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L-Type Amino Acid Transporter 1 Immunoreactivity as a Possible Diagnostic and Prognostic Marker of Thymic Carcinoma

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L-type amino acid transporter 1 (LAT1) functions to transport large neutral amino acids, such as leucine, isoleucine, valine, phenylalanine, tyrosine, tryptophan, methionine, and histidine. These amino acids are essential for cell growth and proliferation. Many studies have demonstrated LAT1 expression in various types of cancer, and its high expression level was associated with poor prognosis. However, the significance of LAT1 expression in thymic epithelial tumors is controversial. We conducted this retrospective study to investigate the LAT1 immunoreactivity in thymic epithelial tumors and its impact on prognosis. We analyzed 32 patients with thymoma and 14 patients with thymic carcinoma who underwent surgery at our institute. Immunohistochemical analysis was performed using formalin-fixed paraffin-embedded surgical tissues and an anti-LAT1 polyclonal antibody. We thus found that LAT1 immunoreactivity was undetectable in all of the thymoma specimens, regardless of the subtypes of thymoma. By contrast, LAT1 immunoreactivity was consistently detected in the cytosol of thymic carcinoma cells; namely, all 14 thymic carcinoma specimens demonstrated LAT1 immunoreactivity in the cytosol. Among these 14 thymic carcinoma specimens, four carcinoma specimens also showed LAT1 immunoreactivity in the cell membrane. Survival analysis indicated that the thymic carcinoma with the LAT1 membrane signal was associated with poor prognosis, compared with the specimens with the LAT1 cytosol signal. We therefore propose that LAT1 is expressed in the cytosol of thymic carcinoma cells, which could be a diagnostic marker of thymic carcinoma. Moreover, LAT1 expression in the cell membrane is a prognostic marker of thymic carcinoma.

Keywords: amino acid transporter 1; immunohistochemistry; prognosis; thymic carcinoma; thymoma
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Introduction

Thymic carcinoma is a well-known malignant type of thymic epithelial tumor, though its occurrence is rare. Treatment of affected patients is challenging, because this tumor is often detected in an advanced stage and involves surrounding organs. Multimodality therapy including complete surgical resection, chemotherapy, and radiotherapy is mandatory to achieve better prognosis. The 5-year overall survival of patients following thymic carcinoma resection is around 60% (Ahmad et al. 2015; Hishida et al. 2016) and is still unsatisfactory. It is important to develop a novel therapeutic strategy based on a mechanism to improve the prognosis of thymic carcinoma patients.

L-type amino acid transporter 1 (LAT1) functions to transport large neutral amino acids into cells, such as leucine, isoleucine, valine, phenylalanine, tyrosine, tryptophan, methionine, and histidine, essential for cell growth and proliferation (Kanai et al. 1998). Among those, leucine (Leu) is not only a source of protein synthesis but also serves as an activator of the mammalian target of rapamycin (mTOR) signaling pathway (Nicklin et al. 2009) to regulate cell proliferation.

Many studies have revealed cancer-specific LAT1 expression in various organs and also demonstrated poor prognosis of patients with high LAT1 expression levels (Kaira et al. 2009a, 2012; Li et al. 2013; Toyoda et al. 2014; Honjo et al. 2016). In regard to thymic carcinoma, two

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studies have presented seemingly inconsistent results (Kaira et al. 2009b; Omatsu et al. 2012); namely, Kaira et al. (2009b) used an anti-LAT1 polyclonal antibody and demonstrated that LAT1 immunoreactivity was frequently detected in thymic carcinoma but was undetectable in thymoma. On the other hand, Omatsu et al. (2012) used an anti-LAT1 monoclonal antibody and reported that LAT1 immunoreactivity was not frequently detected in both thymic carcinoma and thymoma tissues. In the present study, to elucidate the association between LAT1 expression in thymic carcinoma and patient prognosis, we conducted a retrospective clinicopathological study.

Patients and Methods

Patients

We analyzed 46 patients (21 males and 25 females) who underwent surgical resection following diagnosis of thymoma or thymic carcinoma from April 2001 to December 2014 at our institution. A survey of medical records included patient age, sex, Masaoka-Koga classification, World Health Organization (WHO) classification, and tumor size, as well as the maximum of standardized uptake value (SUV max) in ^{18}F-2-fluoro-2-deoxy-D-glucose (FDG)-positron emission tomography (PET) findings when available. This retrospective study was conducted with the approval of the Ethical Committee of Dokkyo Medical University Hospital (#28057).

Immunohistochemical analysis

Immunostaining with an anti-LAT1 antibody was performed according to a previous report (Kaira et al. 2009b). Briefly, formalin-fixed and paraffin-embedded thymic tissues were deparaffinized, and heated with microwave oven for antigen retrieval. Then tissues were reacted with an affinity-purified polyclonal rabbit anti-LAT1 antibody (KE026, TransGenic Inc., Kobe, Japan). The antibody was raised against an oligopeptide corresponding to amino acid residues 497-507 of human LAT1 (CQKLMQVVPQET). Detailed methods were described elsewhere (Yanagida et al. 2001). Immunoreactivity was visualized using the ABC method, and independently evaluated by two researchers (S.M. and Y.N.). Positive signals were determined when the cytosol or the membrane staining was observed more than 10% of a tumor area (Kaira et al. 2009b). Normal placenta tissue was used as a positive control, and the tissue section untreated with the anti-LAT1 antibody was used as a negative control (Fig. 1). In the positive control, LAT1 immunoreactivity was detected in the membrane of syncytiotrophoblasts. The cytosol of cytotrophoblasts and capillary endothelial cells was also weakly positive. The negative control showed no signal.

Survival analysis and statistics

The JMP 12.2 software package (SAS Institute Japan., Tokyo, Japan) was utilized to perform the statistical analyses. Each continuous variable in patients' characteristics among groups was presented in the mean ± standard error of the mean (SEM). Kruskal-Wallis test, Mann-Whitney U test, or Fisher's exact test, whichever appropriate, was used for a statistical test because of small sample number. Overall survival of thymic carcinoma patients was estimated using the Kaplan-Meier method. Survival estimates of two groups were compared with a log-rank test. The statistical significance threshold was set at 0.05.

Results

Patient characteristics are summarized in Table 1. There was a greater number of patients with advanced Masaoka-Koga stage tumor among those with type B2, type B3, or thymic carcinoma according to the WHO classification. There were no significant differences in regard to age and sex among the WHO classification groups. Tumor size was significantly larger in the thymic carcinoma group ($p = 0.0484$, Kruskal-Wallis test). The values of SUV max were higher in the thymic carcinoma group, although the number of patients was limited ($p = 0.0066$, Kruskal-Wallis test).

LAT1 immunostaining revealed completely negative immunoreactivity in thymoma cells and surrounding

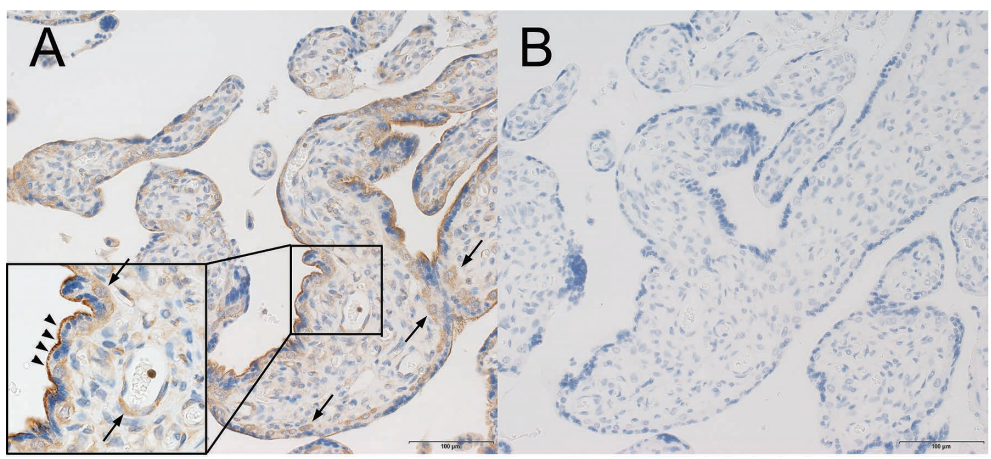

Fig.1. LAT1 Immunostaining in the placenta tissue.
Normal placenta tissue was used as positive and negative control for LAT1 immunostaining.
A) Syncytiotrophoblasts (the outer cells of placental villi) showed positive signal in the membrane (arrowheads in an inset). Some cytotrophoblasts (the inner cells of placenta villi) and capillary endothelial cells showed weak signal in the cytosol (arrows) (Magnification × 200. The inset: Magnification × 400).
B) Negative control shows no signal (Magnification × 200).

Table 1. Patients' characteristics.

	Total	WHO classification					
		A	AB	B1	B2	B3	Carcinoma
Cases	46	4	10	8	6	4	14
Sex							
M:F	21:25	3:1	3:7	3:5	3:3	2:2	7:7
Age							
yrs	62.3 ± 2.0	73.5 ± 1.5	61.4 ± 3.7	61.6 ± 3.8	57.3 ± 9.0	64.5 ± 5.5	61.5 ± 3.6
Myasthenia gravis	7	0	3	3	0	1	0
Masaoka-Koga							
I		2	5	5	1	1	0
II		2	4	3	2	0	1
III		0	1	0	1	2	3
IVa		0	0	0	1	1	0
IVb		0	0	0	1	0	10
Tumor size							
mm		44.8 ± 4.4	48.3 ± 8.6	38.9 ± 9.3	49.7 ± 14.2	55.0 ± 19.4	69.0 ± 7.6†
FDG-PET							
SUV max		2.2 ± 0.7	3.6 ± 0.2	3.6 ± 0.9	4.7 ± 1.2	5.0 ± 2.2	12.9 ± 2.8†
Cases*	30	3	6	5	5	3	8

The Age, tumor size, and SUV max are presented in the mean ± SEM. Cases* means the number of patients who underwent FDG-PET.

†$p < 0.05$, Kruskal-Wallis test.

immature T-lymphocytes, irrespective of the WHO classification type (Fig. 2). In contrast, LAT1 immunoreactivity was detected in the cytosol and membrane of thymic carcinoma cells (Fig. 2L). The thymic carcinoma specimen untreated with the anti-LAT1 antibody showed no immunoreactivity (data not shown).

The clinicopathological features of the 14 patients with thymic carcinoma are summarized in Table 2. There were eight patients with squamous cell carcinoma of the thymus and six patients with non-squamous cell carcinoma. The LAT1 immunoreactivity was consistently detected in the cytosol among all 14 thymic carcinoma specimens (Table 2, Fig. 3). Moreover, four thymic carcinoma specimens showed positive signals in the membrane as well as the cytosol of carcinoma cells (see Figs. 2L and 3D).

According to the LAT1 signal location, we divided thymic carcinomas into two groups (Table 3): membrane/cytosol signal group that showed the signals in both membrane and cytosol (n = 4) and the cytosol signal group (n = 10). There was no noticeable association between the LAT1 signal location and sex, age, tumor size, histology, or the value of SUV max. Incidentally, however, thymic carcinoma with the LAT1 membrane signal was classified as squamous cell carcinoma. In regard to Masaoka-Koga classification and treatment options, statistical test was not appropriate because of the small sample number.

Survival of the 14 thymic carcinoma patients was poor in the membrane/cytosol signal group as compared with the cytosol signal group ($p = 0.034$, log-rank test) (Fig. 4A). In addition, a sub-analysis for a major histological group, eight patients with squamous cell carcinoma, was performed (Fig. 4B). Survival of the membrane/cytosol signal group was also poor as compared to the cytosol signal group ($p = 0.0083$, log-rank test) (Fig. 4B).

Discussion

The present results demonstrate a clear difference between thymoma and thymic carcinoma in terms of immunoreactivity for LAT1. The thymoma specimens showed completely negative immunoreactivity for LAT1 regardless of the WHO classification. In contrast, all 14 thymic carcinoma specimens showed LAT1 positive signal in the cytosol, and four of them also presented positive signal in the cell membrane. We therefore divided thymic carcinoma patients into two groups according to the LAT1 signal location: the cytosol signal group and the membrane/cytosol signal group. Importantly, the prognosis of patients in the membrane/cytosol signal group was worse, as compared with the cytosol signal group. Together, our findings suggest that the LAT1 cytosol signal is a useful marker for diagnosis of thymic carcinoma, and the presence of the membrane signal is predictive of poor prognosis among affected patients.

LAT1 expression has been intensively investigated in other types of malignancies located such as in the lungs, pancreas, and liver, with results demonstrating that patients with LAT1-immunopositive malignancies have poor prognosis (Kaira et al. 2009a, 2012; Li et al. 2013; Toyoda et al. 2014; Honjo et al. 2016). Moreover, to the best of our knowledge, two studies have been presented in English

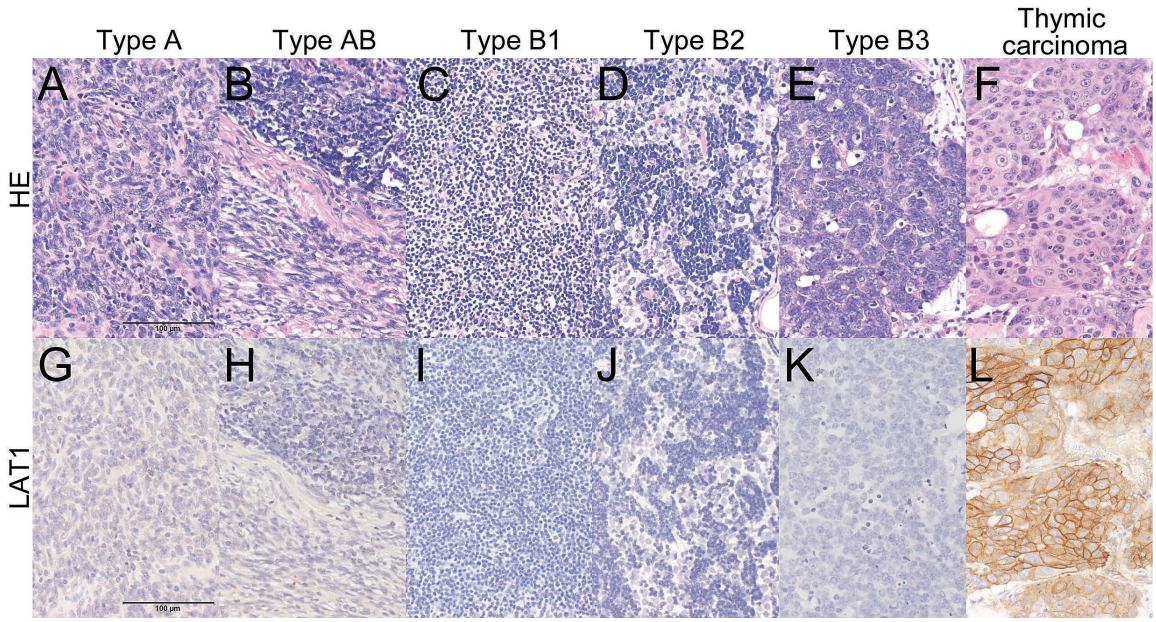

Fig. 2. Histopathology and LAT1 immunostaining of thymoma and thymic carcinoma specimens.
Upper panels (A-F) show Hematoxylin and Eosin (HE) staining of each type of representative thymic epithelial tumors, based on the WHO classification, and lower panels (G-L) show LAT1 immunostaining of respective tissue specimens (A-L: Magnification × 200).
A) and G) Type A thymoma. The tumor consists of bland spindle and/or oval cells with few lymphocytes.
B) and H) Type AB thymoma. The tumor is a mixture of lymphocyte-poor type A thymoma component and lymphocyte-rich Type B component. Upper part is Type B component, and lower part is Type A component separated with fibrous tissue.
C) and I) Type B1 thymoma. Neoplastic epithelial cells (pale pink cells) are scattered, small, with little atypia, and surrounded with lymphocytes (dark cells).
D)and J) Type B2 thymoma. The tumor is composed with clustered neoplastic epithelial cells (pale pink cells). Surrounding lymphocytes (dark cells) are dominant in number.
E) and K) Type B3 thymoma. The tumor cells are polygonal and medium sized with round nuclei. Infiltrating lymphocytes are quite few in number.
F) and L) Thymic carcinoma (squamous cell carcinoma). Tumor cells show nuclear atypia and growth in nests and cords. Lymphocytes are absent among tumor cells. Thymic carcinoma shows LAT1 positive immunoreactivity.
Note the absence of LAT1 positive signals in any types of thymoma (G-K). Positive signals are observed in the cytosol as well as in the membrane of tumor cells (L).

Table 2. Clinical summary of 14 thymic carcinoma patients.

Age	Sex	Masaoka-Koga	preoperative metastasis	Histology	Preoperative Treatment	Resection	Postoperative Treatment	Survival (Months)	Outcome	LAT1 signal location
52	F	III	none	SQ	CRT	Complete	CRT	40	dead	M/C
60	F	IVb	Brain, LNs	SQ	none	Incomplete	Chemo	6	dead	M/C
42	M	IVb	LNs	SQ	none	Complete	CRT	22	dead	M/C
71	F	IVb	LNs	SQ	none	Complete	RT	6	dead	M/C
63	M	III	none	UNDIFF	CRT	Complete	Chemo	26	dead	C
52	M	III	none	UNDIFF	CRT	Complete	Chemo	10	dead	C
74	F	IVb	LNs	UNDIFF	CRT	Incomplete	CRT	42	dead	C
73	F	IVb	LNs	SQ	none	Complete	CRT	135	censoring*	C
72	M	II	none	SQ	none	Complete	RT	27	censoring*	C
58	F	IVb	LNs	SQ	none	Complete	RT	78	dead	C
80	F	IVb	LNs	SQ	CRT	Complete	none	51	censoring*	C
36	M	IVb	LNs	UNDIFF	none	Complete	RT	18	dead	C
75	M	IVb	Liver, LNs	SARC	none	Complete	Chemo	59	dead	C
53	M	IVb	Liver, LNs	UNDIFF	none	Incomplete	Chemo	16	dead	C

UNDIFF, undifferentiated carcinoma; SQ, squamous cell carcinoma; SARC, sarcomatoid carcinoma; CRT, chemo-radiotherapy; Chemo, chemotherapy; RT, radiotherapy; M/C, membrane/cytosol; C, cytosol.
Censoring* means that the patient was lost for follow up.

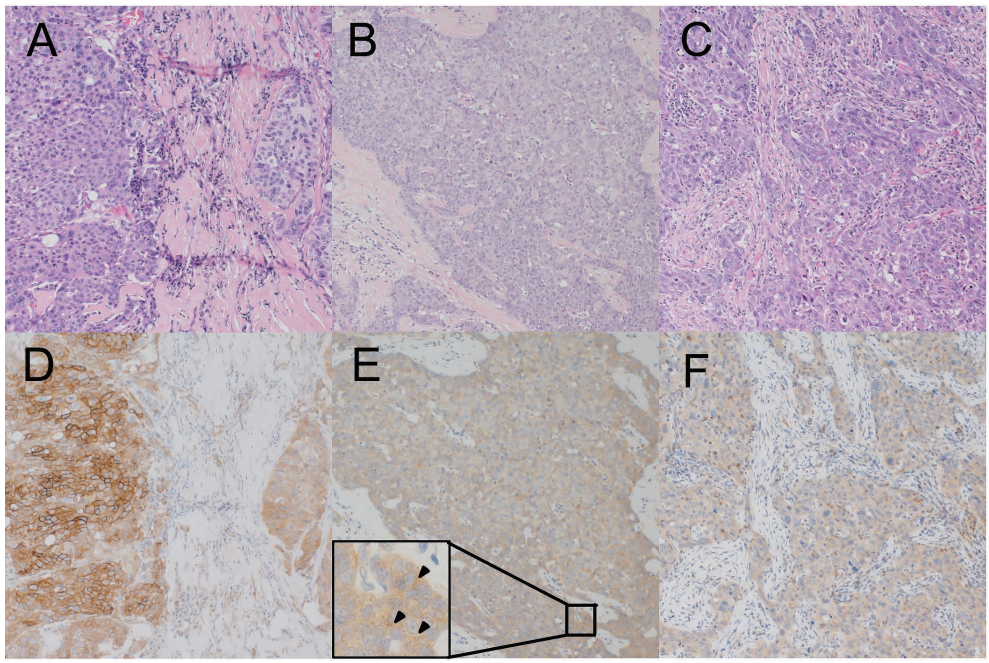

Fig. 3. LAT1 signal location of thymic carcinoma specimens.
Upper panels (A-C): HE staining of representative thymic carcinoma specimens, and Lower panels (D-F): LAT1 immunostaining of respective tissue specimens (A-F: Magnification × 100).
A) and D) Squamous cell carcinoma with the membrane/cytosol signal. Thymic carcinoma cells show the cytosol signal, and more than half of them also present the membrane signal.
B) and E) Squamous cell carcinoma with the cytosol signal. An inset in Panel E is a higher magnification (× 400) image showing the cytosol signal in fine granular appearance (arrowheads).
C) and F) Undifferentiated carcinoma with the cytosol signal.

regarding LAT1 and thymic carcinoma, with seemingly controversial results (Kaira et al. 2009b; Omatsu et al. 2012). These authors considered that LAT1 immunoreactivity was positive only when distinct membrane signal was present. Kaira and coworkers (2009b) reported that LAT1 was frequently expressed in thymic carcinoma but absent in thymoma, whereas Omatsu et al. (2012) reported that LAT1 was rarely positive both in thymoma and thymic carcinoma. The inconsistent results may be due to the difference in the antibodies used for LAT1 immunohistochemistry; namely, Kaira et al. (2009b) used a polyclonal antibody, while Omatsu et al. (2012) used a monoclonal antibody. In the present study, we used the polyclonal antibody raised against the oligopeptide that was also recognized by the antibody used in previous studies (Yanagida et al. 2001; Kaira et al. 2009b). Under our conditions, the LAT1 cytosol signal was detected in all of the 14 thymic carcinoma specimens examined but not in the 32 thymoma specimens. Importantly, the LAT1 membrane signal was detected in only four thymic carcinoma specimens. Incidentally, the thymic carcinoma with the LAT1 membrane signal was categorized as squamous cell carcinoma. Clinical characteristics including preoperative treatment and histology of the examined patients might be associated with the difference of the results.

All thymic carcinoma investigated in the present study showed positive LAT1 staining in the cytosol, although only the LAT1 membrane signal was considered to be important in previous studies (Kaira et al. 2009a, b, 2012; Omatsu et al. 2012; Li et al. 2013; Toyoda et al. 2014; Honjo et al. 2016). We, therefore, propose that the LAT1 cytosol signal is important especially for diagnosis of thymic carcinoma. In fact, none of the thymomas showed positive staining in either location. A recent study found that LAT1 existed in the lysosomal membrane and transported Leu into the lysosomes to activate mTOR complex 1 (mTORC1), leading to cell proliferation (Milkereit et al. 2015). Incidentally, the LAT1 cytosol signals showed fine granular appearance (Fig. 3E). We speculate that the cytosol signals indicate the presence of LAT1 in the lysosomal membrane or upregulation of LAT1 protein synthesis before recruitment to the cell membrane. Thymic carcinoma cells might proliferate using intracellular Leu as a signal transmitter of mTOR signaling pathway in the lysosome. When the tumor increased its malignant nature, they would require much more Leu and other amino acids, and then LAT1 would be recruited on the cell membrane to take amino acids from outside of the tumor cells. Further studies are required to clarify the meaning of LAT1 cytosol signals in terms of tumor biology of thymic carcinoma.

The diagnosis of thymic carcinoma can be difficult (Morinaga et al. 1987), especially when the examined specimen is a small fragment obtained by needle biopsy, which

Table 3. Characteristics of 14 thymic carcinoma patients.

LAT1 signal location		membrane/cytosol	cytosol	p value
Cases		4	10	
Sex	M	1	6	0.5594[a]
	F	3	4	
Age		56.3 ± 6.1	63.6 ± 4.4	0.2288[b]
Masaoka-Koga	I	0	0	
	II	0	1	
	III	1	2	N/A[c]
	IVa	0	0	
	IVb	3	7	
Tumor size	mm	77.5 ± 21.7	65.7 ± 7.1	0.7228[b]
Histology	SQ	4	4	0.0849[a]
	non-SQ	0	6	
FDG-PET	SUV max	18.4 ± 1.2	11.1 ± 3.5	N/A[c]
	Cases*	2	6	
Treatment				
Preoperative	Chemo-radiotherapy	1	4	1.0000
Surgery	Incomplete resection	1	3	1.0000
Postoperative	Chemotherapy	1	4	
	Radiotherapy	1	3	N/A[c]
	Chemo-radiotherapy	2	2	

The Age, tumor size, and SUV max are presented in the mean ± SEM.
Cases* means the number of patients who underwent FDG-PET.
SQ, squamous cell carcinoma of the thymus; non-SQ, other kinds of histology in thymic carcinomas (5 undifferentiated carcinomas, 1 sarcomatoid carcinoma).
[a]Fisher's exact test; [b]Mann-Whitney U test; [c]Statistical analysis was inappropriate because of the small sample number.

is inappropriate for morphological evaluation. Meanwhile, immunoreactivities for CD5 (Hishima et al. 1994) and c-Kit (Pan et al. 2004) have been used for differential diagnosis of thymic carcinoma, and a recent study reported that a combination of CD5, CEA, and GLUT-1 can distinguish thymic carcinoma from type B3 thymoma with high sensitivity and high specificity (Kojika et al. 2009). Our results suggest that LAT1 cytosol signal alone may be a marker with high reliability for differential diagnosis of thymic carcinoma.

In this study, prognosis of thymic carcinoma in the group of LAT1 membrane/cytosol signal was poor compared with the group of cytosol signal; however, we could not show any difference between the two groups in regard to clinical characteristics including staging and the value of SUV max, partly because of small number of patients. We do not have a clear answer to explain why LAT1 membrane signal was associated with poor prognosis of thymic carcinoma. In this context, Kaira and colleagues suggested that LAT1 expression in thymic carcinomas was related with Ki-67 labeling index, vascular endothelial growth factor expression, and microvessel density compared with thymomas (Kaira et al. 2009b). Further studies will be required to answer this question.

A recent *in vitro* study noted the possibility of LAT1 molecular targeted therapy for thymic carcinoma patients; namely, we demonstrated that a specific inhibitor of LAT1 had an anti-proliferative effect toward thymic carcinoma cells induced by apoptosis and G1 arrest (Hayashi et al. 2016). Based on the present findings of LAT1 expression in thymic carcinomas, anti-LAT1 therapy may be promising as treatment for thymic carcinoma.

This study has several limitations. First, it was a retrospective study at a single institution and the number of patients analyzed in this study was small. In addition, LAT1 protein and mRNA analyses were not performed because of a lack of specimens for such examinations. Since thymic carcinoma is a rare disease, a multi-institutional study is expected to confirm the usefulness of LAT1 immunoreactivity as a diagnostic marker and prognostic marker of thymic carcinoma.

In conclusion, the present study shows that LAT1 immunoreactivity in the cytosol is helpful to distinguish thymic carcinoma from thymoma. Furthermore, LAT1 immunoreactivity in the cell membrane is associated with poor prognosis. We propose that LAT1 is a possible diagnostic and prognostic marker for thymic carcinoma.

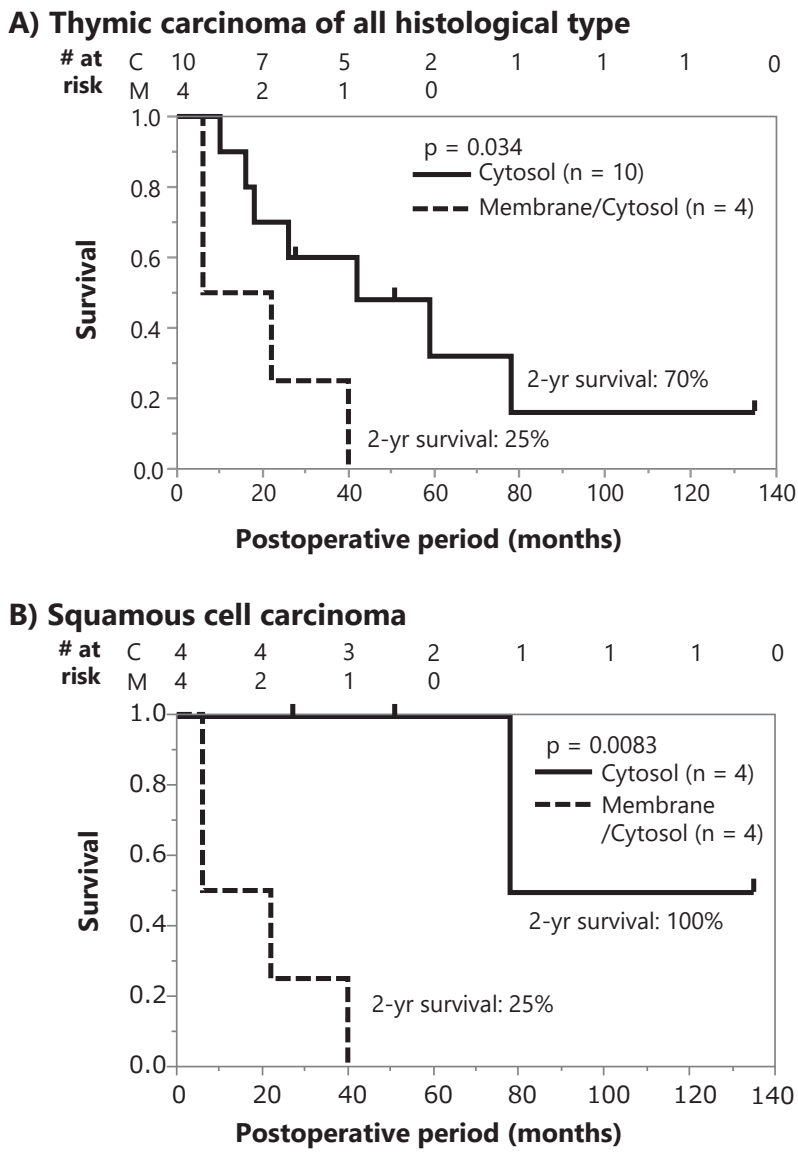

Fig. 4. Survival analysis for thymic carcinoma patients.
A) Survival of all 14 patients with thymic carcinoma. Patients in the LAT1 membrane/cytosol signal group showed significantly worse prognosis as compared with those in the LAT1 cytosol signal group (rog-rank test p = 0.034). Censoring is indicated by the small upward bars.
B) A sub-analysis of the eight patients with squamous cell carcinoma of the thymus. Patients in the LAT1 membrane/cytosol signal group showed poor prognosis as compared with those in the LAT1 cytosol signal group (rog-rank test p = 0.0083).

Acknowledgments

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Conflicts of Interest

The authors declare no conflict of interest.

References

Ahmad, U., Yao, X., Detterbeck, F., Huang, J., Antonicelli, A., Filosso, P.L., Ruffini, E., Travis, W., Jones, D.R., Zhan, Y., Lucchi, M. & Rimner, A. (2015) Thymic carcinoma outcomes and prognosis: results of an international analysis. *J. Thorac. Cardiovasc. Surg.*, **149**, 95-100, 101 e101-102.

Hayashi, K., Jutabha, P., Maeda, S., Supak, Y., Ouchi, M., Endou, H., Fujita, T., Chida, M. & Anzai, N. (2016) LAT1 acts as a crucial transporter of amino acids in human thymic carcinoma cells. *J. Pharmacol. Sci.*, **132**, 201-204.

Hishida, T., Nomura, S., Yano, M., Asamura, H., Yamashita, M., Ohde, Y., Kondo, K., Date, H., Okumura, M. & Nagai, K.; Japanese Association for Research on the Thymus (JART) (2016) Long-term outcome and prognostic factors of surgically treated thymic carcinoma: results of 306 cases from a Japanese Nationwide Database Study. *Eur. J. Cardiothorac. Surg.*, **49**, 835-841.

Hishima, T., Fukayama, M., Fujisawa, M., Hayashi, Y., Arai, K., Funata, N. & Koike, M. (1994) CD5 expression in thymic carcinoma. *Am. J. Pathol.*, **145**, 268-275.

Honjo, H., Kaira, K., Miyazaki, T., Yokobori, T., Kanai, Y., Nagamori, S., Oyama, T., Asao, T. & Kuwano, H. (2016) Clinicopathological significance of LAT1 and ASCT2 in patients with surgically resected esophageal squamous cell carcinoma. *J. Surg. Oncol.*, **113**, 381-389.

Kaira, K., Oriuchi, N., Imai, H., Shimizu, K., Yanagitani, N., Sunaga, N., Hisada, T., Ishizuka, T., Kanai, Y., Endou, H., Nakajima, T. & Mori, M. (2009a) L-type amino acid transporter 1 (LAT1) is frequently expressed in thymic carcinomas but is absent in thymomas. *J. Surg. Oncol.*, **99**, 433-438.

Kaira, K., Oriuchi, N., Imai, H., Shimizu, K., Yanagitani, N., Sunaga, N., Hisada, T., Ishizuka, T., Kanai, Y., Nakajima, T. & Mori, M. (2009b) Prognostic significance of L-type amino acid transporter 1 (LAT1) and 4F2 heavy chain (CD98) expression in stage I pulmonary adenocarcinoma. *Lung Cancer*, **66**, 120-126.

Kaira, K., Sunose, Y., Arakawa, K., Ogawa, T., Sunaga, N., Shimizu, K., Tominaga, H., Oriuchi, N., Itoh, H., Nagamori, S., Kanai, Y., Segawa, A., Furuya, M., Mori, M., Oyama, T., et al. (2012) Prognostic significance of L-type amino-acid transporter 1 expression in surgically resected pancreatic cancer. *Br. J. Cancer*, **107**, 632-638.

Kanai, Y., Segawa, H., Miyamoto, K., Uchino, H., Takeda, E. & Endou, H. (1998) Expression cloning and characterization of a transporter for large neutral amino acids activated by the heavy chain of 4F2 antigen (CD98). *J. Biol. Chem.*, **273**, 23629-23632.

Kojika, M., Ishii, G., Yoshida, J., Nishimura, M., Hishida, T., Ota, S.J., Murata, Y., Nagai, K. & Ochiai, A. (2009) Immunohistochemical differential diagnosis between thymic carcinoma and type B3 thymoma: diagnostic utility of hypoxic marker, GLUT-1, in thymic epithelial neoplasms. *Mod. Pathol.*, **22**, 1341-1350.

Li, J., Qiang, J., Chen, S.F., Wang, X., Fu, J. & Chen, Y. (2013) The impact of L-type amino acid transporter 1 (LAT1) in human hepatocellular carcinoma. *Tumour Biol.*, **34**, 2977-2981.

Milkereit, R., Persaud, A., Vanoaica, L., Guetg, A., Verrey, F. & Rotin, D. (2015) LAPTM4b recruits the LAT1-4F2hc Leu transporter to lysosomes and promotes mTORC1 activation. *Nat. Commun.*, **6**, 7250.

Morinaga, S., Sato, Y., Shimosato, Y., Sinkai, T. & Tsuchiya, R. (1987) Multiple thymic squamous cell carcinomas associated with mixed type thymoma. *Am. J. Surg. Pathol.*, **11**, 982-988.

Nicklin, P., Bergman, P., Zhang, B., Triantafellow, E., Wang, H., Nyfeler, B., Yang, H., Hild, M., Kung, C., Wilson, C., Myer, V.E., MacKeigan, J.P., Porter, J.A., Wang, Y.K., Cantley, L.C., et al. (2009) Bidirectional transport of amino acids regulates mTOR and autophagy. *Cell*, **136**, 521-534.

Omatsu, M., Kunimura, T., Mikogami, T., Hamatani, S., Shiokawa, A., Masunaga, A., Kitami, A., Suzuki, T., Kadokura, M. & Morohoshi, T. (2012) Immunohistochemical analysis of thymic carcinoma focusing on the possibility of molecular targeted and hormonal therapies. *Gen. Thorac. Cardiovasc. Surg.*, **60**, 803-810.

Pan, C.C., Chen, P.C. & Chiang, H. (2004) KIT (CD117) is frequently overexpressed in thymic carcinomas but is absent in thymomas. *J. Pathol.*, **202**, 375-381.

Toyoda, M., Kaira, K., Ohshima, Y., Ishioka, N.S., Shino, M., Sakakura, K., Takayasu, Y., Takahashi, K., Tominaga, H., Oriuchi, N., Nagamori, S., Kanai, Y., Oyama, T. & Chikamatsu, K. (2014) Prognostic significance of amino-acid transporter expression (LAT1, ASCT2, and xCT) in surgically resected tongue cancer. *Br. J. Cancer*, **110**, 2506-2513.

Yanagida, O., Kanai, Y., Chairoungdua, A., Kim, D.K., Segawa, H., Nii, T., Cha, S.H., Matsuo, H., Fukushima, J., Fukasawa, Y., Tani, Y., Taketani, Y., Uchino, H., Kim, J.Y., Inatomi, J., et al. (2001) Human L-type amino acid transporter 1 (LAT1): characterization of function and expression in tumor cell lines. *Biochim. Biophys. Acta*, **1514**, 291-302.