Prognostic values of L-type amino acid transporter 1 and CD98hc expression in breast cancer

Masaaki Ichinoe [ORCID],[1] Tetuo Mikami,[2] Nobuyuki Yanagisawa,[3] Tsutomu Yoshida,[1] Kiyomi Hana,[4] Hitoshi Endou,[4] Isao Okayasu,[1] Norihiko Sengoku,[5] Hideaki Ogata,[6] Makoto Saegusa,[1] Kazutoshi Shibuya,[7] Yoshiki Murakumo[1]

ABSTRACT

Aims L-type amino acid transporter 1 (LAT1) is a major Na^+-independent neutral amino acid transporter, forming a complex with CD98hc. The aim of this study is to investigate the significance of LAT1 and CD98hc in invasive breast cancer.

Methods LAT1 and CD98hc expression was immunohistochemically assessed in 280 invasive breast cancers and analysed for association with clinicopathological features.

Results High levels of LAT1 and CD98hc were observed in triple-negative breast cancers (TNBCs) possessing negative immunoreactivity with oestrogen receptor, progesterone receptor and human epidermal growth factor receptor 2, compared with non-TNBCs (NTNBCs), and were associated with lymph-node metastasis and higher nuclear grade. The high-LAT1-expression group showed a poor prognosis in NTNBC and TNBC, however, high-CD98hc-expression group showed a poor prognosis only in NTNBC. LAT1 and CD98hc expression could be the prognostic factors in univariate analyses, but not in multivariate analyses. Further, we found that invasive tumour components showed higher LAT1 and CD98hc expression than non-invasive tumour components.

Conclusions LAT1 and CD98hc may possess prognostic values in invasive breast cancer. LAT1 may be linked with cancer cell activities and disease progression in breast cancer.

INTRODUCTION

Breast cancer is a second leading cause of death for women in the USA, and the incidence of breast cancer is gradually increasing.[1] The most common type of breast cancer is ductal carcinoma, which is classified into four categories according to oestrogen receptor (ER), progesterone receptor (PgR) and human epidermal growth factor receptor 2 (HER2) expression. The first category, luminal A type, expresses ER and/or PgR without HER2 expression; the second category, luminal B type, expresses ER and/or PgR and HER2; the third category, HER2 type, expresses HER2 without hormone receptor expression; and the final category, triple-negative type, expresses none of these markers.[2] ER-positive tumours are considered an indication for hormone therapy, whereas HER2-positive tumours are considered for the HER2-targeted agent trastuzumab.[3] Triple-negative breast cancer (TNBC) accounts for 10%–20% of breast cancers and is characterised by clinically aggressive behaviour and limited therapeutic strategies compared with other types of breast cancers because of an ineffectiveness of HER2-targeted therapy and hormone therapy.[4 5] Thus, TNBC has a poor prognosis.[5 6]

Amino acid transporters are required for cell growth and proliferation.[7 8] L-type amino acid transporter 1 (LAT1/SLC7A5) is the first isoform of the system L amino acid transporter, which functions as an essential cellular component to provide large neutral amino acids through the cytoplasmic membrane.[9] LAT1 forms a heterodimeric complex with the heavy chain of the 4F2 antigen (4F2hc or CD98hc). LAT1 is expressed in several organs including renal distal tubules, epithelia of the oesophagus and intestine, and endothelia of cerebral vessels in adults, as well as in the thymic epithelia, hepatic cells and haematopoietic cells in fetuses, whereas CD98hc is expressed ubiquitously in most organs in adults.[10 11] In the last decade, upregulation of LAT1 has been reported in several solid tumours, such as brain tumour, gastric cancer, pancreas cancer, prostate cancer, lung cancer, cholangiocarcinoma, colorectal cancer and pulmonary pleomorphic carcinoma.[12–19] Similar results for CD98hc have also been reported in non-small cell lung cancer, biliary tract cancer, hypopharyngeal squamous cell carcinoma and gastric cancer.[19–23] In breast cancer, Furuya et al reported that TNBC showed positive LAT1 and CD98hc expression at a higher frequency than non-TNBC (NTNBC), and CD98hc expression, but not LAT1 expression, was associated with prognosis in TNBC.[24]

In this study, we investigated LAT1 and CD98hc expression in a large population of invasive ductal breast cancer and analysed their clinical significance. Our results indicate the prognostic values of LAT1 and CD98hc in invasive ductal breast cancer.

MATERIALS AND METHODS
Patients

We sought patients with invasive ductal carcinoma of the breast that had been surgically resected at Kitasato University Hospital and Toho University Medical Center Omori Hospital between 2006 and 2010. All 78 identified TNBC cases and 202 randomly selected NTNBC cases were enrolled in this study. Patients who received preoperative chemotherapy and/or irradiation were excluded. Information about the study and the option to opt-out at any time were posted on the bulletin board of each hospital and announced to all prospective participants. We used clinical data and pathological

Table 1 Antibodies used in this study

Antigen	Antibody or kit	Dilution	Antigen retrieval methods	Supplier
ER	Monoclonal, 1D5	Prediluted	Water bath, 99°C, 40 min	Nichirei (Tokyo, Japan)
PgR	mouse monoclonal, PgR636	×40	Water bath, 99°C, 40 min	Thermo Fisher Scientific (Waltham, Massachusetts, USA)
HER2	HercepTest	Kit	Water bath, 99°C, 20 min	DAKO (Glostrup, Denmark)
Ki-67	Monoclonal, MIB-1	×100	Microwave treatment for 15 min	DAKO (Glostrup, Denmark)
CD98hc	Polyclonal, H300	×200	not done	Santa Cruz biotechnology (Santa Cruz, California, USA)
LAT1	Monoclonal, 4A2	Prediluted	Microwave treatment for 15 min	J Pharma (Yokohama, Japan)

ER, estrogen receptor; HER2, human epidermal growth factor receptor-2; LAT1, L-type amino acid transporter 1; PgR, progesterone receptor.

specimens from patients who had not opted-out. The day of surgery was determined as the starting day for postoperative disease free survival (DFS) and overall survival (OS) periods. The follow-up duration ranged from 1 to 90 months.

Immunohistochemical staining
Surgically resected specimens were fixed with 10% formalin and embedded in paraffin. The paraffin blocks were sliced into 4 μm-thick sections. The primary antibodies used are listed in table 1. Immunohistochemical staining for ER, PgR, Ki-67, LAT1 and CD98hc was performed using an Envision$^+$ kit (DAKO, Glostrup, Denmark). De-paraffinised and re-hydrated sections were treated with 0.3% H_2O_2 in methanol for 30 min to block endogenous peroxidase activity. Antigen retrieval for ER and PgR was performed with a hot water bath, and for Ki-67 and LAT1 with a microwave oven. Then, the sections were incubated with the primary antibodies overnight at 4°C. After washing with phosphate-buffered salin (PBS), the sections were processed with the secondary antibody. I-VIEW DAB Universal Kit (Roche Diagnostic, Japan) were used for ER and PgR. LAT1, CD98hc and Ki-67 were used Histofine Simple Stain Max-PO MULTI (Nichirei Bioscience, Japan). The peroxidase reaction was performed with 3-3′-diaminobenzidine. Nuclear counter staining were performed with haematoxylin. HER2 was stained using the HercepTest (DAKO) according to the manufacturer's manual.

Immunohistochemical staining results were judged as follows. For ER and PgR, cases were considered as positive when over 10% of cancer cells showed positive immunoreactivity. For HER2, cases were considered as positive when over 30% of cancer cells showed positive immunoreactivity, and cases were considered as negative when less than 10% of cancer cells showed positive immunoreactivity. In cases where between 10% and 30% of cancer cells were observed as positive, *HER2* gene amplification was assessed using the FISH (Fluorescence In Situ Hybridization) method. In this study, FISH was performed in two cases, both of which did not show *HER2* gene amplification. If these three markers were all negative, the cases were judged as TNBC. Evaluation of immunoreactivity for LAT1 and CD98hc was performed according to Sinicrope's method with minor modifications.[13–15 25] Based on the immunointensity of the carcinoma cell membrane, four categories of intensity were defined: 0, no staining; 1, weakly or patchily positive; 2, moderate staining; intensity 3, intense staining. The stained area was also evaluated as a percentage of the whole carcinoma area, and classified as follows: 0, none; 1, 1%–10%; 2, 11%–30%; 3, >30%. Immunoreactive scores were calculated by multiplication of the values for two parameters, intensity and area. The Ki-67 labelling index (LI) was assessed by counting at least 1000 cancer cells and indicated by the percentage value of positive cancer cells per total cells counted.

Statistical analyses
Statistical analyses were performed using StatView software (Abacus concepts, Berkeley, California, USA) and SPSS software V.22.0.0.0 (IBM SPSS). Spearman's rank correlation coefficient and Wilcoxon-Mann-Whitney and Kruskal-Wallis tests were used to analyse associations and differences in locations for continuous variables. Categorical variables were analysed using χ^2 tests.

Differences in DFS and OS periods were tested with the log-rank test. Univariable and multivariable Cox proportional hazard regression models were fitted to identify prognostic factors. All significance tests were carried out at the 0.05 level.

RESULTS
Patients' characteristics
Patients' characteristics are shown in table 2. A total 280 patients with invasive ductal carcinoma of the breast were enrolled, with a mean age of 59 (range 32–92). Seventy-eight lesions were TNBC (28%) and 202 were NTNBC (72%) including 151 luminal A type (54%), 17 luminal B type (6%) and 34 HER2 type (12%). They were composed of 140 tumours with nuclear grade (NG) 1 or 2 and 140 with NG3, 91 tumours with lymph node metastasis and 189 without lymph node metastasis, and 94 tumours

Table 2 Clinicopathological characteristics

Patients		All (280)	TNBC (78)	NTNBC (202)	P value
Age	Mean	59	60	58	0.0585
	Range	32–92	32–82	33–92	
Intraductal component	Present	171	30	141	0.0004
	Absent	109	48	61	
Nuclear grade	1 or 2	140	12	128	0.0001
	3	140	66	74	
Ki-67 LI (%)	Mean	26.9	41.5	21.3	0.0001
	Range	0–90.3	5.7–88.1	0–90.3	
pT	1	134	26	108	0.0175
	2	129	47	82	
	3	17	5	12	
	4	0	0	0	
lymph node metastasis	Positive	91	30	61	0.1857
	Negative	189	48	141	
pStage	I	94	15	79	0.0049
	II	174	59	115	
	III	12	4	8	
	IV	0	0	0	

NTNBC, non-triple-negative breast cancer; TNBC, triple-negative breast cancer.
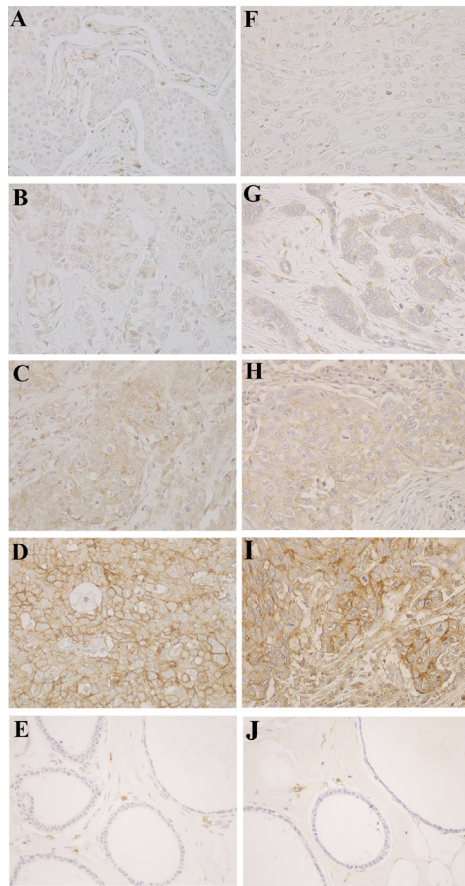
Figure 1 Representative immunoreactivity of L-type amino acid transporter 1 (A–E) and CD98hc (F–J) in breast cancer tissues. Based on the immunointensity of the carcinoma cell membranes, four categories were defined. intensity 0, no immunoreactivity (A and F); intensity 1, weakly positive (B and G); intensity 2, moderately positive (C and H); intensity 3, strong expression (D and I). Normal epithelia with negative staining (E, J). Original magnifications ×400.

Figure 2 Association of LAT1 and CD98hc expression with clinicopathological parameters. (A–D) Analyses of LAT1 expression in all cases. (E and F) Analyses of LAT1 expression in NTNBC cases. (G–J) Analyses of CD98 expression in all cases. (K and L) Analyses of CD98hc expression in NTNBC cases. Number of cases in each group is indicated in parentheses. *P value <0.05. LAT1, L-type amino acid transporter 1; LN(+), with lymph node metastasis; LN(−), without lymph node metastasis; NG, nuclear grade; NTNBC, non-triple-negative breast cancer; TNBC, triple-negative breast cancer.

with pathological stage I, 174 with stage II and 12 with stage III. Between TNBC and NTNBC cases, statistical differences were observed for intraductal component, NG, Ki-67 LI and pathological stage (table 2).

LAT1 expression in breast cancer

LAT1 immunoreactivity was detected in the cytoplasmic membrane of cancer cells, whereas it was not detected in non-neoplastic ductal epithelia. Representative LAT1 expression is shown in figure 1A–E. LAT1 scores are summarised in table 3.
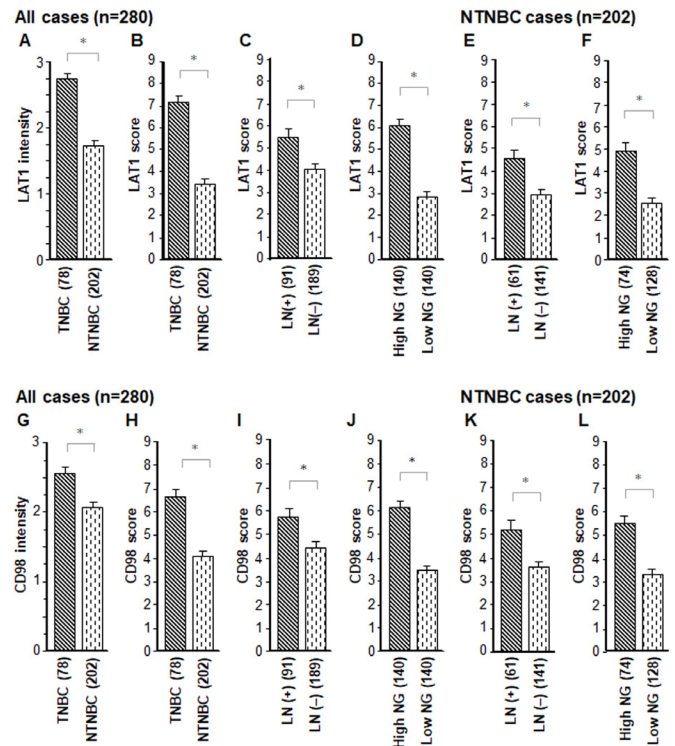
Median of LAT1 intensities and scores were significantly higher in TNBC than in NTNBC (figure 2A,B, table 3). LAT1 scores were also significantly high in tumours with lymph node metastasis or with high NG (NG3) compared with those in tumours without lymph node metastasis or with low NG (NG1 and NG2), respectively (figure 2C,D). Extraductal invasive regions exhibited enhanced LAT1 expression compared with intraductal non-invasive regions (figure 3A,B). When limited to NTNBC, cases with lymph node metastasis or with high NG showed significantly higher LAT1 scores than those without lymph node metastasis or with low NG, respectively (figure 2E,F). In comparison, when limited to TNBC, no significant differences in LAT1 scores were observed between cases with lymph node metastasis

Table 3 LAT1 and CD98hc scores in all cases and various category

| Subtype | LAT1 score | | | | | | | | | CD98hc score | | | | | | | |
	N	0	1	2	3	4	6	9	Median (IQR)	0	1	2	3	4	6	9	Median (IQR)
All cases	280	54	21	20	52	5	47	81	3 (1–9)	23	30	17	63	13	56	78	4 (2.8–9)
TNBC	78	2	3	0	8	2	15	48	9 (6–9)*	3	4	2	6	6	15	42	9 (4–9)*
NTNBC	202	52	18	20	44	3	32	33	3 (0–6)*	20	26	15	57	7	41	36	3 (2–6)*
Luminal A	151	45	17	18	33	3	20	15	2 (0–3.5)	17	24	12	50	7	25	16	3 (1–6)
Luminal B	17	3	0	2	5	0	4	3	3 (2–6)	1	1	2	3	0	4	6	6 (3–9)
HER2	34	4	1	0	6	0	8	15	6 (3–9)	2	1	1	4	0	12	14	6 (6–9)

*P value <0.0001.
HER2, human epidermal growth factor receptor-2; LAT1, L-type amino acid transporter1; NTNBC, non-triple-negative breast cancer; TNBC, triple-negative breast cancer.
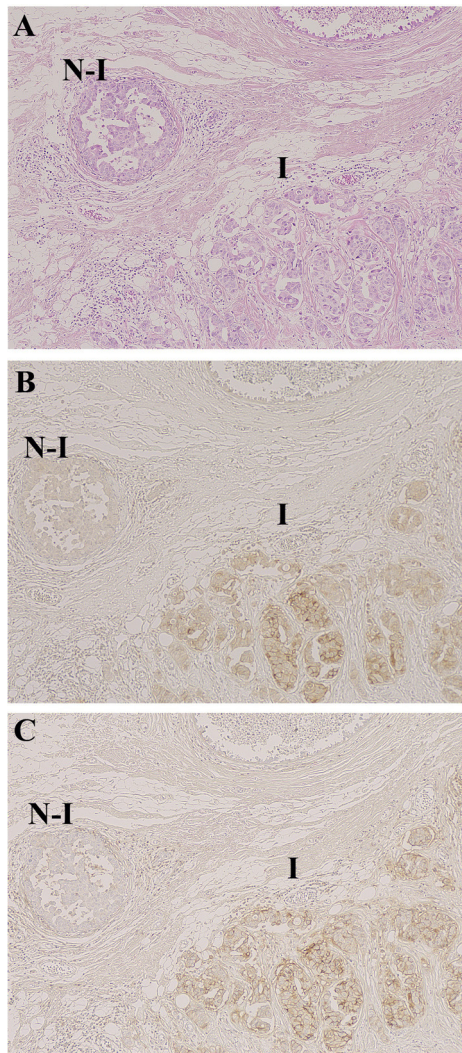
Figure 3 L-type amino acid transporter 1 (LAT1) and CD98hc expression is upregulated in invasive lesions compared with non-invasive lesions. A representative area for both invasive and non-invasive lesions is shown. (A) H&E staining. (B) Immunohistochemical staining for LAT1. (C) Immunohistochemical staining for CD98hc. The right lower part indicates invasive lesions (I), whereas the left upper part indicates non-invasive lesions (N–I). Original magnifications ×100.

and those without lymph node metastasis, and between cases with high NG and those with low NG (data not shown). Statistical analysis was using Wilcoxon-Mann-Whitney tests.

CD98hc expression in breast cancer

Similar to LAT1, CD98hc immunoreactivity was also detected in the cytoplasmic membrane of cancer cells, but not in normal ductal epithelia (figure 1F–J), and means of CD98hc intensities and scores were significantly higher in TNBC than in NTNBC (figure 2G,H, table 3). The mean CD98hc score was also significantly higher in cases with lymph node metastasis or with high NG (figure 2I,J). Invasive regions exhibited more enhanced CD98hc expression than non-invasive regions (figure 3A,C). High CD98hc score was significantly correlated with lymph node metastasis or with high NG in NTNBC cases (figure 2K,L), but not in TNBC cases (data not shown). Statistical analysis were using Wilcoxon-Mann-Whitney tests.

Correlation among LAT1 expression, CD98hc expression and Ki-67 LI

We investigated correlation among LAT1 expression, CD98hc expression and Ki-67 LI with the Spearman's rank correlation coefficient test. A strong positive correlation was observed between LAT1 and CD98hc scores ($\rho=0.729$, $p<0.0001$) in all cases. Relatively weak but significant correlations were also observed between LAT1 score and Ki-67 LI ($\rho=0.436$, $p<0.0001$), and between CD98hc score and Ki-67 LI ($\rho=0.411$, $p<0.0001$).

Prognosis analysis

We tested OS and DFS in association with LAT1 expression with a log-rank test. Cases were divided into low-LAT1-expression and high-LAT1-expression groups according to the LAT1 score (0–4 as low, 6–9 as high). The high-LAT1-expression group showed significantly poor prognosis in both OS and DFS compared with the low-LAT1-expression group analysed in all cases (OS, $p=0.0007$, DFS, $p=0.0004$) (figure 4A and online supplementary figure S1A). When cases were limited to NTNBC, there was a significant difference in DFS, but not in OS, between low-LAT1-expression and high-LAT1-expression groups (OS, $p=0.0869$, DFS, $p=0.0172$) (figure 4B and online supplementary figure S1B). When limited to TNBC, no significant difference was observed in DFS between the two groups ($p=0.3710$) (online supplementary figure S1C), however, an apparent difference was observed in OS between the two groups, although the p value could not be calculated (figure 4C). In cases with advanced stage (stage II or III), high LAT1 expression was linked to a poor prognosis (OS, $p=0.0045$, DFS, $p=0.0048$) (figure 4D and online supplementary figure S1D).

Similar results were obtained in the analyses of prognostic significance of CD98hc. High CD98hc expression was linked to poor OS ($p=0.0067$) and DFS ($p=0.0112$) in all cases (figure 4E and online supplementary figure S1E), poor OS (p value was not available) and DFS ($p=0.0056$) in NTNBC cases (figure 4F and online supplementary figure S1F) and poor OS ($p=0.0335$) in cases with advanced stages (figure 4H), however, it was not linked to OS ($p=0.8413$) or DFS ($p=0.3548$) in TNBC (figure 4G and online supplementary figure S1G), or to DFS ($p=0.0851$) in cases with advanced stages (online supplementary figure S1H).

When combined positive and negative expression of LAT1 and CD98hc was analysed for OS and DFS, high-LAT1/high-CD98hc groups showed apparently poor prognosis compared with low-LAT1/low-CD98hc groups in all the patients categories except for DFS in TNTBC ($p=0.8435$) (figure 4I–L and online supplementary figure S1I–L).

The results indicate that both LAT1 and CD98hc possess prognostic values in invasive breast cancer, and suggest that their expression is likely more useful for a prognosis predictive factor in NTNBC than in TNBC.

Cox proportional hazard regression models

We confirmed proportionality of hazards in the Cox models before the analyses. Univariable Cox proportional hazard methods revealed that poor prognosis was significantly associated with TNBC (DFS, $p=0.0013$; OS, $p=0.0029$), high NG (DFS, $p=0.0209$; OS, $p=0.0199$), positive lymph-node metastasis (DFS, $p=0.0014$; OS, $p=0.0047$), high pathological stage (stage II and III; DFS, $p=0.0157$; OS, $p=0.0461$) and high Ki-67 LI ($>20\%$, DFS, $p=0.0026$; OS, $p=0.0263$) (table 4). LAT1 score could be one of the prognostic factors in univariate
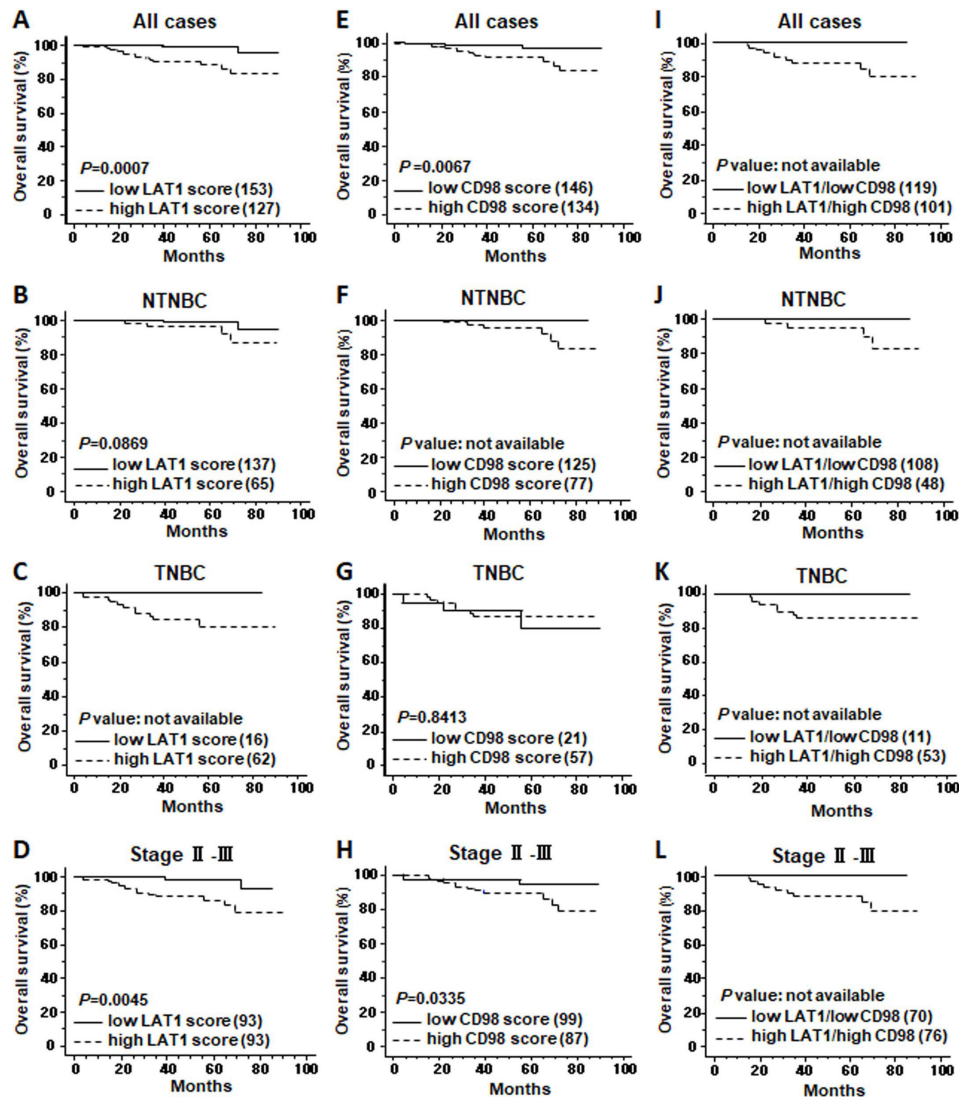
Figure 4 Overall survival curves in association with LAT1 and CD98hc expression. Cases were divided into high-expression and low-expression groups according to LAT1 and CD98hc scores (0–4 as low and 6–9 as high). Kaplan-Meier curves of the groups in all cases (A, E, I), in NTNBC cases (B, F, J), in TNBC (C, G, K) and incases with pathological stage II and III (D, H, L) are shown. LAT1, L-type amino acid transporter 1; NTNBC, non-triple-negative breast cancer; TNBC, triple-negative breast cancer.

analyses (score 0–4 vs 6–9; DFS, p=0.0011; OS, p=0.0046) as well as CD98hc score (score 0–4 vs 6–9; DFS, p=0.0146; OS, p=0.0143) (table 4). We analysed the multivariable models only those covariates which were significant in the univariable models.

However, in multivariate analyses, both LAT1 and CD98hc were revealed not to be independent prognostic factors (LAT1, DFS, p=0.887; OS, p=0.3792; CD98, DFS, p=0.7743; OS, p=0.8489) (table 4).

DISCUSSION

Breast cancer is classified into four subtypes according to the expression of hormone receptors and HER2, and the classification is important to determine the therapeutic strategy. NTNBC, which is positive for at least one of the three markers, has an indication for hormone therapy or HER2-targeted therapy, whereas TNBC, which is negative for all of the three markers, has an indication for neither hormone therapy nor HER2-targeted therapy. Therefore, NTNBC is supposed to have a favourable

prognosis compared with TNBC, however, not all cases show adequate therapeutic responses.

LAT1 is a tumour type-amino acid transporter that shows high levels of expression in several malignant tumours but a limited expression in normal tissues, and is reported to be an independent prognostic factor in some tumours.[13–16] LAT1 requires a binding partner to form a heterodimer, CD98hc, for its sufficient transporting ability.[9] In this study, we investigated the expression of LAT1 and CD98hc in a large population of patients with invasive breast cancer, focusing on the correlation between their expression and prognosis-related factors. We showed that TNBC exhibited higher LAT1 and CD98hc expression than NTNBC. Tumours with lymph node metastasis, high NG, or high Ki-67 LI showed enhanced LAT1 and CD98 expression compared with tumours without lymph node metastasis, with low NG, or with low Ki-67 LI, respectively. These observations are compatible with a previous publication.[24] In contrast, we found that invasive tumour components showed higher LAT1 and CD98hc expression than non-invasive tumour components, whereas no obvious

Table 4 Cox hazard analysis of cause-specific survival in all cases

Variable	Univariate analysis		Multivariate analysis	
	HR (95% CI)	P value	HR (95% CI)	P value
(A) Disease free survival				
Age	1.015 (0.985 to 1.046)	0.3295		
Size	1.445 (1.265 to 1.650)	<0.0001	1.422 (1.221 to 1.657)	<0.0001
Nuclear grade (1–2 vs 3)	0.413 (0.195 to 0.874)	0.0209	1.255 (0.506 to 3.112)	0.6233
TNBC or NTNBC	3.129 (1.560 to 6.276)	0.0013	2.490 (1.068 to 5.806)	0.0346
LAT1 score (0–4 vs 6–9)	3.798 (1.706 to 8.457)	0.0011	0.989 (0.844 to 1.159)	0.887
CD98hc score (0–4 vs 6–9)	2.538 (1.201 to 5.362)	0.0146	1.153 (0.435 to 3.056)	0.7743
Ki-67 LI (≤20% vs >20%)	3.641 (1.572 to 8.432)	0.0026	2.274 (1.014 to 5.097)	0.046
lymph node metastasis	3.170 (1.565 to 6.420)	0.0014	2.708 (1.177 to 6.233)	0.0192
pStage (I vs II-III)	3.640 (1.276 to 10.380)	0.0157	0.810 (0.231 to 2.845)	0.7424
(B) Overall survival				
Age	1.00 (0.959 to 1.043)	0.997		
Size	1.561 (1.302 to 1.870)	<0.0001	1.485 (1.206 to 1.830)	0.0002
Nuclear grade 1–2 vs 3	0.225 (0.64 to 0.790)	0.0199	0.825 (0.102 to 3.535)	0.7951
TNBC or NTNBC	4.655 (1.690 to 12.826)	0.0029	3.150 (0.988 to 10.037)	0.0523
LAT1score 0–4 vs 6–9	8.502 (1.932 to 37.420)	0.0046	2.339 (0.352 to 15.555)	0.3792
CD98hc score 0–4 vs 6–9	4.804 (1.368 to 16.869)	0.0143	1.167 (0.238 to 5.728)	0.8489
Ki-67 LI (≤20% vs >20%)	4.151 (1.182 to 14.575)	0.0263	1.952 (0.442 to 8.626)	0.3778
lymph node metastasis	4.594 (1.596 to 13.227)	0.0047	3.015 (0.881 to 10.318)	0.0788
pStage (I vs II-III)	7.845 (1.036 to 59.408)	0.0461	1.202 (0.119 to 12.142)	0.876

HR, Hazard ratio; LAT1, L-type amino acid transporter 1; LI, labelling index; NTNBC, non-triple-negative breast cancer; TNBC, triple-negative breast cancer.

differences were detected between the invasive and non-invasive components in the previous study.[24]

It was reported that positive rates of LAT1 and CD98hc in metastatic sites were significantly high compared with primary sites in several human neoplasms, and that gastric carcinomas with lymph node metastasis showed higher LAT1 expression than those without lymph node metastasis.[13 26] Therefore, LAT1 expression, as well as CD98hc expression, may be linked with cancer cell activities and disease progression, such as invasion and metastasis, in breast cancer. It is possible that invading carcinoma cells in the stroma have a higher demand for nutrition than non-invading cells, and the LAT1 complex might be required for its supply. To clarify this point, further in vitro studies should be necessary.

The previous report showed that high-level expression of CD98hc was correlated with poor prognosis in TNBC, but that of LAT1 was not.[24] In this study, high-LAT1-expression or high-CD98hc-expression groups showed significantly poor prognosis in all cases and in NTNBC cases. In TNBC cases, there was no association between CD98hc expression and prognosis, whereas a positive association between high-level expression of LAT1 and poor OS was observed. In addition, in advanced cases, high CD98hc expression was weakly associated with poor OS, whereas high LAT1 expression showed a relatively strong association with poor DFS and OS. These results suggest that LAT1 might be more useful as a prognosis predictive factor than CD98hc in invasive breast cancer, especially in TNBC and advanced cases. One possible reason for the discrepancy between the two studies might be the methods used for evaluation of LAT1 and CD98hc immunoreactivity (see the Materials and methods section). In the previous study, 64% and 44% of TNBCs were classified as LAT1- or CD98hc-positive, respectively, whereas in this study, 79% and 73% of TNBCs were classified into high-LAT1-score and high-CD98hc-score groups, respectively. Another possible reason might be the number of participants in each study.

It has been reported that LAT1 and CD98hc expression are independent prognostic factors in some tumours, including gastric cancer, lung cancer, bile duct cancer, hypopharyngeal squamous cell carcinoma and breast cancer.[13 16 17 22–24 27 28] However, LAT1 and CD98hc were not found to be independent prognostic factors in this study. CD98hc can bind with another transporter such as xCT or ASCT2 besides LAT1 and form a CD98 complex. It is possible that CD98 signal does not overlap with LAT1 signal well. In tongue cancer, it was reported that the expression of LAT1 is a significant independent factor for predicting poor prognosis and positive expression of ASCT2 yielded a significant relationship with worse prognosis, but not xCT.[29]

Up to now, expression of LAT1 and CD98hc has been correlated with cancer cell survival and proliferation in several malignant tumours, and the development of anticancer therapy targeting LAT1 has been studied. Inhibition of system L by 2-am inobicyclo-(2,2,1)-hepatane-2-carboxylic acid, a broad system L inhibitor, suppressed cell growth and promoted cell cycle arrest and apoptosis in several cancer cells.[30 31] JPH203, an LAT1-selective compound, also induced apoptosis in human cancer cells.[32 33] Further, there was a report JPH203 reduces growth of thyroid carcinoma in mouse model.[34]

However, the precise mechanism of the relationship between LAT1 and cell proliferation is still unknown. In lung cancer, it has been reported that LAT1 is related to hypoxic marker expression and mammalian target of rapamycin (mTOR) pathway, and LAT1 is thought to play important roles in multiple cellular activities including tumour cell proliferation.[35] There might be a possibility of a similar relationship between the mTOR pathway and LAT1 expression in breast cancer.

In conclusion, in invasive ductal carcinoma of the breast, LAT1 and CD98hc are possible candidates for prognosis predictive factors. LAT1 offers a potential target for anticancer therapy in invasive breast cancer.

Take home messages

► L-type amino acid transporter 1 (LAT1) and CD98hc expression was analysed in invasive breast cancer.
► High levels of both LAT1 and CD98hc expression were observed in triple-negative breast cancer.
► Both LAT1 and CD98hc possess prognostic values in invasive breast cancer.
► LAT1 may be linked with cancer cell activities and disease progression in breast cancer.

ORCID iD
Masaaki Ichinoe http://orcid.org/0000-0002-4622-0565

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