Prognostic significance of L-type amino acid transporter 1 (LAT1) and 4F2 heavy chain (CD98) expression in early stage squamous cell carcinoma of the lung

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The purpose of this study was to evaluate the prognostic value of L-type amino acid transporter 1 (LAT 1) and 4F2 heavy chain (CD98) in patients with stage I squamous cell carcinoma of the lung. A total of 84 consecutive patients with completely resected pathologic stage I squamous cell carcinoma of the lung were retrospectively reviewed. All patients underwent resection of the tumor and the immunohistochemical analysis was done to determine the expression of LAT 1, CD98, Ki-67 labeling index, vascular endothelial growth factor, and microvessel density. These pathological parameters were correlated with the prognosis of patients after complete resection of the tumor. A positive rate of LAT 1 expression (87%; 73/84) was significantly higher than that of CD98 expression (65%; 55/84) ($P = 0.0018$). Cooperative expression of LAT 1 and CD98 was recognized in 62% (52/84). LAT 1 expression was significantly correlated with CD98, Ki-67 labeling index, vascular endothelial growth factor, and microvessel density. The 5-year survival rates of the LAT 1-positive and LAT1-negative patients were 59% and 88%, respectively ($P = 0.2186$). Tumor cell proliferation and angiogenesis were not also (a) prognostic factor. However, the 5-year survival rate of patients with both LAT 1 and CD98-positivity (57%) was significantly worse than that of other patients (88%; $P = 0.0136$). Multivariate analysis confirmed that positive cooperative expression of LAT 1 and *CD98* was an independent factor for predicting a poor prognosis. A cooperative expression of LAT 1 and CD98 is a significant pathological factor for predicting the poor prognosis in patients with resectable stage I squamous cell carcinoma of the lung. (*Cancer Sci* 2009; 100: 249–254)

Lung cancer is the leading cause of cancer death in the United States and other countries, including Japan.[1] Despite the potential benefits of surgical resection, patients with the same pathologic stage of disease display marked variability in the possibility of recurrence and the survival. Moreover, the outcome after treatment has been very dismal in many cases. At present, no predictive factors have been demonstrated in patients with stage I non-small-cell lung cancer (NSCLC) who are at increased risk of disease recurrence.

Amino acid transporters are essential for the proliferation of normal and transformed cells.[2,3] Among amino acid transporters system L is a Na^+-independent large and neutral amino acid transport agency.[2,4] L-type amino acid transporter 1 (LAT1) is one of the L-type amino acid transporters, and transports large neutral amino acids such as leucine, isoleucine, valine, phenylalanine, tyrosine, tryptophan, methionine, and histidine.[4,5] LAT1 is widely expressed in primary human cancers and plays essential roles in the growth of tumor and the survival of patients.[6,7] LAT1 requires covalent association with the heavy chain of 4F2 cell surface antigen (CD98) for its functional expression in plasma membrane.[4] LAT1 and CD98 form a heterodimeric complex via a disulfide bond. The CD98 mRNA is ubiquitously expressed in embryonic and adult tissues; however, the LAT1 mRNA is expressed only in the restricted organs such as brain, spleen, placenta, and testis.[5] Recently, we described that positive expression of LAT1 could be a significant factor for predicting poor prognosis in NSCLC.[8] We also found that LAT1 expression was significantly higher in squamous cell carcinoma (SCC) than in adenocarcinoma (AC), and was associated with lymph node metastasis and disease stage in NSCLC.[8,9] However, it is unclear whether overexpression of LAT1 is associated with CD98 expression in SCC. In the present study, the prognostic significance of LAT1 expression was investigated in patients with stage I SCC of the lung. In addition, LAT1 expression was correlated with CD98, Ki-67 labeling index, and angiogenic markers.

Materials and Methods

Patients. We analyzed 102 consecutive patients with stage I squamous cell lung cancer who underwent resection lobectomy with mediastinal lymph node dissection at Gunma University Hospital between April 1998 and June 2004. Seven patients who received induction of chemotherapy or radiation therapy were excluded. Specimens of 11 patients were not available. A total of 84 patients (81 men, three women) were evaluated. The study protocol was approved by the institutional review board.

The age of the patients ranged from 52 to 84 years, and the mean age at the time of surgery was 70 years. The tumor specimens were histologically classified according to the criteria of the World Health Organization. Postsurgical pathologic stage was determined by the current tumor-node-metastasis classification.[10] As postoperative adjuvant therapies, platinum-based chemotherapy, radiation, and oral administration of tegafur (a fluorouracil derivative drug) were administered to six, two, and eight patients, respectively. Intraoperative therapy was not performed on any patients. The postoperative clinical course was assessed by analyzing outpatient medical records and by making telephone inquiries. The day of surgery was considered

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the starting day for counting postoperative survival. The follow-up duration ranged from 8 to 100 months (median, 49 months).

Immunohistochemical staining

LAT1 and CD98. LAT1 expression was determined by immunohistochemical staining with an affinity-purified rabbit polyclonal antihuman LAT1 antibody.[11] An oligopeptide corresponding to amino acid residues 497–507 of human LAT1 (CQKLMQVVPQET) was synthesized. The N-terminal cysteine residue was introduced for conjugation with keyhole limpet hemocyanine. Antipeptide antibody was produced as described elsewhere.[12] For immunohistochemical analysis, antiserum was affinity-purified as described previously.[12]

Immunohistochemical staining was performed on paraffin sections using a polymer peroxidase method (Envision+/HRP; Dako Cytomation, Denmark). Briefly, deparaffinized, rehydrated sections were treated with 0.3% hydrogen peroxide in methanol for 30 min to block endogenous peroxidase activity. To expose antigens, sections were autoclaved in 10 mmol/L sodium citrate buffer (pH 6.0) for 5 min, and cooled for 30 min. After rinsing in 0.05 M tris-buffered saline containing 0.1% tween-20, the sections were incubated with affinity purified anti-LAT1 antibody (1:3200) overnight at 4°C. The LAT1 antibody at concentration of 0.375 μh/mL was used to stain for LAT1. Thereafter, they were incubated with Envision (+) rabbit peroxidase (Dako, Carpinteria, CA, USA) for 30 min. The peroxidase reaction was performed using 0.02% 3,3′-diaminobenzidine tetrahydrochloride and 0.01% hydrogen peroxide in 0.05 mol/L tris-HCl buffer, pH 7.4. Finally, nuclear counterstaining was performed with Mayer's hematoxylin. For negative control, incubation step with the primary antibody was omitted. The specificity of immunoreactions using the anti-LAT1 antibody was established in previous studies.[13,14]

CD98 is an affinity purified goat polyclonal antibody raised against a peptide mapping at the carboxy terminus of CD98 (1:200 dilution; Santa Cruz Biotechnology) of human origin. Immunohistochemical staining for CD98 was performed by the avidin-biotin-peroxidase complex (ABC) method.

LAT1 and CD98 expression were considered positive only if distinct membrane staining was present. Staining intensity was scored as follows: 1, <10% of tumor area stained; 2, 11–25% stained; 3, 26–50% stained; and 4, ≥51% stained. The tumors in which stained tumor cells made up more than 10% of the tumor were graded as positive. According to this scoring protocol, two investigators from the author group, without prior knowledge of the clinical data, independently graded the staining intensity in all cases. To test the intraobserver variability, each section was reassessed by the same investigators after the first assessment had been completed. The time interval between the first and second assessments was at least 4 weeks. The interobserver variability was also determined by comparing the values of the first measurements of two investigators.

Ki-67. The detailed protocol for immunostaining has been published elsewhere.[15] Briefly, formalin-fixed and paraffin-embedded sections of resected specimens were dewaxed, rehydrated, trypsinized, and boiled in 0.01 mol/L citrate buffer for 20 min. For immunostaining, the murine monoclonal antibody MIB-1 (Dako, Denmark), specific for human nuclear antigen Ki-67, was used in a 1:40 dilution. The sections were lightly counterstained with hematoxylin. Sections of a normal tonsil were used as positive control for proliferating cells.

A highly cellular area of the immunostained sections was evaluated. All epithelial cells with nuclear staining of any intensity were defined as positive. Approximately 1000 nuclei were counted on each slide. Proliferative activity was assessed as the percentage of MIB-1-stained nuclei (Ki-67 labeling index) in the sample. Sections were evaluated by two investigators separately and in case of discrepancies both would evaluate the slide simultaneously and would reach an agreement in their final assessment.

Vascular endothelial growth factor (VEGF), CD31, and CD34. Immunohistochemical staining for VEGF, CD31, and CD34 was performed by the ABC method. In brief, sections were deparaffinized with xylene and rehydrated with ethanol. For VEGF, the sections were trypsinized, and incubated with blocking serum. For CD31, antigen retrieval was done by placing the specimen in 0.01 mol/L of citrate buffer at pH 6.0 and exposed to microwave heating of 20 min at 450 W. For CD34, the sections were treated by protease.

The antibodies used were: a monoclonal antibody against VEGF (1:100 dilution; Immuno-Biological Laboratories Co., Japan); a mouse monoclonal antibody against CD31 (1:50 dilution; Dako); and a mouse monoclonal antibody against CD34 (1:200 dilution; Nichirei, Tokyo, Japan).

The expression of VEGF was assessed according to the percentage of immunoreactive cells in a total of 1000 neoplastic cells (quantitative analysis).

MVD was assessed using the criteria of Weidner *et al*.[16] The areas of highest neovascularization were identified as regions of invasive carcinoma with the highest numbers of discrete microvessels stained for CD31 and CD34. Any brown stained endothelial cell or endothelial cell cluster that was clearly separate from adjacent microvessels, tumor cells, and other connective tissue elements was considered a single, countable microvessel. Microvessels in sclerotic areas within the tumor, where microvessels were sparse, and immediately adjacent areas of unaffected lung tissue were not considered in vessel counts. The number of CD31- and CD34-positive vessels was counted in four selected hotspots in a ×400 field (0.26 mm^2 field area). The mean value of the two independent readings of the same specimen was calculated, and MVD was defined as the mean count of microvessels per 0.26 mm^2 field area.[17]

Statistical analysis. Probability values of <0.05 indicated a statistically significant difference. Fisher's exact test was used to examine the association of two categorical variables. Correlation between LAT1 and CD98, Ki-67, VEGF, CD31, or CD34 was analyzed using the non-parametric Spearman's rank test. Survival time was determined as the time from tumor resection to death from any cause. For survivors, survival times were censored on the last date that patients were known to be alive. The Kaplan–Meier method was used to estimate survival as a function of time, and survival difference were analyzed by the log-rank test. Multivariate analyses were performed using the stepwise Cox proportional hazards model to identify independent prognostic factors. Statistical analysis was performed using StatView J-4.5 for Windows.

Results

Immunohistochemical findings. LAT1, CD98, Ki-67, VEGF, CD31, and CD34 immunohistochemical staining were evaluated for the surgically resected 84 primary lesions.

LAT1 and CD98. Immunohistochemical staining revealed that the expression of LAT1 and CD98 was localized predominantly on their plasma membrane (Fig. 1). All positive cells revealed strong membranous immunostaining. In the present study, no expression of LAT1 and CD98 protein were observed in any normal epithelium cells of the lung, including bronchial epithelial, and alveolar cells. A positive LAT1 and CD98 expression of primary tumors were recognized in 87% (73/84) and 65% (55/84), respectively ($P = 0.0018$). The average score of the LAT1 and CD98 expression was 2.9 ± 1.0 and 1.9 ± 0.8 on a scale of 1–4, respectively ($P < 0.001$). There was no expression of CD98 in eight (73%) of 11 patients without LAT1 expression, whereas, a positive CD98 expression was recognized in 52 (71%) of 73 patients with LAT1 expression. The relationship between LAT1 and CD98 was examined because it is well

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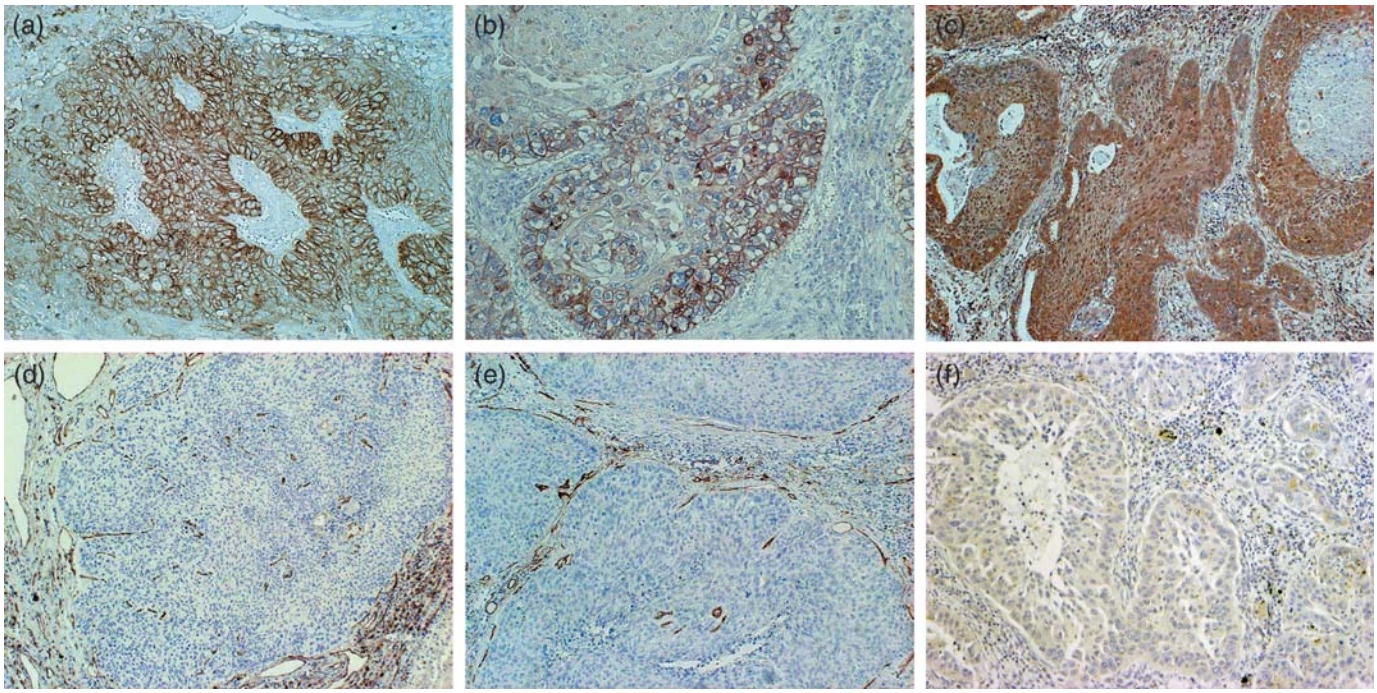

Fig. 1. Immunohistochemical analysis of the resected tumor. The score of LAT1 (a) and CD98 (b) immunostaining was grade 4 and grade 4, respectively, and their immunostaining pattern was membranous. Immunostaining for VEGF (c). More than 75% of tumor cells showed a positive reaction for anti-VEGF antibody. Immunostaining for CD31 (d) and CD34 (e). Many small vessels were positive for CD31 and CD34 in the stroma of tumor tissue. The figure of negative staining for LAT1 (f).

Table 1. Clinicopathological features according to LAT1 and CD98 expression

Parameter	No.	LAT1 positive		CD98 positive	
		No. (%)	*P*-value	No. (%)	*P*-values
Total	84	73 (87%)		55 (65%)	
Age					
≤65/>65	14/70	14 (100)/60 (86)	0.113	10 (71)/45 (64)	0.762
Gender					
Male/Female	81/3	70 (86)/3 (100)	1.000	52 (64)/3 (100)	0.548
Disease stage					
Ia/Ib	40/44	36 (90)/37 (84)	0.525	25 (63)/30 (68)	0.649
Lymphatic permeation					
±	34/50	29 (85)/44 (88)	0.520	21 (62)/34 (68)	0.642
Vascular invasion					
±	24/60	20 (83)/49 (61)	1.000	14 (58)/41 (68)	0.449
Pleural involvement					
±	19/65	16 (84)/57 (88)	0.705	10 (53)/45 (69)	0.272

LAT1, L-type amino acid transporter 1.

known that LAT1 requires CD98 for its functional expression. A cooperative expression of LAT1 with CD98 ('LAT1 with CD98') was recognized in 62% (52/84).

Expression of LAT1 and CD98 according to patient's characteristics is listed in Table 1. In this study, LAT1 and CD98 expression was not significantly associated with gender, disease stage, lymphatic permeation, vascular permeation, or pleural involvement.

Ki-67. The median rate of Ki-67 labeling index was 36% (range, 8–84%), and the value of 35 was chosen as the cut-off point. High expression was seen in 54 (64%) of 84 patients.

VEGF, CD31, and CD34. The staining pattern of VEGF was uniformly localized in the cytoplasm and/or membrane of neoplastic cells as shown in Fig. 1. The median rate of VEGF positivity was 35% (range, 9–75%), and the value of 35 was chosen as the cut-off point. High expression was seen in 61 (73%) of 84 patients. The median rate of MVD as assessed by CD31 was 32% (2–80%) and the value of 30 was chosen as the cut-off point. High expression was seen in 42 (50%) of 84 patients. The median rate of MVD as assessed by CD34 was 39% (5–70%) and the value of 40 was chosen as the cut-off point. High expression was seen in 50 (60%) of 84 patients. The analysis of the relationship between VEGF and the number of microvessels in the areas of the highest vascularization showed a significant association between mean microvessel count and VEGF expression in the primary tumors. As shown in Fig. 2, an increase in the VEGF expression was statistically associated with the number of microvessel count determined by the expression of CD31 ($\gamma = 0.5816$, $P < 0.0001$) and CD34 ($\gamma = 0.5075$, $P < 0.0001$).

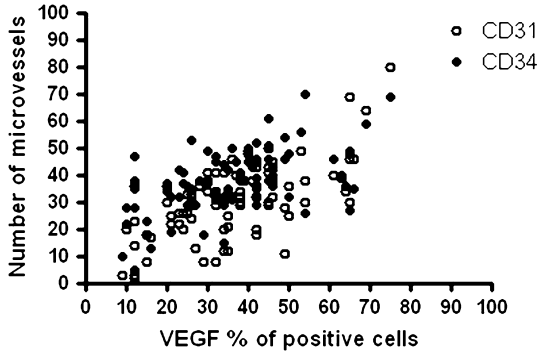

Fig. 2. Microvessel count was correlated with VEGF expression in all patients (CD31-MVD [$\gamma = 0.5816$, $P < 0.0001$] and CD34-MVD [$\gamma = 0.5075$, $P < 0.0001$]).

Table 2. Correlation between the expression of LAT1 and other immunohistochemical markers

Markers	Spearman γ	95% confidence interval	*P*-values
CD98	0.5117	0.3282–0.6580	<0.0001
Ki-67	0.6780	0.5378–0.7816	<0.0001
VEGF	0.4262	0.2269–0.5912	<0.0001
CD31	0.5101	0.3262–0.6568	<0.0001
CD34	0.4529	0.2582–0.6123	<0.0001

LAT1, L-type amino acid transporter 1; VEGF, vascular endothelial growth factor.

Table 3. Correlation between the coexpression of LAT1 with CD98 and other immunohistochemical markers

Markers	Spearman γ	95% confidence interval	*P*-values
Ki-67	0.3899	0.1853–0.5623	0.0002
VEGF	0.2449	0.0256–0.4416	0.0248
CD31	0.2521	0.0333–0.4478	0.0207
CD34	0.1849	–0.0371–0.3896	0.0921

LAT1, L-type amino acid transporter 1; VEGF, vascular endothelial growth factor.

Correlation between LAT1, CD98, cell proliferation, and angiogenesis. LAT1 expression was significantly correlated with CD98, Ki-67 labeling index, VEGF, CD31, and CD34 (Table 2); whereas cooperative expression of LAT1 and CD98 expression was significantly correlated with LAT1, Ki-67 labeling index, VEGF, and CD31 (Table 3).

Immunohistochemical markers and postoperative survival. In all patients, the 5-year survival rates of the LAT1-positive and LAT1-negative patients were 59% and 88%, respectively ($P = 0.2186$) (Fig. 3a). In the limited patients with pathological stage IA disease, the 5-year survival rates of LAT1-positive and LAT1-negative patients were 72% and 100%, respectively ($P = 0.3424$). For pathological stage IB disease, the 5-year survival rates of LAT1-positive and LAT1-negative patients were 50% and 83%, respectively ($P = 0.6901$); whereas the 5-year survival rate of patients with both LAT1 and CD98-positive was significantly worse than the other patients (57% and 88%, $P = 0.0136$) (Fig. 3b). For pathological stage IA disease, the 5-year survival rates of patients with both LAT1 and CD98-positive were not significantly different from the other patients (57% and 91%, respectively, $P = 0.1094$). For pathological stage

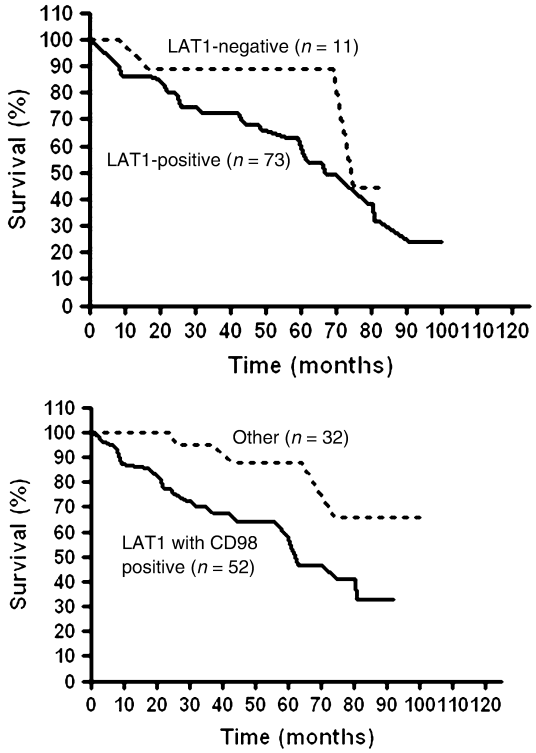

Fig. 3. Postoperative survival of patients with completely resected pathologic stage I squamous cell carcinoma of the lung. Comparison of postoperative survival rates was based on LAT1 and CD98 expression. (a) The 5-year survival rates of patients with LAT1-positivity and LAT1-negativity were 59% and 88%, respectively ($P = 0.2186$). (b) The 5-year survival rate of patients with both LAT1 and CD98-positive was significantly worse than that of the other patients (57% and 88%, respectively; $P = 0.0136$).

IB disease, the 5-year survival rates of patients with both LAT1 and CD98-positive were significantly worse than the other patients (51% and 83%, respectively, $P = 0.0320$). However, no significant difference in the prognosis was demonstrated between the patients with both LAT1 and CD98-positivity and the other patients according to the age, gender, and disease stage. Moreover, there was no significant difference in the prognosis of patients between Ki-67-positivity and Ki-67 negativity, between VEGF-positivity and VEGF-negativity, between CD31-positivity and CD31-negativity, and between CD34-positivity and CD34-negativity.

Multivariate analysis of prognostic factors. Mutivariate analysis confirmed that positive expression of both LAT1 and CD98 was an independent and significant factor for predicting a poor prognosis (Table 4).

Discussion

The present study evaluated the clinical significance of LAT1 and CD98 expression in stage I SCC. Our results demonstrated that a cooperative expression of LAT1 and CD98 was a significant independent factor for predicting a poor prognosis in patients with stage I SCC. LAT1 expression was closely associated with CD98, cell proliferation, and angiogenesis. However, the cooperative expression of LAT1 with CD98 was weakly associated with cell proliferation and angiogenesis. Moreover, the positive rate of LAT1 expression was significantly higher than that of CD98 expression. In patients with SCC, a positive LAT1 expression was recognized in 87%;

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Table 4. Multivariate analysis of the prognostic factors in stage I SCC

Prognostic factor	Hazard ratio	95% confidence interval	*P* values
Age (≤65 years/>65 years)	1.710	0.643–4.549	0.2829
Gender (male/female)	0.683	0.082–4.905	0.6619
Disease stage (IA/IB)	1.280	0.629–2.604	0.4954
LAT1 (positive/negative)	1.764	0.393–7.915	0.4586
LAT1 with CD98 (positive/negative)	2.780	1.111–6.955	0.0289

Note: Hazard ratios, 95% confidence intervals, and two-side *P*-values were obtained from the Cox proportional hazards models.
LAT1, L-type amino acid transporter 1.

however, the overexpression of LAT1 without expression of CD98 does not seem to be associated with poor prognosis.

Our recent study demonstrated the potential of LAT1 expression as a prognostic factor of NSCLC.[8] In the report, a prognostic significance was not analyzed according to the histological types. Moreover, the association between the expression of CD98 and LAT1, and the predictive value of the expression of LAT1 and CD98 for the prognosis were not investigated. Recently, Nakanishi *et al*. reported that a cooperative expression of LAT1 and CD98 was significantly correlated with both overall and disease-free survival rates in transitional cell carcinoma of the upper urinary tract.[18] In their study, a cooperative expression of LAT1 with CD98 was recognized in 71.7% of all samples. The incidence of the cooperative expression was also similar to our results (74%). Nawashiro *et al*. also described that the grading of LAT1 staining was significantly correlated with CD98 staining in human astrocytic tumors.[13] They found that LAT1 staining was a significant independent factor for predicting a poor prognosis and CD98 staining was also a significant prognostic factor for survival. Our study is the first report on the prognostic significance of the cooperative expression of LAT1 with CD98 in patients with SCC.

CD98 is a disulfide-linked 125-kDa heterodimeric membrane glycoprotein, which is found on the cell surface of most normal cells. However, CD98 has been shown to be involved in cellular proliferation, transformation, fusion, and adhesion, and also in the LAT system, in addition to regulating integrin activation, and therefore integrin signaling and anchorage independent growth. CD98 is strongly expressed on human embryonic and newborn fibroblasts but the expression gradually diminishes from 100% to 1% on adult fibroblasts. CD98 is reconstituted and expressed at high levels on the surface of many types of tumor cells. Furthermore, the overexpression of CD98 has been shown to result in cellular transformation.[19,20] The expression patterns suggest that the function of CD98 is coupled to cellular activation. These properties of CD98 would support the roles of this transporter in providing cells with essential amino acids for increased protein synthesis of the cellular proliferation. Previous studies have shown that overexpression of CD98 increases cell adhesion and migration.[18–20] Ohkame *et al*. reported the overexpression of CD98 in the metastatic liver tumor of rat models.[21] In the present study, no expression of CD98 protein was observed in any normal epithelial cells of the lung, including bronchial epithelium and alveolar cells. These results suggest that CD98 may have a role in tumor progression and metastasis, and CD98 is expressed above a normal threshold value. Therefore, the up-regulation of both LAT1 and CD98 may be required for poor prognosis rather than LAT1 alone.

Overexpression of LAT1 protein in NSCLC is shown to associate with Ki-67 labeling index, indicating an up-regulation of metabolic activity.[8,9] Our result also showed a close association between Ki-67 labeling index and LAT1 expression. However, a meta-analysis indicated that the expression of Ki-67 is not a prognostic factor for poor survival in patients with stage I NSCLC.[22] Overexpression of VEGF was reported to be associated with a poor prognosis in patients with all stages (I–III) of squamous cell lung cancer.[23] The expression of VEGF has been shown to correlate with a poor prognosis in stage I NSCLC.[24] However, there was no statistically significant association of VEGF as a prognostic factor of stage I SCC. In the present study, cell proliferation and angiogenesis seemed to be more closely correlated with LAT1 and CD98 expression. Ki-67 labeling index and VEGF expression were not significantly associated with poor prognosis in stage I SCC.

The rate of overexpression of LAT1 is not clearly understood in patients with SCC. Fuchs *et al*. hypothesize that LAT1 provides the essential amino acids that act as signal to enhance growth of cancer cells via mammalian target-of-rapamycin (mTOR)-stimulated translation. Likewise, mTOR regulates amino acid transporter gene expression and trafficking to the plasma membrane in response to the growth signal.[7] However, the mechanism of LAT1 expression in association with the mTOR pathway is unknown. The expression profiles of LAT1 according histological type have not been investigated in human neoplasms. We have found the difference of the LAT1 expression profiles between AC and SCC.[8,9] The results of the present study suggest that a cooperative expression of LAT1 and CD98 is essential for the progression of tumor in patients with SCC.

Several clinical investigations showed the increased uptake of radiolabeled amino acids in human neoplasms.[25–27] L-[3^{18} -F]-methyltyrosine (^{18}F-FMT) is transported via LAT1, and the uptake correlates with LAT1 expression in NSCLC.[9,25,28] Future studies are warranted in order to clarify the prognostic significance of ^{18}F-FMT uptake as compared with the cooperative expression of LAT1 and CD98 in patients with SCC.

The major role of prognostic markers in early stage NSCLC is to identify patients at high risk such as recurrence or metastasis after surgical treatment and possibly to guide adjuvant therapy. In cases with stage II–IIIA NSCLC, adjuvant chemotherapy after resection of the tumor with cisplatin-based regimens is now the standard of care based on the results of phase III trials.[29–31] However, adjuvant chemotherapy with uracil-tegafur for patients with stage IB adenocarcinoma of the lung remains controversial.[32,33] There was no evidence of adjuvant chemotherapy in patients with stage I SCC. Therefore, we should investigate possible markers to determine whether adjuvant therapy is needed in patients with stage I SCC of the lung.

The limitation of this study must be addressed. Our sample size was small and the number of LAT1-negative samples was only 11. In our previous study with various histology of NSCLC including 69 patients with SCC, LAT1 expression alone was statistically significant. The observation that LAT1 expression alone did not reach statistical significance with regard to prognostic survival rates may be grounded in disparities in population sample size in the present study. A large number of LAT1-negative samples may be evaluated to determine whether LAT1 expression alone is an independent prognostic factor in patients with stage I SCC of the lung.

In conclusion, a cooperative expression of LAT1 and CD98 is a significant factor for predicting poor prognosis, and it may be an important clinical marker of adjuvant therapy for stage I SCC of the lung. LAT1 and CD98 expression were correlated with tumor cell proliferation and angiogenesis. However, tumor cell proliferation and angiogenesis were not a prognostic factor in stage I SCC of the lung. A cooperative inhibiting of LAT1 and CD98 function may provide a new and effective therapeutic target for stage I SCC of the lung.

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