L-type amino acid transporter 1 and CD98 expression in primary and metastatic sites of human neoplasms

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(Received June 25, 2008/Revised August 3, 2008; August 4, 2008/Accepted August 5, 2008/Online publication November 19, 2008)

The significance of L-type amino acid transporter (LAT) 1 expression remains unclear in the metastatic process of human neoplasms, whereas experimental studies have demonstrated that LAT1 is associated with the metastatic process of cancer cells. We compared the immunohistochemical expression of LAT1 and CD98 between the primary site and a concordant pulmonary metastatic site in 93 cancer patients, all of whom had undergone thoracotomy. LAT1, CD98, Ki-67 labeling index, vascular endothelial growth factor (VEGF), CD31, and CD34 were analyzed by immunohistochemical staining in the resected tumors of 93 cancer patients: 45 colon cancers; nine breast cancers; eight head and neck cancers; 11 genital cancers; 14 soft-tissue sarcomas; and six other cancers. The expression of these markers was significantly higher in the metastatic sites than in the primary sites. In total, the positive rates of LAT1, CD98, Ki-67, VEGF, CD31, and CD34 were 40, 24, 56, 41, 45, and 39%, respectively, in the primary sites and 65, 45, 84, 67, 73, and 61%, respectively, in the metastatic sites. LAT1 expression was closely correlated with CD98 expression, angiogenesis, and cell proliferation. The association between LAT1 and CD98 expression was strongest in the primary and metastatic sites. The present study suggests that overexpression of LAT1 and CD98 has an important role to play in the metastatic process of variable human neoplasms. Moreover, LAT1 expression was significantly correlated with cell proliferation and angiogenesis. (*Cancer Sci* 2008; 99: 2380–2386)

Amino acid transporters are essential for the growth and proliferation of normal and transformed cells.[1,2] Amino acid transporter system L is a Na-independent large and neutral amino acid transport agency.[1,3] L-type amino acid transporter (LAT) 1 is an L-type amino acid transporter that transports large neutral amino acids, such as leucine, isoleucine, valine, phenylalanine, tyrosine, tryptophan, methionine, and histidine.[2–4] LAT1 requires a covalent association with the heavy chain of the 4F2 cell surface antigen (CD98) for its functional expression in plasma membrane.[3] Previous studies have shown LAT1 to be expressed highly in proliferating tissues, many tumor cell lines (T24 bladder carcinoma cells, RERF-LC-MA lung small-cell carcinoma cells, and HeLa uterine cervical carcinoma cells) and primary human tumors.[4] Recent studies have demonstrated the overexpression of LAT1 in lung cancer and esophageal carcinoma.[5–7] Positive expression of LAT1 is reported to be a significant factor in predicting poor prognosis in non-small cell lung cancer (NSCLC), and tends to increase from low-grade to high-grade neuroendocrine tumors of the lung.[7,8] Nakanishi *et al*. reported that the cooperative expression of LAT1 and CD98 is significantly correlated with both overall and disease-free survival rates in transitional-cell carcinoma of the upper urinary tract.[9] Nawashiro *et al*. reported that overall immunoreactivity for LAT1 correlates well with the prognosis of patients with astrocytic tumors, and high CD98 immunoreactivity also correlates with high LAT1 expression.[10] An experimental study demonstrated that LAT1 expression is closely related to tumor cell growth of liver metastases in a rat model,[11] and a previous study has shown that LAT1 expression is significantly associated with lymph node metastasis, disease stage, and cell proliferation in NSCLC.[7] However, it is unknown whether LAT1 and CD98 expression are associated with the development of metastases in human neoplasms. Although several authors have investigated the correlation between LAT1 expression and the development of metastases in a rat model,[11,12] no studies have compared LAT1 and CD98 expression between primary and metastatic sites in human neoplasms.

To elucidate the roles of both LAT1 and CD98 expression in the development of human cancers, we undertook an immunohistochemical examination of LAT1 and CD98 expression in both primary and pulmonary metastatic sites. LAT1 expression was correlated with the proliferative activity of the tumor as assessed by Ki-67 labeling index and the angiogenesis of the tumor as assessed by vascular endothelial growth factor (VEGF) and microvessel density (MVD).

Materials and Methods

Patients. We analyzed 106 consecutive patients who underwent complete resection of primary malignant tumors and thoracotomy for pulmonary metastases due to the primary tumors at Gunma University Hospital between June 1992 and December 2007. Seven patients who received induction chemotherapy or radiation therapy were excluded. Specimens of six patients were not available. A total of 93 patients were evaluated. The study protocol was approved by the institutional review board.

Immunohistochemical staining.

LAT1 and CD98. LAT1 expression was determined by immunohistochemical staining with an affinity-purified rabbit polyclonal antihuman LAT1 antibody.[4] 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.[13] For immunohistochemical analysis, antiserum was affinity purified as described previously.[13]

Immunohistochemical staining was carried out on paraffin sections using a polymer peroxidase method (Envision+/horseradish peroxidase; Dako Cytomation, Glostrup, Denmark). Briefly, deparaffinized, rehydrated sections were treated with 0.3%

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doi: 10.1111/j.1349-7006.2008.00969.x

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 mol/L Tris-buffered saline containing 0.1% Tween-20, the sections were incubated with affinity-purified anti-LAT1 antibody (1.2 mg/mL; 1:3200) overnight at 4°C. The LAT1 antibody was used at a concentration of 0.375 μg/mL to stain for LAT1. Thereafter, they were incubated with Envision$^+$ rabbit peroxidase for 30 min. The peroxidase reaction was carried out 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 carried out using Mayer's hematoxylin. For the negative control, the incubation step with the primary antibody was omitted. The specificity of immunoreactions using the anti-LAT1 antibody was established in previous studies.[10,14]

CD98 is an affinity-purified goat polyclonal antibody raised against a peptide mapping to the carboxyl terminus of CD98 of human origin. Immunohistochemical staining for CD98 was carried out using the avidin–biotin method. Briefly, formalin-fixed and paraffin-embedded sections of resected specimens were dewaxed and rehydrated. The sections were incubated with affinity-purified goat polyclonal antibody against CD98 (1:200 dilution; Santa Cruz Biotechnology, Santa Cruz, CA, USA) overnight at 4°C.

L-type amino acid transporter 1 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% tumor area stained; 3, 26–50% tumor area stained; and 4, ≥51% tumor area 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 of the authors, without prior knowledge of the clinical data, independently graded the staining intensity in all cases. To test the intra-observer 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 the two investigators.

Ki-67. The detailed protocol for Ki-67 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 Cytomation), specific for human nuclear antigen Ki-67, was used at a dilution of 1:40. The sections were counterstained lightly with hematoxylin. Sections of a normal tonsil were used as a 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 cases of discrepancies, both would evaluate the slide simultaneously and agree in their final assessment. Neither investigator had knowledge of patient outcome.

Vascular endothelial growth factor, CD31, and CD34. Immunohistochemical staining for VEGF, CD31, and CD34 was carried out using the avidin–biotin 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 citrate buffer at pH 6.0 and exposing it to microwave heating of 20 min at 450 W. For CD34, the sections were treated with protease.

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

Expression of VEGF and vessel count were evaluated by two investigators without knowledge of patient outcome. The expression of VEGF was assessed according to the percentage of immunoreactive cells in a total of 1000 neoplastic cells (quantitative analysis).

Microvessel density 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 hot spots in a ×400 field (0.26-mm^2 field area). The mean value of 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. Mann–Whitney's *U* test and paired two-group *t*-test were used to examine the association of two categorical variables. Statistical analysis of LAT1 and CD98 scores was carried out using Mann–Whitney's *U* test. In Ki-67, VEGF, and microvessel counts for CD31 and CD34, paired two-group *t*-tests were carried out. Correlations between LAT1, CD98 expression, Ki-67 labeling index, VEGF, CD31, and CD34 were analyzed using the non-parametric Spearman's rank test. A *P*-value less than 0.05 was considered to be indicative of statistical significance. Statistical analysis was carried out using StatView J-4.5 for Windows (SAS Inst., Cary, NC, US).

Results

Patient characteristics. The patients' characteristics are listed in Table 1. The patients comprised 54 men and 39 women, ranging in age from 10 to 81 years (median, 62.0 years). The organ types of the primary site were as follows: 45 colon cancers, nine breast cancers, eight head and neck cancers, 11 genital cancers, 14 soft-tissue sarcomas, and six other cancers. The histological types of primary tumors were as follows: 50 adenocarcinomas, 10 squamous cell carcinomas, 14 sarcomas, and 19 others.

Immunohistochemical analysis. LAT1, CD98, Ki-67, VEGF, CD31, and CD34 immunohistochemical staining were evaluated for the surgically resected 93 primary sites and 93 pulmonary metastatic sites.

Table 1. Patient characteristics

Characteristic	No. patients
Patients enrolled	93
Median age (years) (Range)	62 (10–81)
Men/Women	54/39
Primary site	
Colon cancer	43
Breast cancer	9
Head and neck cancer	8
Genital cancer	11
Soft-tissue sarcoma	14
Other	6
Gastric cancer	1
Hepatocellular carcinoma	1
Thyroid cancer	2
Thymic cancer	2

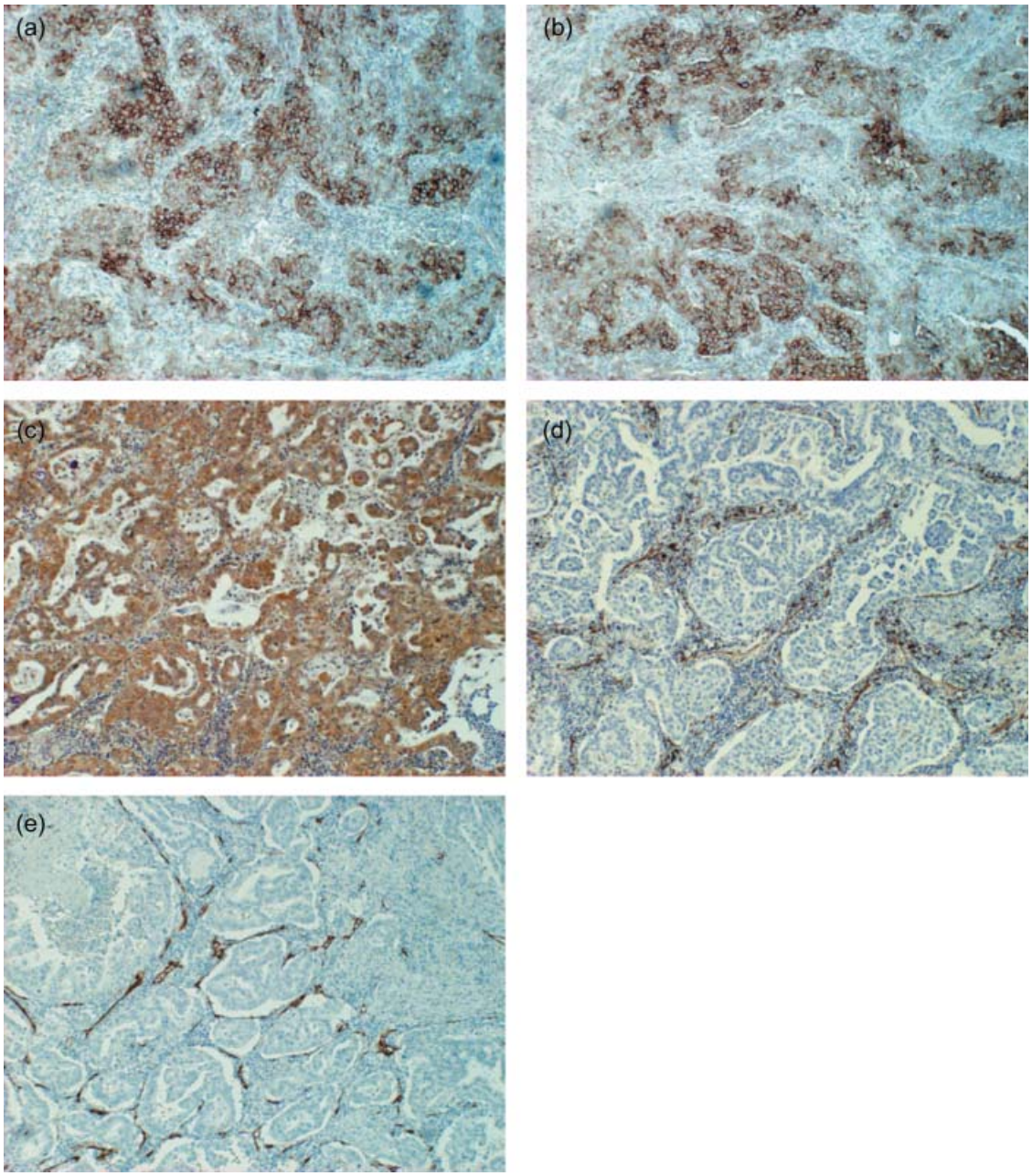

Fig. 1. Immunohistochemical analysis of a resected metastatic tumor in a 49-year-old woman with otolaryngeal adenosquamous cell carcinoma: comparison of LAT1, CD98, VEGF, CD31, and CD34. The immunostaining scores of (a) LAT1 and (b) CD98 were both grade 4, and their immunostaining patterns were membranous. (c) Immunostaining for vascular endothelial growth factor (VEGF). More than 75% of tumor cells showed a positive reaction for anti-VEGF antibody. Immunostaining for (d) CD31 and (e) CD34. Many small vessels positive for CD31 and CD34 were seen in the stroma of the tumor tissue.

LAT1 and CD98. LAT1 and CD98 immunostaining was detected in carcinoma cells from tumor tissues and was localized predominantly to the plasma membranes (Fig. 1). Positive LAT1 expression in the primary and metastatic sites was recognized in 40 (37/93) and 65% (60/93), respectively ($P = 0.0012$). Positive CD98 expression in the primary and metastatic sites was recognized in 24 (24/93) and 45% (42/93), respectively ($P = 0.0032$) (Table 2). LAT1 and CD98 expression was significantly higher in the metastatic sites than in the primary sites. Next, we analyzed the expression of LAT1 and CD98 in patients with the same histological type of primary site (Table 3). Both LAT1 and CD98 expression at the metastatic sites were significantly higher than those of the primary sites in 50 patients with adenocarcinoma. The scoring of both LAT1 and CD98 expression of the metastatic sites was significantly higher than those of the primary sites in 10 patients with squamous cell carcinoma. Although the positive rates of LAT1 and CD98 expression were higher in the metastatic sites than in the primary sites in patients with squamous cell carcinoma and sarcoma, no statistical significance was observed. As shown in Tables 4 and 5, LAT1 expression was significantly correlated with CD98 expression in the primary and metastatic sites.

Ki-67. The median rate of the Ki-67 labeling index was 26% (range, 3–60%), and the value of 25 was chosen as the cut-off point. High expression in the primary and metastatic sites was seen in 56 (52/93) and 84% (78/93), respectively ($P < 0.001$)

doi: 10.1111/j.1349-7006.2008.00969.x

Table 2. Positive rate of immunohistochemical markers in primary and metastatic sites

Marker	Total (%) (n = 93)	Colon cancer (%) (n = 45)	Breast cancer (%) (n = 9)	Head and neck cancer (%) (n = 8)	Genital cancer (%) (n = 11)	Soft-tissue sarcoma (%) (n = 14)	Other cancer (%) (n = 6)
LAT1							
Primary site	40 (37/93)	35 (16/45)	56 (5/9)	75 (6/8)	45 (5/11)	21 (3/14)	33 (2/6)
Metastatic site	65 (60/93)	60 (27/45)	78 (7/9)	88 (7/8)	91 (10/11)	43 (6/14)	50 (3/6)
CD98							
Primary site	24 (22/93)	22 (10/45)	11 (1/9)	63 (5/8)	36 (4/11)	0 (0/14)	17 (1/6)
Metastatic site	45 (42/93)	46 (21/45)	11 (1/9)	88 (7/8)	27 (3/11)	21 (3/14)	50 (3/6)
Ki-67							
Primary site	56 (52/93)	51 (23/45)	56 (5/9)	63 (5/8)	36 (4/11)	29 (4/14)	50 (3/6)
Metastatic site	84 (78/93)	84 (38/45)	89 (8/9)	88 (7/8)	82 (9/11)	86 (12/14)	67 (4/6)
VEGF							
Primary site	41 (35/93)	29 (13/45)	67 (6/9)	50 (4/8)	36 (4/11)	36 (5/14)	50 (3/6)
Metastatic site	67 (62/93)	51 (23/45)	78 (7/9)	75 (6/8)	91 (10/11)	86 (12/14)	67 (4/6)
CD31							
Primary site	45 (42/93)	31 (14/45)	56 (5/9)	75 (6/8)	64 (7/11)	50 (7/14)	50 (3/6)
Metastatic site	73 (68/93)	51 (23/45)	89 (8/9)	100 (8/8)	91 (10/11)	93 (13/14)	100 (6/6)
CD34							
Primary site	39 (36/93)	20 (9/45)	67 (6/9)	63 (5/8)	36 (4/11)	57 (8/14)	50 (3/6)
Metastatic site	61 (57/93)	38 (17/45)	89 (8/9)	75 (6/8)	73 (8/11)	86 (12/14)	100 (6/6)

LAT, L-type amino acid transporter; NS, not significant; VEGF, vascular endothelial growth factor.

Table 3. Expression of L-type amino acid transporter (LAT) 1 and CD98 according to the histological type of various primary sites

Cancer	LAT1			CD98		
	Primary site	Metastatic site	P-value	Primary site	Metastatic site	P-value
Adenocarcinoma (n = 50)						
Mean scoring	1.44	2.14	0.0007	1.22	1.70	0.0025
Positive rate	34% (17/50)	64% (32/50)	0.0048	22% (10/50)	53% (24/50)	0.0043
Squamous cell carcinoma (n = 10)						
Mean scoring	2.11	3.22	0.0377	1.50	2.50	0.0382
Positive rate	60% (6/10)	100% (10/10)	ns	40% (4/10)	70% (7/10)	ns
Sarcoma (n = 14)						
Mean scoring	1.21	1.78	ns	1.00	1.50	ns
Positive rate	21% (3/14)	43% (6/14)	ns	0% (0/14)	21% (3/14)	ns

(Table 2). The percentage of Ki-67 labeling index for the primary and metastatic sites achieved a significant difference in all cancers except breast cancer.

Vascular endothelial growth factor, CD31, and CD34. The staining pattern of VEGF was localized uniformly to the cytoplasm and membrane of neoplastic cells (Fig. 1). The median rate of VEGF positivity was 41% (range, 2–90%), and the value of 40 was chosen as the cut-off point. High expression in the primary and metastatic sites was seen in 41 (35/93) and 67% (62/93), respectively ($P < 0.001$) (Table 2). The median rate of MVD as assessed by CD31 was 31 (1–99) and the value of 30 was chosen as the cut-off point. High expression in the primary and metastatic sites was seen in 45 (42/93) and 73% (68/93), respectively ($P < 0.001$) (Table 2). The median rate of MVD as assessed by CD34 was 40 (2–102) and the value of 40 was chosen as the cut-off point. High expression in the primary and

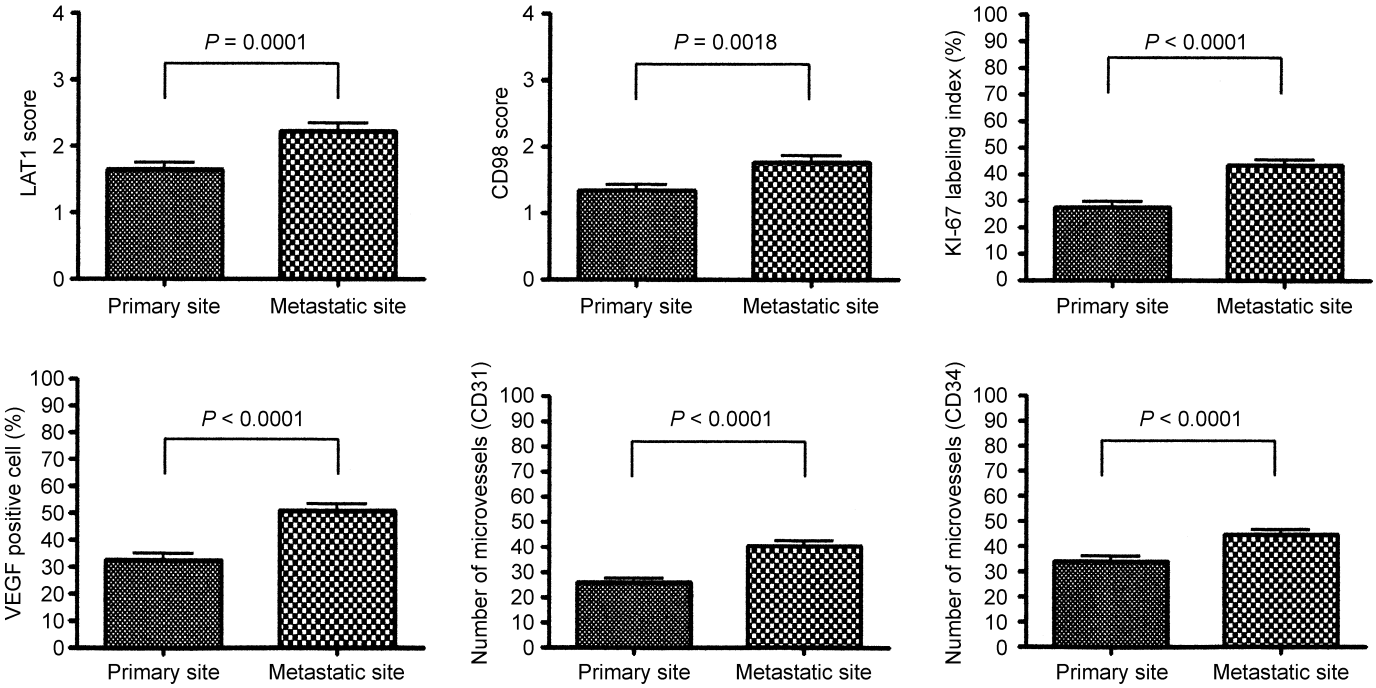

Fig. 2. Comparison of the immunohistochemical analysis between primary and metastatic sites in 82 cancer patients. The expression incidence of all markers, including LAT1, CD98, Ki-67, VEGF, CD31, and CD34, was significantly higher in metastatic sites than in primary sites.

Table 4. Correlation between the expression of L-type amino acid transporter (LAT) 1 and immunohistochemical markers in the primary sites

Marker	Spearman γ	95% confidence interval	*P*-value
CD98	0.6628	0.5263–0.7660	<0.0001
Ki-67	0.6311	0.4858–0.7425	<0.0001
VEGF	0.3547	0.1567–0.5151	0.0008
CD31	0.3547	0.1567–0.5252	0.0005
CD34	0.3690	0.1728–0.5371	0.0003

VEGF, vascular endothelial growth factor.

Table 5. Correlation between the expression of L-type amino acid transporter (LAT) 1 and immunohistochemical markers in the metastatic sites

Marker	Spearman γ	95% confidence interval	*P*-value
CD98	0.6009	0.4477–0.7199	<0.0001
Ki-67	0.5976	0.4436–0.7174	<0.0001
VEGF	0.4361	0.2492–0.5916	<0.0001
CD31	0.2900	0.0856–0.4710	0.0048
CD34	0.3572	0.1596–0.5273	0.0004

VEGF, vascular endothelial growth factor.

metastatic sites was seen in 39 (39/93) and 61% (57/93), respectively ($P = 0.0124$) (Table 2). VEGF expression and the number of CD31- and CD34-positive vessels was significantly higher in the metastatic sites than in the primary sites (Table 3). The percentage of VEGF-positive cells in the primary and metastatic sites achieved a significant difference in all cancers except the group of other cancers. The number of CD31-positive vessels in the primary and metastatic sites achieved a significant difference in all cancers. The number of CD34-positive vessels in the primary and metastatic sites achieved a significant difference in colon, genital, and other cancers (Table 3). As shown in Tables 4 and 5, LAT1 expression correlated significantly with VEGF expression and MVD in the primary and metastatic sites. Analysis of the relationship between VEGF and the number of microvessels in the areas of highest vascularization showed a significant association between mean microvessel count and VEGF expression in the primary and metastatic sites. An increase in VEGF expression was statistically associated with the microvessel count (CD31-MVD [$\gamma = 0.7242$, $P < 0.0001$] and CD34-MVD [$\gamma = 0.7053$, $P < 0.0001$]).

In total, the expression of all markers was significantly higher in the metastatic sites than in the primary sites (Fig. 2). Although LAT1 expression was significantly associated with CD98, Ki-67, VEGF, CD31, and CD34, the association between LAT1 and CD98 expression was strongest among Ki-67, VEGF, CD31, and CD34 in the primary and metastatic sites (Tables 3, 4).

Discussion

The present study is the first to compare the expression of LAT1 and CD98 in primary and metastatic sites in human neoplasms. In this study, the expression of LAT1 and CD98 was significantly higher in the metastatic sites than in the primary sites. LAT1 expression correlated closely with the expression of CD98, and was also significantly associated with angiogenesis and cell proliferation. Our results suggest that cooperative overexpression of LAT1 and CD98 in addition to cell proliferation and angiogenesis is essential for the progression and metastasis of human neoplasms. In particular, overexpression of LAT1 and CD98 seems to be necessary for the development of metastases in patients with colon cancer.

It is widely known that amino acid transport systems play an important role in the regulation of cellular proliferation, whereas the details of their function in promoting tumor cell proliferation have not been clarified.[2] LAT1 is expressed widely in primary human cancers and several cancer cell lines, where it has been shown to play essential roles in growth and survival.[18]

doi: 10.1111/j.1349-7006.2008.00969.x

Although it is currently unclear why LAT1 is necessary for transformed cells, Fuchs and Bode hypothesize that LAT1 provides the essential amino acids that act as a signal to enhance the growth of cancer cells via mammalian target of rapamycin-stimulated translation.[18] Likewise, mammalian target of rapamycin regulates amino acid transporter gene expression and trafficking to the plasma membrane in response to the growth signal.[18] Moreover, overexpression of LAT1 was reported to be associated with metastasis *in vivo*.[11] When colon cancer RCN-9 cells were injected into the spleens of rats, the size of the resultant metastatic liver tumors was directly correlated with LAT1 expression.[11,12] Thus, inhibition of LAT1 function could be a potential therapeutic strategy for many types of cancer.[18,19]

In the present study, we found that overexpression of LAT1 and CD98 has an important role to play in the progression and metastasis of various human neoplasms. Angiogenesis and cell proliferation have been reported to be associated with the metastatic process in human cancer, and our study indicates similar results. The correlation between LAT1 and VEGF expression in the metastatic sites seems to be higher than in the primary sites. However, the correlation between LAT1 and CD98 and between LAT1 and Ki-67 was not higher in the metastatic sites than in the primary sites. The correlation between LAT1 and CD98 expression was unremarkable in the primary and metastatic sites; however, the level of LAT1 and CD98 expression was increased markedly in the metastatic sites compared with the primary sites. Although LAT1 is essential in order for CD98 to be functional, the cooperative overexpression of LAT1 and CD98 may be necessary for the development of metastases in human neoplasms. Ohkame *et al*. reported that tumor size in the LAT1-positive and CD98-positive group was significantly larger than in the LAT1-negative and CD98-negative group in liver metastatic tumor lesions of rat models.[11] Their results suggest that LAT1 can enhance its ability to promote tumor growth in cooperation with CD98. Fenczik *et al*. also reported that CD98 is an important regulator of integrin activation, which is involved in cell growth.[20] It is possible that CD98 has some influence on tumor cell growth. In metastatic sites, CD98 may be necessary in order for LAT1 to facilitate tumor growth.

Several clinical investigations have revealed increased uptake of radiolabeled amino acids in human neoplasms.[21–23] We have used L-[3-^{18}F]-α-methyltyrosine (^{18}F-FMT) as a tracer for amino acid transport using positron emission tomography imaging[24] and investigated the clinical utility of ^{18}F-FMT in several tumors, including brain tumor, lung cancer, head and neck cancer, soft-tissue sarcoma, and lymphoma.[21,22,25,26] ^{18}F-FMT is transported via an L-type amino acid transporter that is specific to cancer cells.[21,22] Recently, we reported a significant correlation between ^{18}F-FMT uptake and LAT1 expression, and the role of LAT1 expression as a significant factor in predicting poor prognosis in NSCLC.[7,22] Moreover, recent studies have demonstrated the clinical significance of LAT1 and CD98 expression in the development of genital cancer and brain tumor.[9,10] LAT1 expression is also reported to be closely associated with Ki-67 labeling index as a proliferative marker of tumor cells in lung cancer.[22] There have been no reports on the association between LAT1 expression and Ki-67 labeling index in human neoplasms except for lung cancer. To our knowledge, the correlation between LAT1 expression and angiogenesis has not been described previously. Although the present study demonstrated a weak correlation between LAT1 expression and angiogenesis, cooperative expression of LAT1 and VEGF may also be essential for the metastatic process in human neoplasms.

One of the limitations of our study is small sample sizes with heterogenous populations. Therefore, the prognostic value of LAT1 and CD98 expression could not be elucidated. A further limitation is that our study did not evaluate the VEGF family of proteins and receptors. This family comprises six secreted glycoproteins, of which VEGF-A, VEGF-C, and VEGF-D are of great significance.[27,28] These VEGF ligands mediate their angiogenic effects via the receptor tyrosine kinases VEGFR-1 (flt-1), VEGFR-2 (KDR or Flk-1), and VEGFR-3 (Flt-4).[29] The relationship between the VEGF family and LAT1 expression should be elucidated.

In conclusion, the present study suggests that overexpression of LAT1 and CD98 has an important role to play in the metastatic process of various human neoplasms. Moreover, LAT1 expression was significantly correlated with cell proliferation and angiogenesis. Inhibition of LAT1 and CD98 function may provide new and effective therapeutic targets for many types of cancer in the future.

Acknowledgments

We thank T. Hikino for technical assistance in the immunohistochemical staining of LAT1, CD98, Ki-67, VEGF, CD31, and CD34.

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doi: 10.1111/j.1349-7006.2008.00969.x