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Role of Amino Acid Transporter Expression as a Prognostic Marker in Patients With Surgically Resected Colorectal Cancer

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Abstract. *Background/Aim: L-type amino acid transporter 1 (LAT1) is highly expressed in various human cancers. However, the clinicopathological significance of LAT1 and 4F2 cell surface antigen (4F2hc) in patients with colorectal cancer (CRC) is unknown. The aim of this study was to clarify the prognostic significance of LAT1 expression in CRC patients who underwent surgical resection. Materials and Methods: Samples from one hundred and forty-seven patients were examined by immunohistochemistry. The expression of LAT1 and 4F2hc, and the Ki-67 labeling index were assessed using resected tumor specimens. Results: The positive expression of LAT1 and 4F2c was 80% (118/147) and 58% (86/147) (p<0.01), respectively. The expression of LAT1 was identified as an independent significant marker linked to worse prognosis in patients with CRC, and was correlated with tumor cell proliferation, tumor aggressiveness, and metastasis. Moreover, LAT1 was closely associated with the expression of 4F2hc and phosphorylation of the mTOR pathway. Conclusion: LAT1 is a significant molecular marker used to predict prognosis after surgical resection of CRC patients.*

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Colorectal cancer (CRC) is one of the major neoplasms that cause cancer-related deaths worldwide. When the initial diagnosis of CRC is made in the advanced stage of the disease, systemic chemotherapy is a suitable treatment in addition to palliative surgery when necessary. Despite recent translational research efforts, there is still no established biomarker to predict prognosis after any treatment.

We investigated the clinicopathological significance of L-type amino acid transporter 1 (LAT1) expression within tumor specimens from patients with human neoplasms and confirmed that LAT1 is highly expressed in many types of cancers (1-9). We found that the increased expression of LAT1 is a clear negative marker predicting worse outcomes in some human cancers (2, 4-7). Although LAT1 works as a membrane transporter of neutral amino acids, 4F2hc (CD98) is required for its function (10). The L-type amino acid transporter consists of several subtypes, such as LAT1, LAT2, LAT3, and LAT4 (1, 3, 10). Recent studies have indicated that LAT1 can be used as a specific marker for malignant lesions, LAT2 is expressed in normal tissues, LAT3 is observed in hormone-producing tumors such as prostate cancer, and the role of LAT4 in tumor growth and progression is unknown (11). It has been established that LAT1 is an important molecule in targeted therapy, and the inhibition of LAT1 contributes to the suppression of tumor growth via the mammalian target of rapamycin (mTOR) pathway (8, 9). The protein expression of LAT1 has been clinically proven to be different according to the histological subtype, and its overexpression is closely linked to tumor proliferation, angiogenesis, and metastasis (2-9). Little is known about the prognostic significance of LAT1 expression in patients with CRC; however, Hayase *et al*. recently reported that LAT1 was highly expressed in 72.4% of CRC patients, and its up-

regulation is closely correlated with venous invasion and tumor depth (11). Although there are some reports concerning the upregulated expression of LAT1 in CRC tumor tissues, no adequate investigation on the prognostic significance of LAT1 has been performed in patients with CRC (11). As described above, it is critical to elucidate the relationship between LAT1 expression and its related markers such as 4F2hc, Ki-67, and mTOR in various types of cancers. Unfortunately, little is known about the association between LAT1 expression and its related markers in patients with CRC. Hence, we conducted a study to elucidate the prognostic significance of LAT1 expression in patients with surgically resected CRC. Moreover, the expression of LAT1 was also correlated with that of 4F2hc and Ki-67, determined by the cell proliferative marker and phosphorylation of mTOR (p-mTOR).

Materials and Methods

Patients. From January 2011 to December 2011, consecutively, 150 patients with histologically confirmed CRC who underwent radical surgery at Gunma University Hospital were registered in this study. Of these 150 patients, 3 received systemic chemotherapy and radiotherapy before the resection of the primary tumor; therefore, they were excluded from this study. One hundred and forty-seven patients were eligible for the current study. One hundred and forty-seven surgically resected primary tumors were analyzed in accordance with the institutional guidelines and the Helsinki Declaration. The institutional review boards of all participating institutions approved this study. The clinicopathological variables were obtained from pathological reports and medical records and included age, gender, histological type, tumor depth, lymph node metastasis, lymphatic invasion, vascular invasion, perineural invasion, pathological disease staging, and outcome.

Immunohistochemical staining. LAT1 expression was determined immunohistochemically by incubating tumor samples with rabbit monoclonal antibody to LAT1 (provided by J-Pharma, Tokyo, Japan) at a dilution of 1:5000 in phosphate buffered saline containing 0.1% bovine serum albumin at 4˚C overnight, followed by incubation at room temperature for 30 min. 4F2hc is an affinity-purified goat polyclonal antibody raised against a peptide located at the carboxy terminus of 4F2hc (1:200 dilution; Santa Cruz Biotechnology, Inc., Tokyo, Japan) of human origin. The reaction was visualized using the Histofine Simple Stain MAX-PO (Multi) Kit (Nichirei, Tokyo, Japan) according to the manufacturer's instructions. The detailed protocol for immunostaining has been published elsewhere (12). Negative controls were incubated without primary antibody, and no detectable staining was evident. The expression of LAT1 and 4F2hc was considered positive only if distinct membrane staining was present. The percentage of staining of LAT1 and 4F2hc was scored as follows, 1=0-10%, 2=11-25%, 3=26-50%, and 4=51-100%. The staining intensity was not considered in assessing staining outcomes. Positive expression was defined when tumors contained cancer cells that were assigned a staining score of 3 or 4, and the tumors in which cancer cells were scored as 4 were defined as having high expression.

For Ki-67 and p-mTOR, immunohistochemical staining was performed according to the procedures described in a previous report (12). The murine monoclonal antibody against Ki-67 (Dako, Glostrup, Denmark; 1:40 dilution) and a rabbit monoclonal antibody against phospho-mTOR (Cell signaling, 80 dilution) were used. For Ki-67, a highly cellular area of the immunostained section was assessed. All epithelial cells with nuclear staining of any intensity were defined as having high expression. Approximately 1000 nuclei were counted on each slide. Proliferative activity was assessed as the percentage of Ki-67-stained nuclei (Ki-67 labeling index) in the sample. The median value of the Ki-67 labeling index was evaluated, and tumor cells with expression greater than the median value were defined as having high expression. p-mTOR was considered positive if membranous and/or cytoplasmic staining was present. For p-mTOR, a semi-quantitative scoring method was used: 1=<10%, 2=10-25%, 3=25-50%, and 4=51-100% positive cells. The tumors in which stained tumor cells made up more than 25% of the tumor were graded as positive.

The sections were evaluated using a light microscope in a blinded fashion by at least two of the authors. In the case of a discrepancy, both investigators simultaneously evaluated the slides until a consensus was reached. Both investigators were blinded to patient outcomes.

Statistical analysis. Statistical analyses were performed using Student's *t*-test and the χ^2-test for continuous and categorical variables, respectively. Correlations were analyzed using the nonparametric Spearman's rank test. The Kaplan–Meier method was used to estimate survival as a function of time, and survival differences were analyzed by the log-rank test. Overall survival (OS) was defined as the time from tumor resection to death from any cause. Disease-free survival (DFS) was defined as the time between tumor resection and the first episode of disease progression or death. Univariate and multivariate survival analyses were performed using the Cox proportional hazards model and a logistic regression model for radical surgery. Values of $p<0.05$ were considered statistically significant. All statistical analyses were performed using GraphPad Prism version 4 software (GraphPad Software, San Diego, CA, USA) and JMP Pro version 12.0 software (SAS Institute Inc., Cary, NC, USA).

Results

Patient demographics. The clinicopathological information regarding a total of 147 patients (nmales=90, nfemales=57; median age=69 years; age range=38-92 years) is listed in Table I. Sixty patients had a smoking history, and pathological staging yielded 35 patients with stage I, 54 patients with stage II, 39 patients with stage III, and 19 patients with stage IV. Forty-nine patients (33%) received adjuvant chemotherapy after surgical resection, and 37 patients developed recurrent disease after surgical resection.

Immunohistochemical analysis. Immunohistochemical assessment was performed using 147 primary sites of CRC. The representative images of LAT1 and 4F2hc are shown in Figure 1. The immunostaining of LAT1 and 4F2hc was detected in the CRC cells, localized predominantly on the plasma membrane. All positive cells displayed strong membranous immunostaining, whereas cytoplasmic staining

Table I. *Patient's characteristics according to the expression of LAT1 and 4F2hc.*

Variables	Total	LAT1 expression						4F2hc expression					
	n=147	Positive n=118	Negative n=29	*p*-Value	High n=61	Low n=86	*p*-Value	Positive n=86	Negative n=61	*p*-Value	High n=36	Low n=111	*p*-Value
Age (≥69/>69)	77/60	63/55	14/15	0.68	28/33	49/37	0.24	51/35	26 /35	0.06	18/18	59/52	0.84
Gender (male/female)	90/57	72/46	18/11	>0.99	40/21	50/36	0.39	53/33	37/24	>0.99	23/13	67/44	0.84
Smoking (Yes/No)	60/87	46/72	14/15	0.40	22/39	38/48	0.39	32/54	28/33	0.31	13/23	47/64	0.56
T factor (T1-2/T3-4)	40/107	26/92	14/15	**<0.01**	11/50	29/57	**0.04**	16/70	24/37	**<0.01**	3/33	37/74	**<0.01**
N factor (present/absent)	92/55	69/49	23/6	0.05	31/30	61/25	**0.01**	49/37	43/18	0.11	21/15	72/39	0.55
Tumor location (Right/Left)	49/98	42/76	7/22	0.27	20/41	29/57	>0.99	30/56	19/42	0.72	12/24	37/74	>0.99
Pathological stage (I-II/III-IV)	89/58	67/51	22/7	0.08	29/32	60/26	**<0.01**	47/39	42/19	0.08	20/16	69/42	0.55
Histology (Well/Not-well)	39/108	28/90	11/18	0.15	13/48	26/60	0.25	18/68	21/40	0.08	8/28	31/80	0.66
Lymphatic permeation (absent/present)	42/105	27/91	15/14	**<0.01**	8/53	34/52	**<0.01**	13/73	29/32	**<0.01**	3/33	39/72	**<0.01**
Vascular invasion (absent/present)	51/96	38/80	13/16	0.27	15/46	36/50	**0.03**	21/65	30/31	**<0.01**	9/27	42/69	0.22
Perneural invasion (absent/present)	92/56	71/47	21/9	0.40	33/28	59/27	0.08	51/35	41/20	0.38	23/13	69/42	>0.99
Adjuvant chemotherapy (absent/present)	98/49	76/42	22/7	0.27	42/19	56/30	0.72	55/31	43/18	0.47	87/11	11/100	**<0.01**
LAT1 (positive/negative)	118/29	-	-	-	-	-	-	85/1	33/28	**<0.01**	36/0	82/29	**<0.01**
LAT1 (high/low)	61/86	-	-	-	-	-	-	49/37	12/49	**<0.01**	31/5	30/81	**<0.01**
4F2hc (positive/negative)	87/60	86/32	1/28	**<0.01**	49/12	38/48	**<0.01**	-	-	-	-	-	-
4F2hc (high/low)	36/111	36/82	0/29	**<0.01**	31/30	5/81	**<0.01**	-	-	-	-	-	-
Ki-67 (positive/negative)	74/73	67/51	7/22	**<0.01**	49/12	25/61	**<0.01**	51/35	23/38	**0.01**	26/10	48/63	**<0.01**
p-mTOR (positive/negative)	53/94	52/66	1/28	**<0.01**	37/24	16/70	**<0.01**	36 /50	17/44	0.11	18/18	35/76	0.07

LAT1: T-type amino acid transporter 1; 4F2hc: 4F2 cell surface antigen; p-mTOR: phosphorylation of mammalian target of rapamycin; values in bold are considered statistically significant.

was rare. The rates of positive expression and average scores for LAT1 and 4F2hc were 80% (118/147) and 58% (86/147) ($p<0.01$), respectively, and 3.2±0.9 and 2.6±1.0 ($p<0.01$), respectively. Moreover, the percentage of high expression for LAT1 and 4F2hc was 41% (61/147) and 24% (36/147) ($p<0.01$), respectively. Figure 2 reveals the comparison between LAT1 and 4F2hc expression according to the scoring of primary tumors, indicating that the high expression of LAT1 was superior to that of 4F2hc. The Ki-67 labeling index averaged 53±18% (median, 53%), ranging from 5 to 90% in all patients. The positive expression of the Ki-67 labeling index was observed in 50% (74/147). The rates of positive expression and average scores for p-mTOR were 36% (53/147) and 2.1±0.8, respectively.

Positive expression of LAT1 was significantly associated with T factor, lymphatic permeation, cell proliferation, and the expression of 4F2hc and p-mTOR, and that of 4F2hc exhibited a significant relationship with T factor, lymphatic permeation, vascular invasion, and cell proliferation (Table I). When the definition of the cut-off value as a scoring of 4 was considered, the high expression of LAT1 displayed a significant association with T factor, lymph node metastasis, advanced disease stage, lymphatic permeation, vascular invasion, cell proliferation, and the expression of 4F2hc and p-mTOR (Table I). Moreover, Spearman's rank test revealed that the expression of LAT1 was significantly correlated with the positive expression of 4F2hc [r=0.59, 95% confidence interval (CI)=0.47-0.69, $p<0.01$], Ki-67 labeling index (r=0.54, 95%CI=0.41-0.65, $p<0.01$), and p-mTOR (r=0.44, 95%CI=0.31-0.57, $p<0.01$).

Survival analysis. The 5-year DFS and OS rates were 73% and 75%, respectively. Of all patients, 41 died after the initial surgery. The median follow-up period was 2072 days, ranging from 46 to 2734 days. Univariate and multivariate analyses were performed in all patients (Tables II and III). Univariate analysis revealed that T factor, N factor, staging, lymphatic permeation, vascular invasion, perineural invasion, LAT1, 4F2hc, Ki-67, and p-mTOR were identified as significantly worse prognostic factors for DFS (Table II) and significant predictors of OS were N factor, staging, lymphatic permeation, vascular invasion, perineural invasion, LAT1, 4F2hc, Ki-67, and p-mTOR (Table III). According to the results of the univariate log-rank test, we screened variables with a cut-off of $p<0.01$ and excluded 4F2hc, Ki-67, and p-mTOR, which were closely correlated with LAT1 expression, and T factor and N factor, which were significantly related to staging from multivariate analysis, because of confounding factors. By multivariate analysis, staging, adjuvant chemotherapy, and positive LAT1 expression were confirmed to be independent prognostic factors of a worse OS, and also lymphatic permeation was a significant prognostic marker for DFS (Tables II and III). Next, we also attempted multivariate analyses using high

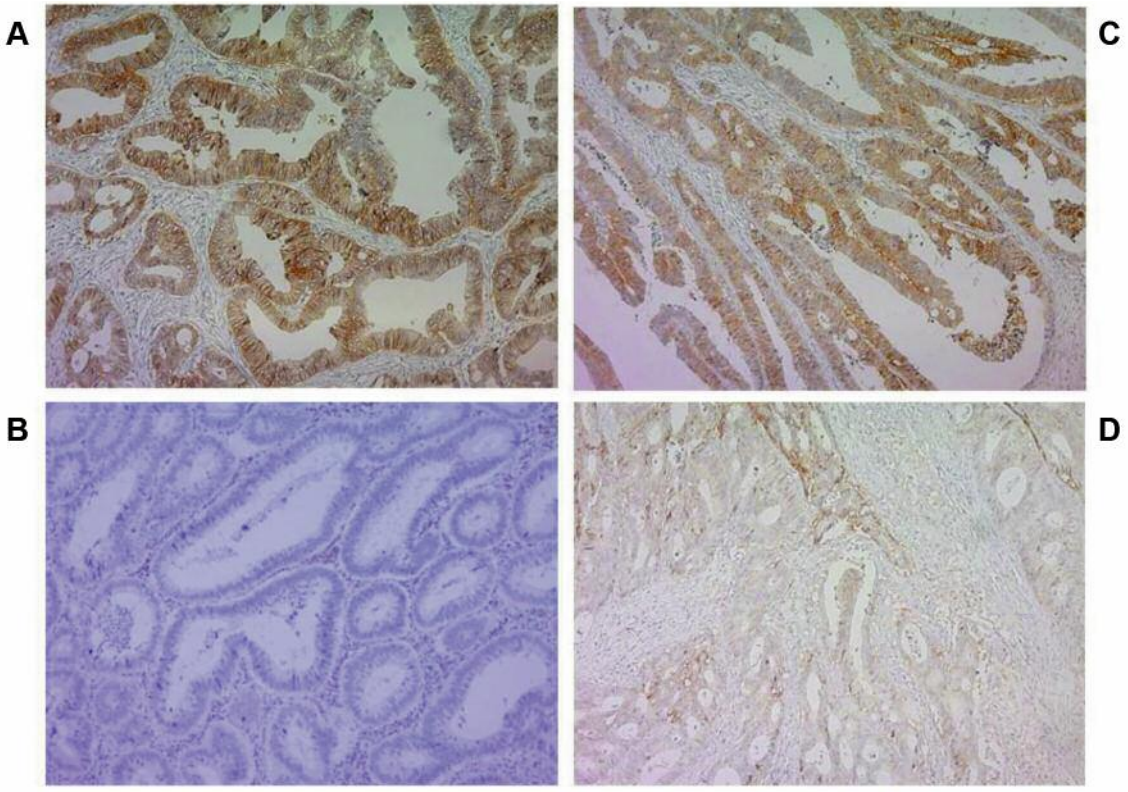

Figure 1. *Immunohistochemical staining for LAT1 and 4F2hc in colorectal carcinoma showing expression at the membrane. Panels A and B show the immunohistochemical staining scores for LAT1 expression: (A) score=4 and (B) score=1, and panels C and D reveal that by 4F2hc expression: (C) score=4 and (D) score=2.*

expression of LAT1. Multivariate analysis also confirmed that the high expression of LAT1, adjuvant chemotherapy, and staging were identified as significant prognostic markers for predicting worse OS and a significant prognostic marker for DFS was the high expression of LAT1 (Tables II and III). Kaplan–Meier survival curves for patients with high and positive expression of LAT1 and those of 4F2hc are shown in Figures 3 and 4.

Discussion

A clinicopathological study was performed to elucidate the prognostic role of LAT1 and its related markers in tumor specimens of human CRC patients. To the best of our knowledge, this study is the first to determine that LAT1 is significantly correlated with the expression levels of 4F2hc, tumor cell proliferation antigen (Ki-67), and p-mTOR in CRC patients. Increased expression of LAT1 was identified as an independent marker predicting a worse outcome in patients with CRC. Our study also indicated that LAT1 is highly expressed in CRC patients and is closely correlated with tumor aggressiveness. Aside from previous studies, the results of our study contributed to the novel findings regarding the prognostic significance of LAT1 and after surgical resection of CRC. A high LAT1 expression was proven to be a powerful prognostic marker predicting worse outcomes after surgical resection of CRC. Our prognostic analysis was supported by a sufficient follow-up period. Future studies should investigate whether LAT1 or 4F2hc is a significant marker to predict the outcome after systemic chemotherapy in patients with advanced CRC.

LAT1 is highly expressed in various human cancers and many tumor cell lines, correlating with tumor cell proliferation, angiogenesis, and mTOR signaling pathway activation (1-10). Its expression is known to vary according to histological types of cancers. In particular, the expression levels of LAT1 in squamous cell carcinoma (SCC) tends to be higher than that in adenocarcinoma (AC) (7, 9). In our previous study, a higher frequency of positive expression of LAT1 in pancreatic ductal adenocarcinoma was observed compared to other cancers with histology of AC, yielding a positive expression rate of 53% (13). Surprisingly, the rate of positive LAT1 expression in CRC was 80%, similar to that of SCC. Hayase *et al.* have also reported a 72.4% positive rate of LAT1 expression in CRC

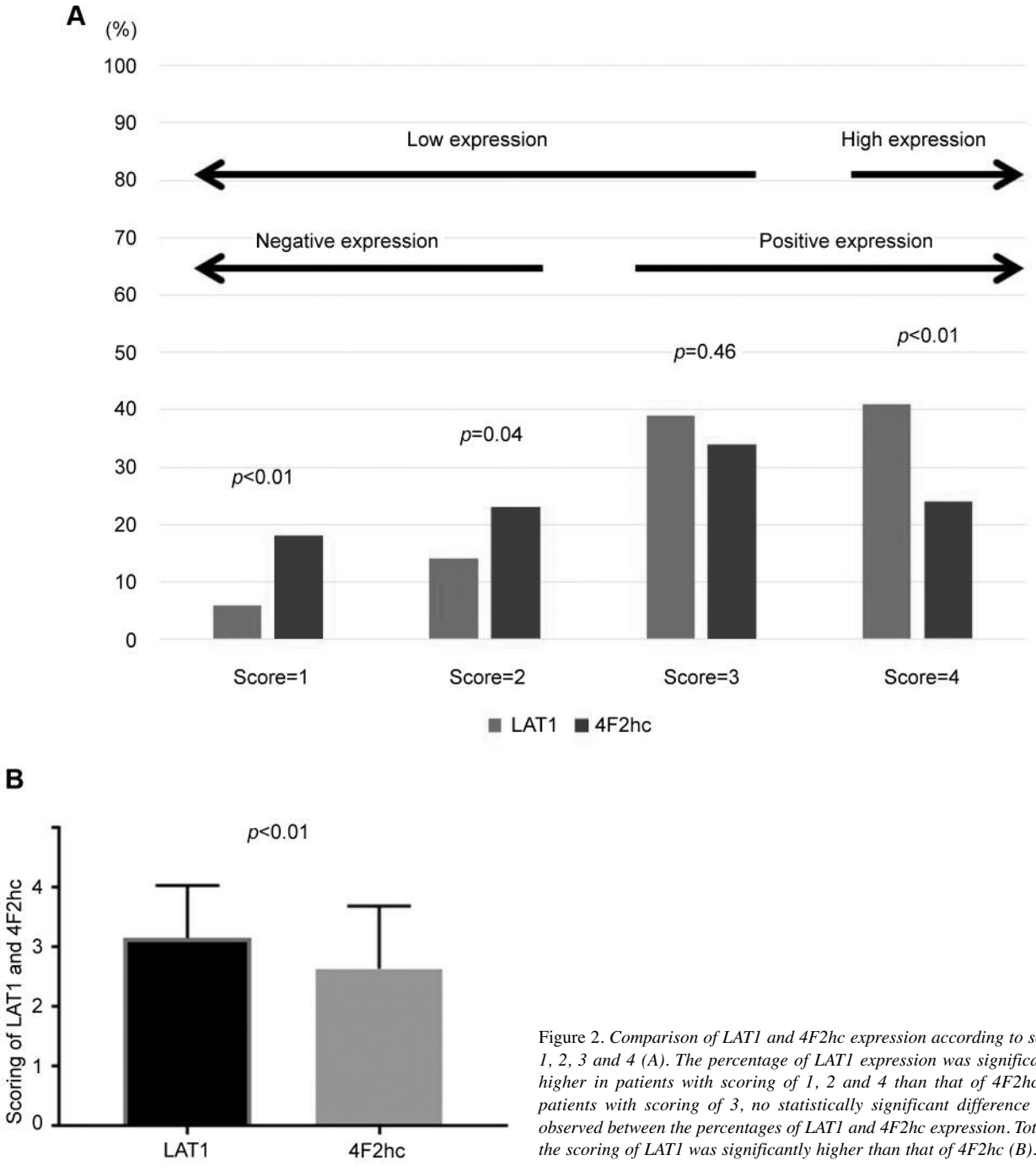

Figure 2. *Comparison of LAT1 and 4F2hc expression according to score 1, 2, 3 and 4 (A). The percentage of LAT1 expression was significantly higher in patients with scoring of 1, 2 and 4 than that of 4F2hc. In patients with scoring of 3, no statistically significant difference was observed between the percentages of LAT1 and 4F2hc expression. Totally, the scoring of LAT1 was significantly higher than that of 4F2hc (B).*

(11). Although little is known about the detailed molecular mechanisms, CRC may be characterized by higher expression of LAT1 compared to other cancers with AC. In their study, however, the anti-LAT1 used for immunohistochemistry was different from that used in our study, and they reported that LAT1staining was observed mainly at the cellular membrane and cytoplasm. Generally, LAT1 is considered a membrane transporter and should be predominantly located on the plasma membrane, not in the cytoplasm, such as in our immunohistochemical staining. LAT1 expression on the cellular membrane and cytoplasm may bias the survival results and clinicopathological analyses. Therefore, the technical limitations of immunohistochemistry may distort the biological and prognostic significance of LAT1.

Table II. *Predictors of disease-free survival*.

Variables	Disease-free survival*		Positive LAT1 expression and Disease-free survival†		High LAT1 expression and Disease-free survival†	
	5-year DFS rate (%)	*p*-Value	HR (95%CI)	*p*-Value	HR (95%CI)	*p*-Value
Age (≤69/>69)	73/71	0.76				
Gender (male/female)	68/79	0.13				
Smoking (Yes/No)	64/75	0.55				
T factor (T1-2/T3-4)	88/67	**0.01**				
N factor (present/absent)	67/89	**0.01**				
Tumor location (Right/Left)	71/73	0.64				
Pathological stage (I-II/III-IV)	86/51	**<0.01**	1.80 (0.84-3.29)	0.11	1.29 (0.61-2.40)	0.46
Histology (Well/Not-well)	78/71	0.29				
Lymphatic permeation (present/absent)	63/97	**<0.01**	2.33 (1.00-0-10.06)	**0.04**	1.98 (0.84-8.60)	0.13
Vascular invasion (present/absent)	61/93	**<0.01**	1.73 (0.99-3.61)	0.05	1.64 (0.91-3.50)	0.10
Perineural invasion (present/absent)	55/83	**<0.01**	1.20 (0.59-2.05)	0.55	10.9 (0.64-2.23)	0.77
Adjuvant chemotherapy (present/absent)	78/69	0.47				
LAT1 (positive/negative)	68/91	**0.02**	1.69 (0.92-4.20)	0.09		
LAT1 (high/low)	42/94	**<0.01**			2.74 (1.81-4.51)	**<0.01**
4F2hc (positive/negative)	61/89	**<0.01**				
4F2hc (high/low)	54/79	**<0.01**				
Ki-67 (positive/negative)	62/83	**<0.01**				
p-mTOR (positive/negative)	52/83	**<0.01**				

*Univariate analysis; †Multivariate analysis; LAT1: L-type amino acid transporter 1; well: well-differentiated; not-well: not well-differentiated; 4F2hc: 4F2 cell surface antigen; p-mTOR: phosphorylation of mammalian target of rapamycin; HR: hazard ratio; 95%CI: 95% confidence interval; DFS: disease-free survival; values in bold are considered statistically significant.

Table III. *Predictors of overall survival*.

Variables	Overall survival*		Positive LAT1 expression and Overall survival†		High LAT1 expression and Overall survival†	
	5-year OS rate (%)	*p*-Value	HR (95%CI)	*p*-Value	HR (95%CI)	*p*-Value
Age (≤69/>69)	78/69	0.12				
Gender (male/female)	69/82	0.18				
Smoking (Yes/No)	69/79	0.53				
T factor (T1-2/T3-4)	79/73	0.62				
N factor (present/absent)	58/83	**<0.01**				
Tumor location (Right/Left)	73/74	0.75				
Pathological stage (I-II/III-IV)	85/58	**<0.01**	2.66 (1.37-4.71)	**<0.01**	2.14 (1.09-3.84)	**0.02**
Histology (Well/Not-well)	81/71	0.10				
Lymphatic permeation (present/absent)	71/85	**0.03**				
Vascular invasion (present/absent)	66/89	**<0.01**	1.38 (0.86-2.36)	0.18	1.32 (0.81-2.27)	0.25
Perineural invasion (present/absent)	63/81	**<0.01**	1.16 (0.62-1.94)	0.59	1.07 (0.57-1.78)	0.80
Adjuvant chemotherapy (present/absent)	85/68	**<0.01**	2.62 (1.72-4.21)	**<0.01**	2.26 (1.50-3.63)	**<0.01**
LAT1 (positive/negative)	71/85	0.06	1.66 (1.04-3.06)	**0.03**		
LAT1 (high/low)	52/90	**<0.01**			1.92 (1.34-2.85)	**<0.01**
4F2hc (positive/negative)	64/89	**<0.01**				
4F2hc (high/low)	58/79	**<0.01**				
Ki-67 (positive/negative)	67/81	**0.02**				
p-mTOR (positive/negative)	59/81	**<0.01**				

*Univariate analysis;†Multivariate analysis; LAT1: L-type amino acid transporter 1; well: well-differentiated; not-well: not well-differentiated; 4F2hc: 4F2 cell surface antigen; p-mTOR: phosphorylation of mammalian target of rapamycin; HR: hazard ratio; 95%CI: 95% confidence interval; OS: overall survival; values in bold are considered statistically significant.

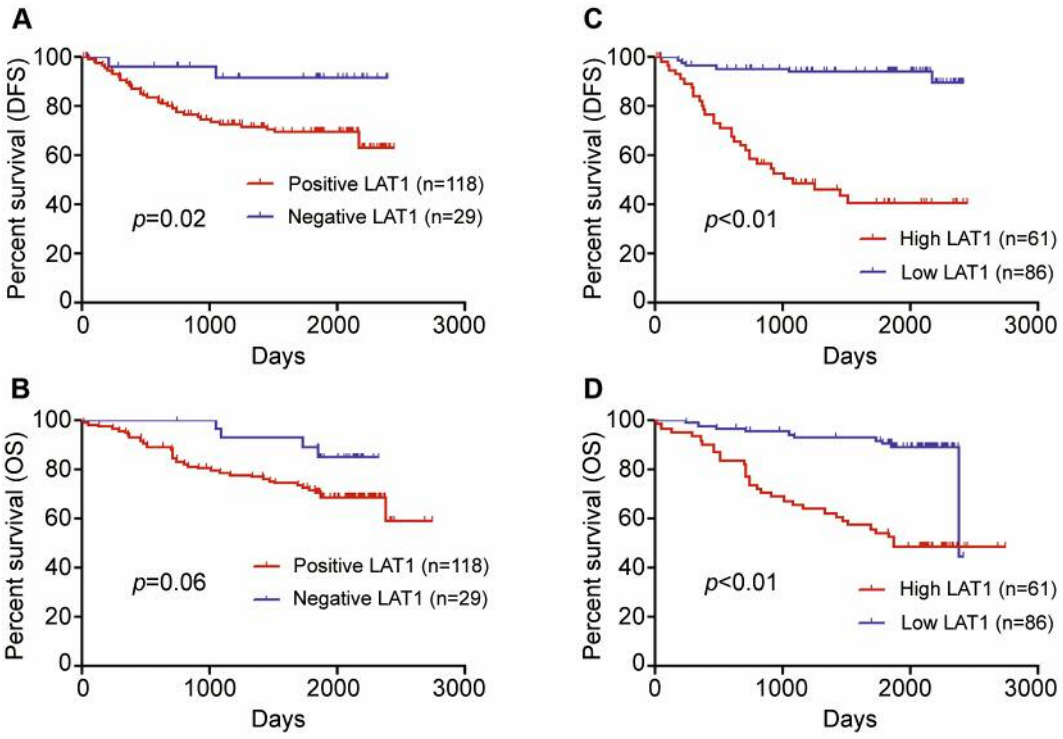

Figure 3. *Kaplan–Meier survival curves for patients with positive and negative, and high and low expression of LAT1. The 5-year DFS and OS rates of LAT1-positive and LAT1-negative patients were 68% and 91%, respectively, (p=0.02) (A) and 71% and 85%, respectively, (p=0.06) (B). The 5-year DFS and OS rates of LAT1-high and LAT1-low patients were 42% and 94%, respectively, (p<0.01) (C) and 52% and 90%, respectively, (p<0.01) (D).*

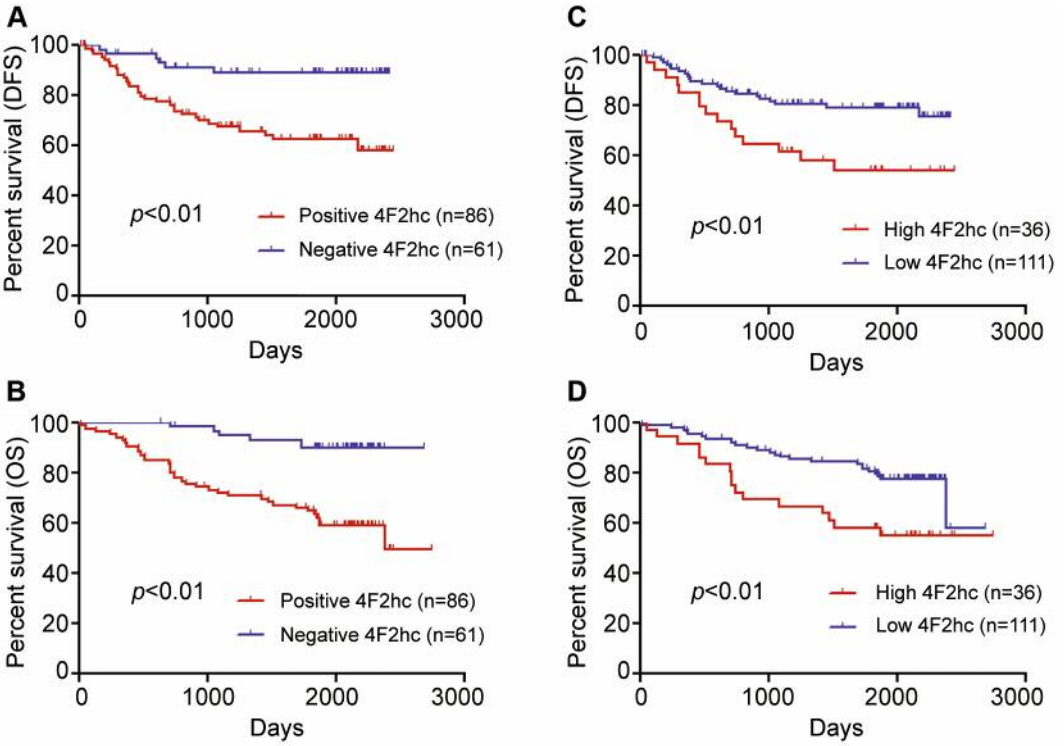

Figure 4. *Kaplan–Meier survival curves for patients with positive and negative, and high and low expression of 4F2hc. The 5-year DFS and OS rates of 4F2hc-positive and 4F2hc-negative patients were 61% and 89%, respectively, (p<0.01) (A) and 64% and 89%, respectively, (p<0.01) (B). The 5-year DFS and OS rates of 4F2hc-high and 4F2hc-low patients were 54% and 79%, respectively, (p<0.01) (C) and 58% and 79%, respectively, (p<0.01) (D).*

To our knowledge, the present study is the first to evaluate the relationship between LAT1 expression and its related markers such as 4F2hc, Ki-67, and mTOR. Expression of 4F2hc was identified as a significant prognostic predictor for patients with CRC. Moreover, expression levels of LAT1 were closely linked to the vascular, lymphatic, and perineural invasion, which leads to the advanced stage. The expression of LAT1 plays a crucial role in tumor progression and aggressiveness. A previous *in vitro* study has demonstrated that inhibition of LAT1 reduced the viability of tumor cells through suppression of phosphorylation of mTOR, p70S6K, and 4EBP1 (8). Although it remains unclear whether LAT1 increases tumor viability via phosphorylation of mTOR in patients with CRC, they may act synergistically to stimulate tumor progression. Hayase *et al*. have suggested that restriction of amino acids induced an antitumor effect through the inhibition of the LAT1/mTOR pathway in CRC cell lines (11). Further investigation is warranted to elucidate the mechanism regarding the inhibition of LAT1/mTOR pathway using *in vivo* models.

In our study, the role of LAT1 as a prognostic marker increased as its expression was increasing. CRC patients with LAT1 expression of higher than 50% were identified as the population having a high risk of recurrence and death after surgical resection. Interestingly, it was also apparent that and increased expression of 4F2hc, Ki-67 labeling index, and p-mTOR within CRC tumor cells was closely correlated with a worse outcome after surgery. The inhibition of LAT1 targeting molecules may synergistically contribute to suppression of tumor growth *via* the inhibition of LAT1. In addition to these markers, other amino acid transporters such as alanine-serine-cysteine transporter 2 (ASCT2) or cystine-glutamate exchanger transporter (xCT), which are related to tumor growth, should also be investigated in a future study (14). It has been reported that the glutamine transporter ASCT2 promotes tumor growth independently of LAT1 (15). A previous study has demonstrated that ASCT2 is positively expressed in patients with CRC and is related to aggressive biological behavior and survival (16). Moreover, Sugano *et al*. have reported that immunoreactivity for xCT was found in approximately 70% of CRC patients, and increased xCT expression was identified as an independent significant predictor of disease recurrence, correlated with tumor invasiveness and lymph node metastasis (17). However, it remains unclear how LAT1 triggers tumor growth in cooperation with ASCT2 or xCT in patients with CRC. Thus, the cooperative expression of LAT1, ASCT2, and xCT should be explored to elucidate the biological role of amino acid transporters in CRC tumor cells.

There are some certain limitations in the present study. First, the results of our study were not validated in an independent cohort; therefore, another study is necessary to confirm the results of our study. Second, the role of LAT1 as a predictor is obscure in patients with advanced CRC who are candidates for systemic chemotherapy. A further study is warranted to investigate the expression of LAT1 in such patients. Finally, exploration of the cooperative expression of LAT1, ASCT2, and xCT within CRC tumor cells would be beneficial. This approach will elucidate the different roles of these amino acid transporters in the development and carcinogenesis of CRC.

In conclusion, high expression of LAT1 was identified as an independent significant marker linked to worse prognosis in patients with CRC, correlated with tumor cell proliferation, tumor aggressiveness, and metastasis. Moreover, LAT1 was closely associated with the expression of 4F2hc and the phosphorylation of the mTOR pathway. LAT1 is a promising molecular marker to predict the prognosis after surgical resection in CRC patients.

Conflicts of Interest

The Authors have no financial or personal relationships with other people or organizations that could inappropriately influence their work.

Authors' Contributions

HO and KK: conception and preparation of the manuscript. YM, TY, TT, RK, KO, RK and CK: management of the patient. OY: statistical analysis and patient's data collection. TO, YK, TY, TA, HK and KS: revising the manuscript. All Authors contributed and agreed with the content of the manuscript.

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