immunostaining for c-Kit was detected in the cytoplasm. The difference between TC and L-SCC was also statistically significant (chi-square test, $p < 0.01$).

CD5

The vast majority cases of type B3 thymoma (16 of 17

Table 2. Summary of the results

Type of tumor	Case number	Age	Sex	c-kit	MIB-1	CLDN-1	CD5	β5t	p53	GLUT-1
Type B3 thymoma	Case 1	61	F	−	5.9	−	−	+++	−	−
	Case 2	58	M	−	3.96	−	−	+++	−	−
	Case 3	57	F	−	5.9	−	−	++	−	−
	Case 4	74	M	−	5.3	−	−	++	+	−
	Case 5	45	F	−	6.5	−	−	++	−	−
	Case 6	71	F	+ c	5.94	−	−	++	−	−
	Case 7	38	F	−	4.1	−	−	++	−	+
	Case 8	53	M	−	6.9	+	−	+++	−	++
	Case 9	41	M	−	6.1	+	−	++	−	++
	Case 10	49	F	−	6.9	+	−	++	+	+
	Case 11	83	F	−	7.2	+	−	++	++	++
	Case 12	46	M	−	16.6	+++	−	+	++	−
	Case 13	35	F	+ c	22.3	+++	−	+	+	++
	Case 14	78	M	−	10.2	+++	−	+	++	−
	Case 15	75	F	+++ m	11.1	+++	−	+	++	+++
	Case 16	62	F	−	16	+++	−	+	+++	−
	Case 17	70	M	−	28.3	+++	+++	+	−	+++
Thymic carcinoma	Case 18	81	M	+ m&c	20.2	+++	+	−	+	+
	Case 19	81	M	++ m&c	25.9	+++	−	−	−	+++
	Case 20	66	M	++ m&c	15.7	+++	+	−	−	+
	Case 21	73	F	−	23.4	+++	−	−	−	−
	Case 22	48	F	++ m&c	22.3	+++	+	−	++	+++
	Case 23	72	F	+++ m&c	41.5	+++	+++	−	−	+++
	Case 24	70	M	+++ m&c	25	+++	++	−	−	+++
	Case 25	74	F	+++ m&c	34.7	+++	+	−	+	++
	Case 26	56	M	+++ m&c	30	+++	−	−	ND	+++
	Case 27	26	M	−	36	+++	−	−	−	−
	Case 28	54	M	+++ m&c	5.9	+++	−	−	++	++
	Case 29	64	M	+++ m&c	40.1	+++	−	−	−	+++
	Case 30	72	M	+++ m&c	14.5	+++	+++	−	++	+++
	Case 31	75	F	+++ m&c	19.5	+++	+	−	−	+++
	Case 32	70	M	+++ m&c	19.5	+++	++	−	++	+++
	Case 33	48	M	+++ m&c	27	+++	+	−	++	+++
	Case 34	65	M	+++ m&c	46.7	++	+	−	++	+++
	Case 35	66	F	+++ m&c	35.2	+++	+++	−	+	+++
Lung cancer	Case 36	74	M	−	35.1	+++	−	−	+++	+++
	Case 37	80	M	+ c	44.3	+++	−	−	+++	+++
	Case 38	69	M	−	33.3	+++	−	−	−	+++
	Case 39	78	M	−	27.4	+++	−	−	++	+++
	Case 40	79	M	+ c	35.7	+++	−	−	−	+++
	Case 41	67	M	−	37.8	+++	−	−	−	+++
	Case 42	65	F	−	31.8	+++	−	−	+++	+++
	Case 43	78	M	−	37.3	+++	−	−	+++	+++
	Case 44	80	M	−	54	+++	−	−	++	+++
	Case 45	58	M	−	35.3	+++	−	−	+++	+++
	Case 46	79	M	−	47.9	+++	−	−	+++	+++
	Case 47	75	M	−	28.6	+++	−	−	+++	+++

−, less than 5% ; +, 5-30% ; ++, 30-60% ; +++, 60-100% of positive cells ; M, male ; F, female ; m, membrane ; c, cytoplasmic ; m&c, membrane and cytoplasmic ; ND, not done

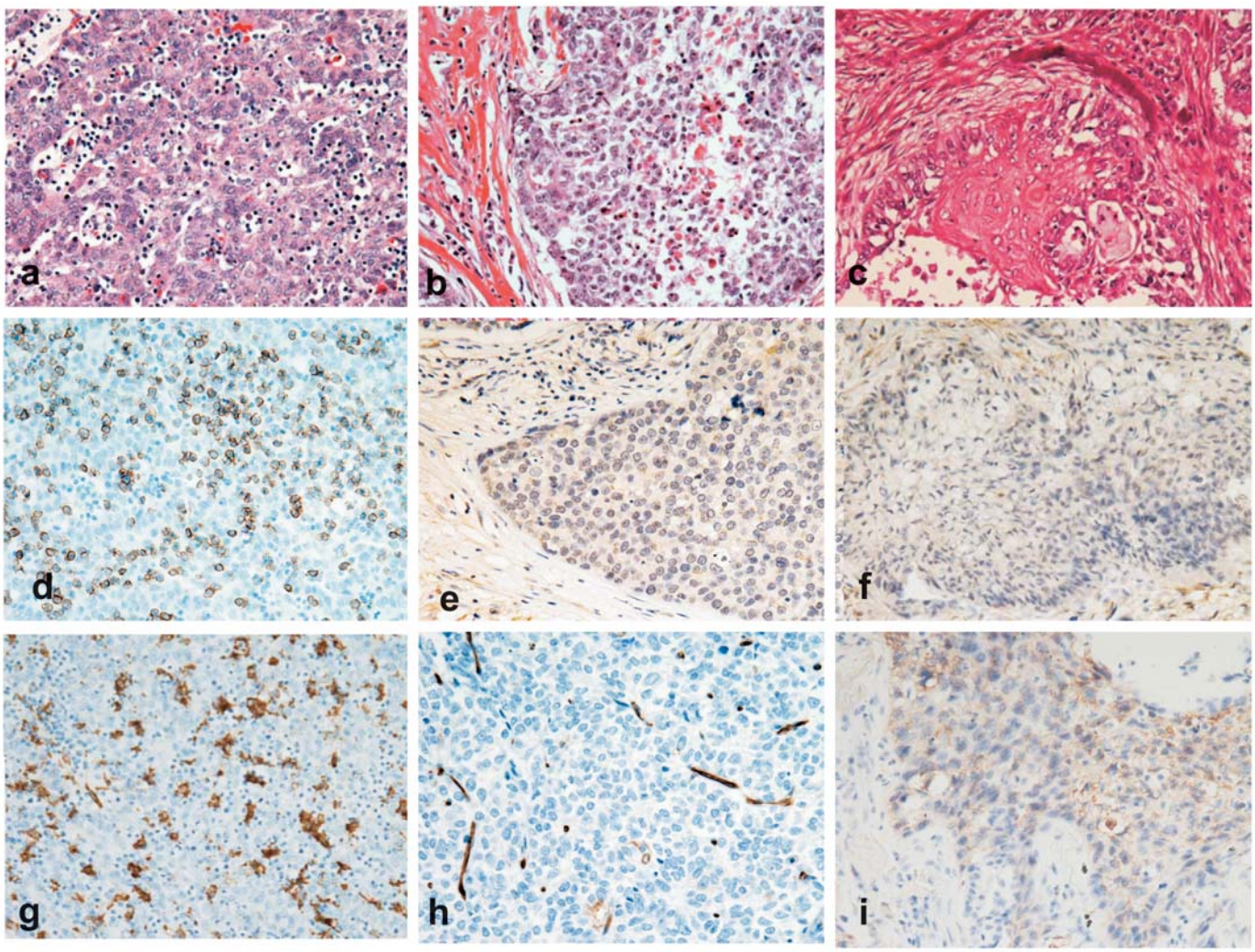

Fig. 1. Hematoxylin-eosin (HE), CD99, and fascin staining of type B3 thymoma, thymic carcinoma (TC), and lung squamous cell carcinoma (L-SCC). (***1a***) HE stain of type B3 thymoma. Case-1, type B3, × 200. (***1b***) HE stain of TC. Case-21, TC, × 200. (***1c***) HE-stain of L-SCC. Case-36, L-SCC, × 200. (***1d***) A considerable number of $CD99^+$ cortical thymocytes (c-Thys) are intermingled with tumor cells of type B3 thymoma. Case-1, type B3, anti-CD99, × 200. (***1e***) $CD99^+$ Thys are found in any case of TC. Case-21, TC, anti-CD99, × 200. (***1f***) $CD99^+$ Thys are found in any case of L-SCC. Case-36, L-SCC, anti-CD99, × 200. (***1g***) $Fascin^+$ thymic cortical dendritic macrophages (TCDMs) are scattered regularly throughout the type B3 thymoma. Case-1, type B3, anti-fascin, × 200. (***1h***) None of $fascin^+$ TCDMs is found in any case of TC. Note that none of cells except for capillary endothelial cells are positive for fascin. TC, anti-fascin, × 200. (***1i***) $Fascin^+$ TCDMs are found in any case of L-SCC. Case-36, L-SCC, anti-fascin, × 200.

cases) were negative for CD5 (Fig. 2d). Intense membranous immunostaining for CD5 was detected in only one of 17 cases of type B3 thymoma. In contrast, intense membranous immunostaining for CD5 was detected in 12 of 18 cases of TC (Fig. 2e). Among these 12 positive cases, 3 cases were strongly positive, 2 cases were moderately positive, and 7 cases were weakly positive for CD5. CD5 was not detected in any 12 cases of L-SCC at all (Fig. 2f). The difference between type B3 thymoma and TC was statistically significant (chi-square test, $p < 0.01$).

GLUT-1

Membranous and cytoplasmic immunostaining for GLUT-1 were detected in 8 of 17 cases of type B3 thymoma (Fig. 2g). GLUT-1 was detected intensely in 2 cases, moderately in 4 cases, and weakly in 2 cases. Membranous and cytoplasmic immunostaining for GLUT-1were detected in 16 of 18 cases of TC (Fig. 2h). GLUT-1 was detected intensely in 12 cases, moderately in 2 cases, and weakly in 2 cases. Intense membranous and cytoplasmic immunostaining for GLUT-1 was detected in all cases (12 of 12 cases) of L-SCC (Fig. 2i).

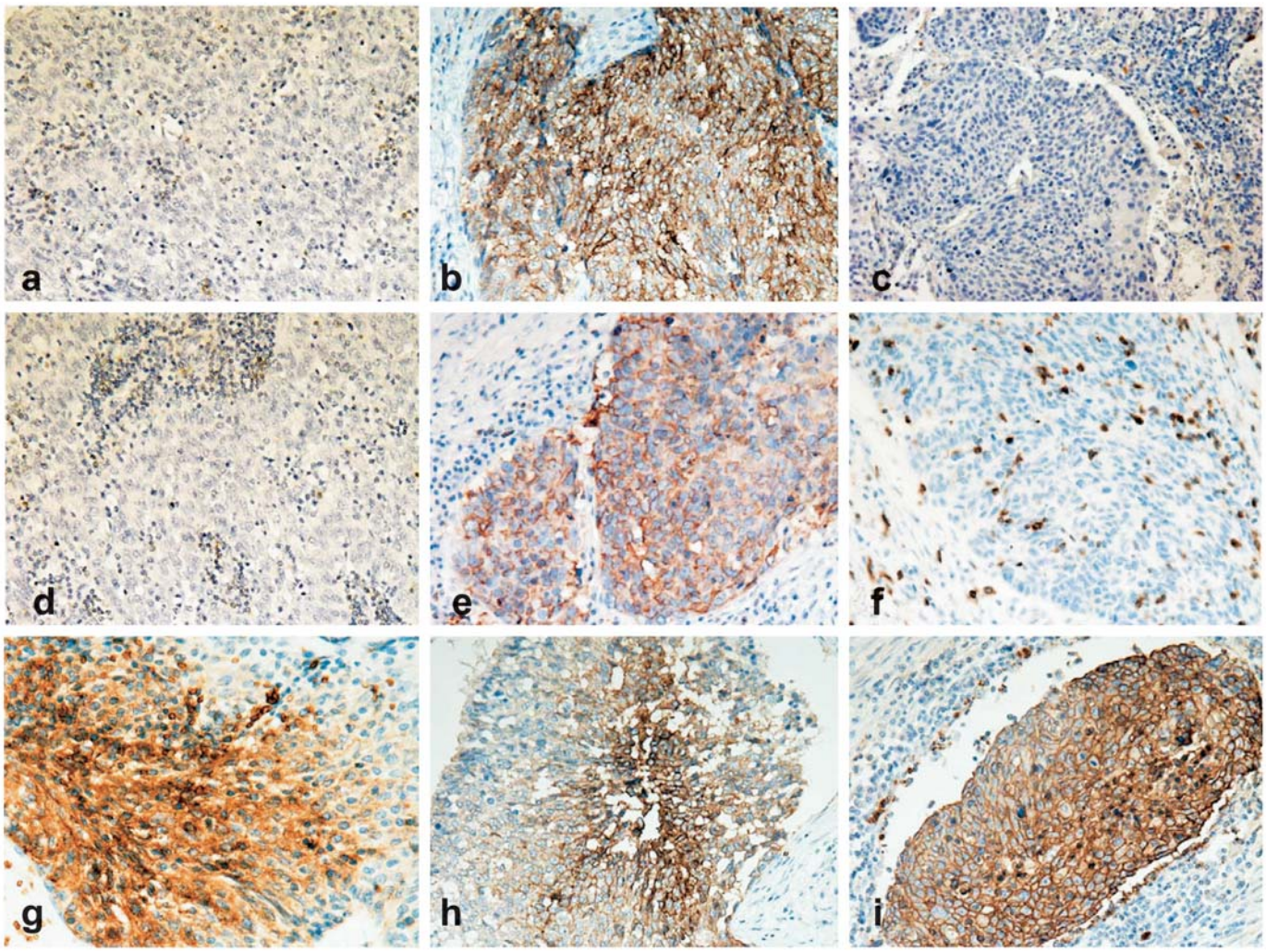

Fig. 2. Immunostaining for thymic carcinoma-markers, c-Kit, CD5, and GLUT-1 of type B3 thymoma, thymic carcinoma (TC), and lung squamous cell carcinoma (L-SCC). (***2a***) The vast majority of the cases of type B3 thymoma (14 of 17 cases) are negative for c-Kit. Case-1, type B3, anti-c-Kit, × 200. (***2b***) Intense membrane immunostaining for c-Kit is detected in the vast majority of the cases of TC (16 of 18 cases). Case-21, TC, anti-c-Kit, × 200. (***2c***) Almost all cases of L-SCC (11 of 12 cases) are negative for c-Kit. Case-36, L-SCC, anti-c-Kit, × 200. (***2d***) Almost all cases of type B3 thymoma (16 of 17 cases) are negative for CD5. Case-1, type B3, anti-CD5, × 200. (***2e***) The majority of cases of TC (12 of 18 cases) are positive for CD5. Note intense immunoreactivity for CD5 is detected at cell membrane. Case-23, TC, anti-CD5, × 200. (***2f***) Almost all cases of type L-SCC (11 of 12 cases) are negative for CD5. $CD5^+$ small cells are mature lymphocytes intermingled with tumor cells. Case-37, L-SCC, anti-CD5, × 200. (***2g***) Some cases of type B3 thymoma (8 of 17 cases) are positive for GLUT-1. Case-15, type B3, anti-GLUT-1, × 200. (***2h***) The vast majority of cases of TC (16 of 18 cases) are positive for GLUT-1. Case-19, TC, anti-GLUT-1, × 200. (***2i***) All 12 cases of L-SCC are positive for GLUT-1. Case-36, L-SCC, anti-GLUT-1, × 200.

The difference between type B3 thymoma and TC was statistically significant (chi-square test, $p < 0.01$). The difference between TC and L-SCC was not statistically significant (chi-square test, $p > 0.05$).

CLDN-1

CLDN-1 was weakly detected in the cell membranes of thymic medullary epithelial cells (m-TEC) in the normal human thymus (data not shown). This protein was not detected in any case of type B1 or type B2 thymoma (unpublished data). CLDN-1 was absent in 7 of 17 cases of type B3 thymoma. Among 10 positive cases, CLDN-1 was detected intensely in 3 cases, moderately in 8 cases, and weakly in 6 cases (Fig 3a). Immunoreactivity for CDLN-1 was detected at cell-cell borders.

In contrast, the overexpression of CLDN-1 was detected in all 18 cases of TC (Fig. 3b). The difference between type B3 thymoma and TC was statistically significant (chi-square test, $p < 0.01$). The overexpression of CLDN-1 was de-

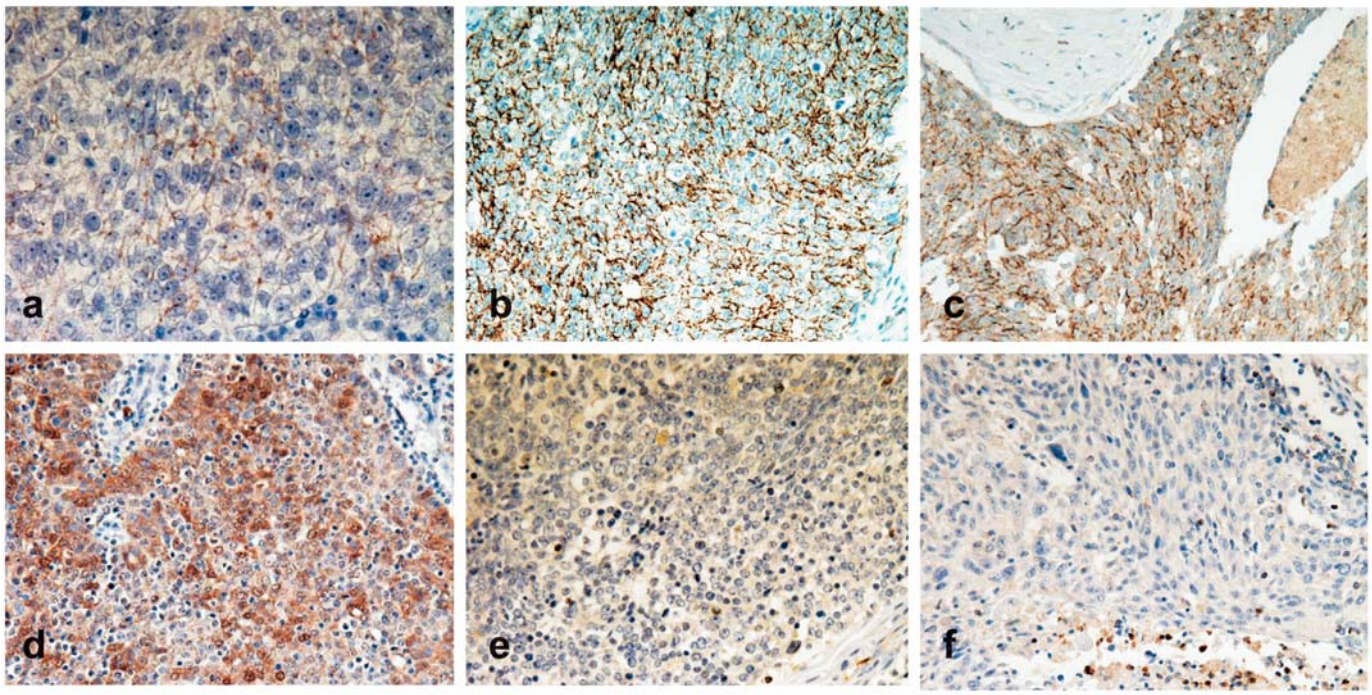

Fig. 3. CLDN-1-staining and thymoproteasome β5t-staining of type B3 thymoma, thymic carcinoma (TC), and lung squamous cell carcinoma (L-SCC). (***3a***) A case of type B3 thymoma faintly expressing CLDN-1. Case-5, type B3, anti-CLDN-1, × 200. (***3b***) CLDN-1 is overexpressed in all cases of TC. Case-21, TC, anti-CLDN-1, × 200. (***3c***) CLDN-1 is overexpressed in all cases of L-SCC. Case-36, L-SCC, anti-CLDN-1, × 200. (***3d***) Thymoproteasome β5t is expressed in all cases of type B3 thymoma. Case-1, TC, anti-β5t, × 200. (***3e***) None of TC express thymoproteasome β5t. Case-21, TC, anti-β5t, × 200. (***3f***) None of L-SCC express thymoproteasome β5t. Case-36, L-SCC, anti-β5t, × 200.

tected in all 12 cases of L-SCC (Fig. 3c).

Thymoproteasome subunit β5t

Thymoproteasome β5t was intensely detected in c-TEC, but not in m-TEC, in the normal human thymus (data not shown). β5t was detected in all cases of type B3 thymoma (Fig 3d). Immunoreaction products for β5t were strongly detected in 11 cases and dimly in 6 cases of type B3 thymoma. In contrast, β5t was not detected in any case of TC (Fig. 3e) or L-SCC (Fig. 3f).

MIB-1 index

The mean MIB-1 index of the 17 cases of type B3 thymoma, the 18 cases of TC, and the 12 cases of L-SCC was 10.0, 26.8 and 37.4, respectively. The mean MIB-1 index of TC was statistically significant higher than that of type B3 thymoma (t-test : $p < 0.05$) and lower than that of L-SCC (t-test : $p < 0.05$).

p53

Nuclear p53 staining was detected in 8 of 17 cases of type B3 thymoma (1 intense, 4 moderate, 3 weak), 8 of 17 cases of TC (6 moderate, 2 weak), and 9 of 12 cases of L-SCC (7 intense, 2 moderate). The difference between type B3 thymoma and TC was not statistically significant (chi-square test, $p > 0.05$). The difference between TC and L-SCC was also statistically not significant (chi-square test, $p > 0.05$).

DISCUSSION

TC is a malignant neoplasm of the anterior mediastinum, Because TC is rare malignant tumor, it should be correctly distinguished from the less malignant but more frequent type B3 thymoma and the usually more malignant primary L-SCC with massive anterior mediastinal involvement. It is, however, often very difficult to distinguish TC from these tumors, especially on small biopsy specimens. In this study, we evaluated the usefulness of several immunohistochemical markers, including c-Kit, CD5, GLUT-1, CLDN-1, thymic proteasome subunit β5t, p53 and MIB-1 in distinguishing TC from type B3 thymoma and L-SCC. We also evaluated the usefulness of thymic microenvironmental cells, c-Thys and TCDMs in distinguishing TC from B3 thymoma.

As shown in this study, TCDMs are more useful as marker cells than c-Thys in distinguishing type B3 thymoma

from TC. However, this study also showed that these thymic cortical microenvironmental marker cells scarcely occur in some cases of type B3 thymoma. Therefore, if these thymic microenvironmental marker cells are absent from certain thymic epithelial tumors, one should examine further immunohistochemical markers to distinguish between type B3 thymomas and TC.

It has been shown that the expression of c-Kit is strongly related to thymic carcinoma, but not to thymomas including type B3 thymoma.[11] c-Kit, the tyrosine kinase receptor protein encoded by proto-oncogene Kit, is a growth factor receptor for stem cell factor.[12] The expression of c-Kit has been documented in a wide variety of human neoplasms, including acute myeloid leukemia, germ cell tumors, ovarian carcinoma, lung small cell carcinoma, gastrointestinal stromal tumors, and breast cancer.[13-19] In the present study, c-Kit was detected in the vast majority of TC cases (16 of 18 cases ; 89%) and in three of 17 cases of type B3 thymoma (18%). These findings indicate that c-Kit is preferentially, but not specifically expressed in TC among the various types of thymic epithelial tumors.

Among types of lung cancer, c-Kit has been reported to be expressed in 27% of L-SCC cases, 27% of lung adenocarcinoma cases and over 70% of small cell carcinoma cases.[20] In this study, c-Kit-immunoreactivity was weakly detected in 2 of 12 cases of L-SCC. Taken together, it seems probable that c-Kit is preferentially, but not specifically, expressed in TC among the various types of mediastinal epithelial tumors. Therefore, one should be careful to distinguish TC from type B3 thymoma or L-SCC on the basis of the positivity for c-Kit only. However, it is noteworthy that in c-Kit$^+$ cases of L-SCC and type B3 thymoma, immunoreaction products for c-Kit were usually detected diffusely and weakly in cytoplasm but not at cell membrane. Such diffuse weak cytoplasmic immunoreactivity for c-Kit has been detected in some cases of various types of tumors by several authors, although its biological significance of diffuse cytoplasmic immunoreactivity for this cell-surface receptor-protein c-Kit remains uncertain.[21,22]

CD5 is a monomeric glycoprotein that is expressed by T cells during the various stages of T cell differentiation in the thymus.[23] CD5 is a type of receptor molecule and is one of the ligands for CD72 on the surface of B cells.[24,25] It has been shown that CD5 is specifically expressed in thymic carcinoma but not in type B3 thymoma, and that CD5 is useful for distinguishing thymic carcinoma from type B3 thymoma.[5,26] In the present study, CD5 was detected in 12 of 18 cases of thymic carcinoma, and in only 1 of 17 cases of type B3 thymoma. The present study also indicated that none of 18 cases of L-SCC expressed CD5. Therefore, the present study confirmed that CD5 is useful for distinguishing TC from type B3 thymoma and L-SCC. The problem is that a considerable number of cases of TC (6 of 18 cases) do not express CD5. Moreover, it has been reported that 15-20% of L-SCCs express CD5. Taken together, one should also be careful to distinguish TC from L-SCC on the basis of positivity for CD5.

GLUT-1 is one of 14 members of the mammalian facilitative glucose transporter family of passive carriers which functions as an energy-independent system for the transport of glucose down a concentration gradient.[27,28] GLUT-1 is expressed in a variety of carcinomas, including breast carcinoma, ovarian carcinoma, renal cell carcinoma, lung cancer and malignant pleural mesothelioma.[4,29-35] It has been reported that GLUT-1 is specifically expressed in thymic carcinoma and not in thymomas.[4] In the present study, however, GLUT-1 was detected in 8 of 17 cases of type B3 thymoma and in the vast majority of cases of TC (16 of 18 cases), indicating that GLUT-1 is not very useful for distinguishing TC from type B3 thymoma.

CLDN-1 has been shown to be specifically expressed on thymic medullary epithelial cells in the normal human thymus. We analyzed the expression of CLDN-1 in various types of thymic epithelial tumors. As shown in this study, CLDN-1 was over expressed in all cases of TC and L-SCC, and in a considerable number of cases (7 of 17 cases) of type B3 thymoma. CLDN-1 is a member of the tight junction protein claudin-family composed of at least 23 different proteins.[36] CLDN-1 regulates the permeability of cell junctions in different types of epithelia and the vascular epithelium. The loss of CLDN and other tight junction proteins in cancer has been interpreted as a mechanism underlying the loss of cell adhesion and is an important step in the progression of cancer to metastasis. Consistently, it has been shown that the expression of CLDN-1 is reduced in various cancers, such as breast cancer and colon cancer.[37-39] CLDN-7 is also downregulated in invasive breast cancer and head and neck cancer.[40,41] Paradoxically, however, other studies have shown that certain CLDN proteins are upregulated in cancer. In fact, the overwhelming majority of studies published thus far report an overexpression of CLDN proteins, such as CLDN-3 and CLDN-4, in various cancers, suggesting that these proteins may exert positive effects on tumorigenesis.[42] CLDN-1 has been shown to be overexpressed in squamous cell carcinoma.[43] In the present findings, an overexpression of CLDN-1 was detected in all cases of TC and L-SCC without exception, and in 6 of 17 cases of type B3 thymoma. In contrast, the overexpression of CLDN-1 was not observed in any of the case of type B1 or B2 thymoma (unpublished data). These findings strongly suggest that the overexpression of CLDN-1 is involved in the malignant conversion of thymic epithelial tumors, although CLDN-1 is not very useful for distinguishing TCC from type B3 thymoma.

It has been documented that thymic proteasome subunit β5t is expressed in type B3 thymoma but not in thymic carcinoma.[44] β5t is a recently discovered β subunit of the 20S

proteasome that is expressed exclusively in thymic cortical epithelial cells in humans and mice. Recently, Yamada *et al*.[44] reported for the first time that β5t is a specific marker for thymomas including type B3 thymoma. The authors stressed that β5t is very useful for distinguishing thymic carcinoma from type B3 thymoma. However, this view is only preliminary because the authors examined only four cases of type B3 thymoma. The present findings that all 17 cases of type B3 thymoma variedly expressed β5t and that none of the18 cases of TC expressed β5t confirm this preliminary view.

It is generally accepted that L-SCC tends to be more malignant than TC to some extent. Consistently, the present study indicated that the average MIB-1 index of L-SCC (37.4 %) was significantly higher than that of TC (26.8%) ($p < 0.05$). MIB-1 is a monoclonal antibody to the Ki-67 protein, which is one of the most sensitive cell proliferation markers.[45-47] The MIB-1 index, which is calculated as the percentage of MIB-1-positive nuclei, has been widely used as a complementary tool to differentiate between the better and worse prognoses groups of various neoplasms, including thymic epithelial tumors. This study also indicated that the MIB-1 index of TC (26.8%) is significantly higher than that of type B3 thymoma (10%). These findings also indicate that the MIB-1 index is, to some extent, useful in differentiating TC from L-SCC and type B3 thymoma.

Similarly, an over expression of nuclear p53 was detected in 7 of 12 cases of L-SCC, none of 18 cases of TC and 1 of 17 cases of type B3 thymoma in this study. A moderate expression of nuclear p53 was detected in 2 of 12 case of L-SCC, 6 of 18 cases of TC, and in 4 of 17 cases of type B3 thymoma. Accumulation of p53 nuclear proteins is one of the most common abnormalities in human cancer.[48] A missense mutation of the p53 gene or the binding of oncoproteins to p53 proteins leads to the accumulation of p53 proteins. This accumulation abrogates the ability of normal p53 to suppress tumor growth.[49-51] This may be an important step in the complex process of carcinogenesis in human cancer. A high frequency of p53 proteins has been reported in thymic carcinomas but not in thymomas.[52] p53 staining is also in some extent useful for distinguishing TC from L-SCC or type B3 thymoma., although the differences were not statistically significant.

In conclusion, as shown in this study, among these immunohistochemical markers the most useful markers for distinguishing thymic carcinoma from type B3 thymoma and L-SCC are β5t, c-Kit, and CD5. Therefore, we present a flow chart for differential diagnosis of these anterior mediastinal tumors as shown in Fig. 4. In addition, thymic cortical microenvironmental markers that included c-Thys and TCDMs, claudin-1, p53, and MIB-1 may be also useful. The proper use of these immunohistochemical markers may make it possible to distinguish correctly between these three morphologically similar anterior mediastinal tumors.

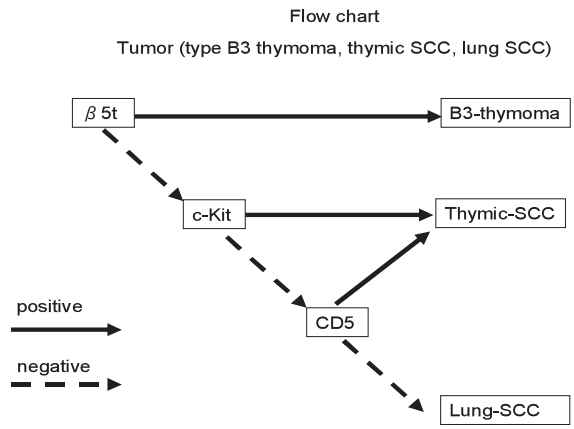

Fig. 4. The flow chart for differential diagnosis of thymic carcinoma, type B3 thymoma, and lung squamous cell carcinoma.

ACKNOWLEDGEMENT

This study was supported by KAKENHI #22590313 (Grant-in-Aid for Science Research of Japanese Government).

CONFLICT OF INTEREST

There is no conflict of interest.

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