Human Pathology (2019) **84**, 142–149

ELSEVIER

Human PATHOLOGY

www.elsevier.com/locate/humpath

Original contribution

Expression of amino acid transporter (LAT1 and 4F2hc) in pulmonary pleomorphic carcinoma ☆

Kyoichi Kaira MD[a,*], Osamu Kawashima MD[b,1], Hedeki Endoh MD[c,1], Kazuyoshi Imaizumi MD[d,1], Yasuhiro Goto MD[d], Mitsuhiro Kamiyoshihara MD[e], Masayuki Sugano MD[f], Ryohei Yamamoto MD[c], Takashi Osaki MD[g], Shigefumi Tanaka MD[h], Atsushi Fujita MD[i], Hisao Imai MD[j], Yoshihito Kogure MD[k], Yukio Seki MD[l], Kimihiro Shimizu MD[m], Akira Mogi MD[m], Yoshinori Shitara MD[n], Tetsunari Oyama MD[o], Yoshikatsu Kanai MD[p], Takayuki Asao MD[q]

[a]*Department of Oncology Clinical Development, Gunma University Graduate School of Medicine, Gunma 371-8511, Japan*
[b]*Department of Respiratory Surgery, Shibukawa Medical Center, Gunma 377-0280, Japan*
[c]*Department of Thoracic Surgery, Saku Central Hospital Advanced Care Center, Nagano 385-0051, Japan*
[d]*Department of Respiratory Medicine, Fujita Health University, Aichi 470-1192, Japan*
[e]*Department of Respiratory Surgery, Japanese Red Cross Maebashi Hospital, Gunma 371-0014, Japan*
[f]*Department of Respiratory Surgery, Takasaki Medical Center, Gunma 370-0829, Japan*
[g]*Department of Respiratory Medicine, Shibukawa Medical Center, Gunma 377-0280, Japan*
[h]*Department of Respiratory Surgery, Isesaki Municipal Hospital, Gunma 372-0817, Japan*
[i]*Department of Respiratory Surgery, Gunma Prefectural Cancer Center, Gunma 373-8550, Japan*
[j]*Department of Respiratory Medicine, Gunma Prefectural Cancer Center, Gunma 373-8550, Japan*
[k]*Department of Respiratory Medicine, Nagoya Medical Center, Aichi 460-0001, Japan*
[l]*Department of Thoracic Surgery, Nagoya Medical Center, Aichi 460-0001, Japan*
[m]*Department of General Surgical Science, Gunma University, Graduate School of Medicine, Gunma 371-8511, Japan*
[n]*Department of Respiratory Surgery, Fujioka General Hospital, Gunma 375-0015, Japan*
[o]*Department of Diagnostic Pathology, Gunma University Graduate School of Medicine, Gunma 371-8511, Japan*
[p]*Department of Bio-system Pharmacology, Graduate School of Medicine, Osaka University, Osaka 565-0871, Japan*
[q]*Big Data Center for Integrative Analysis, Gunma University Initiative for Advance Research, Gunma 371-8511, Japan*

Received 30 May 2018; revised 26 September 2018; accepted 29 September 2018

☆ Disclosures: The authors have no financial or personal relationships with other people or organizations that could inappropriately influence their work. The authors report no funding/support.

* Corresponding author at: Department of Oncology Clinical Development, Gunma University Graduate School of Medicine, Showa-machi, Maebashi, Gunma 371-8511, Japan.
E-mail address: kkaira1970@yahoo.co.jp (K. Kaira).

https://doi.org/10.1016/j.humpath.2018.09.020

Keywords:
LAT1;
4F2hc;
Pleomorhic carcinoma;
Lung;
Immunohistochemistry;
Prognosis

Summary Amino acid transporters are necessary for tumor growth, metastasis, and survival of various neoplasms; however, the clinicopathological significance of L-type amino acid transporter 1 (LAT1) and 4F2 cell surface antigen (4F2hc) in patients with pulmonary pleomorphic carcinoma (PPC) remains unknown. The aim of this study is to clarify the prognostic impact of these amino acid transporters in PPC. One hundred five patients with surgically resected PPC were assessed by immunohistochemistry. The expression of LAT1 and 4F2hc, and Ki-67 labeling index were investigated using specimens of the resected tumors. LAT1 and 4F2hc were highly expressed in 35% and 53% of all patients (n = 105, $P <$ .01), 25% and 48% of patients with an adenocarcinoma component (n = 48, $P = .02$), and 44% and 58% of patients with a nonadenocarcinoma component (n = 57, $P = .18$), respectively. A high LAT1 expression was significantly related to advanced disease, lymphatic permeation, tumor cell proliferation, and 4F2hc expression. By multivariate analysis, LAT1 and 4F2hc were identified as significant independent markers for predicting a worse prognosis. LAT1 is highly expressed in PPC, and high LAT1 expression can serve as a significant predictor linked to a worse prognosis in patients with PPC.

1. Introduction

Pulmonary pleomorphic carcinoma (PPC) is a rare epithelial tumor with aggressive characteristics. Its incidence ranges from 0.1% to 0.4% of all lung cancers [1]. PPC contains carcinomatous and sarcomatoid components and is classified as a subtype of sarcomatoid carcinoma of the lung in the World Health Organization histologic classification of lung neoplasms [2,3]. It has been reported that PPC has more aggressive malignant potential than any other non–small cell lung cancer (NSCLC) and is closely linked to a worse prognosis and poorer response to systemic chemotherapy [4-6]. Because of its rarity, little is known about useful biomarkers to predict the outcome of treatment in patients with PPC.

Amino acid transporters are required for tumor growth and proliferation [7-9]. L-type amino acid transporter 1 (LAT1), a systemic L amino acid transporter, requires covalent association with the heavy chain of 4F2 cell surface antigen (4F2hc) for its functional expression in the plasma membrane [9,10]. LAT1 is highly expressed in cancer cells and is closely associated with tumor metastases, cell proliferation, and a shorter survival time in several human neoplasms [9-12]. Although the underlying mechanism of LAT1 expression in various human neoplasms is still a matter of debate, it is known that LAT1 provides cancer cells with the amino acids essential for protein synthesis as well as for the stimulation of cancer cell growth via the mammalian target of rapamycin [13,14]. Recently, we described the clinicopathological significance of LAT1 expression on tumor aggressiveness and as a worse prognostic predictor in patients with NSCLC [11,12]. Moreover, Koshi et al [15] reported that LAT1 is closely correlated with the degree of malignancies of soft and tissue sarcoma. LAT1 may be responsible for tumor progression in cancer patients with both epithelial and sarcomatous components. 4F2hc plays an important part in the function of LAT1 as an amino acid transporter. Clinically, 4F2hc seems to be a stronger predictive marker in cancer patients with advanced disease and metastases than LAT1 [16-18]. We previously reported the prognostic role of LAT1 and 4Fhc expression in patients with thoracic neoplasms, including lung cancer, thymic tumors, and malignant pleural mesothelioma [11,12,18-20].

Based on this background, we conducted a clinicopathological study to investigate the expression of LAT1 and 4F2hc in patients with surgically resected PPC. Because of its rare incidence and the difficulty diagnosing it using biopsy samples, tumor samples of surgically resected PPC were collected from multiple institutions.

2. Materials and methods

2.1. Patients

One hundred five patients with histologically confirmed PPC who underwent surgical resection at multiple institutions between August 2001 and October 2015 were enrolled in this study. Pleomorphic carcinoma was diagnosed according to the 2015 World Health Organization classification of tumors [2]. Diagnoses were confirmed using light microscopy and immunohistochemistry. PPC was defined as NSCLC containing at least 10% sarcomatoid components. One hundred five surgically resected primary tumors were included in this study in accordance with institutional guidelines and the Declaration of Helsinki. The institutional review boards of all participating institutions approved this study.

2.2. Immunohistochemical staining

LAT1 expression was determined immunohistochemically by incubating tumor samples with rabbit monoclonal antibody

Table 1 Patient's demographics according to LAT1 and 4F2hc expression

Variables	LAT1 expression				4F2hc expression		
	Total (n = 105)	High (n = 37)	Low (n = 68)	*P*	High (n = 56)	Low (n = 49)	*P*
Age: <69 y/≥69 y	54/51	23/14	31/37	.15	32/24	22/27	.24
Sex: male/female	79/26	28/9	51/17	>.99	40/16	39/10	.370
Smoking: yes/no	84/21	29/8	55/13	.80	42/14	42/7	.220
T factor: T1-2/T3-4	65/40	20/17	45/23	.29	16/40	49/0	<.01 *
N factor: absent/present	72/33	23/14	49/19	.37	34/22	38/11	.090
Pathological stage: I-II/III-IV	71/34	19/18	52/16	.02 *	32/24	39/10	.02 *
Lymphatic permeation: absent/present	41/64	9/28	32/36	.04 *	22/34	19/30	>.99
Vascular invasion: absent/present	31/74	11/26	20/48	>.99	17/39	14/35	>.99
Pleural invasion: absent/present	49/56	16/21	33/35	>.99	26/30	23/26	>.99
Adjuvant chemotherapy: absent/present	78/27	25/12	53/15	.25	41/15	37/12	.82
LAT1: high/low	37/68	–	–	–	30/26	7/42	<.01 *
4F2hc: high/low	56/49	30/7	26/42	<.01 *	–	–	–
Ki-67 labeling index: high/low	50/55	24/13	26/42	.01 *	28/28	22/27	.69

* $P < .05$ is considered statistically significant. *t* Test score for continuous variables and χ^2 test for categorical variables.

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 minutes. 4F2hc is an affinity-purified goat polyclonal antibody raised against a peptide mapping at the carboxy terminus of 4F2hc (1:200 dilution; Santa Cruz Biotechnology, 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 already been published elsewhere [16]. 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 to 10%; 2, 11% to 25%; 3,

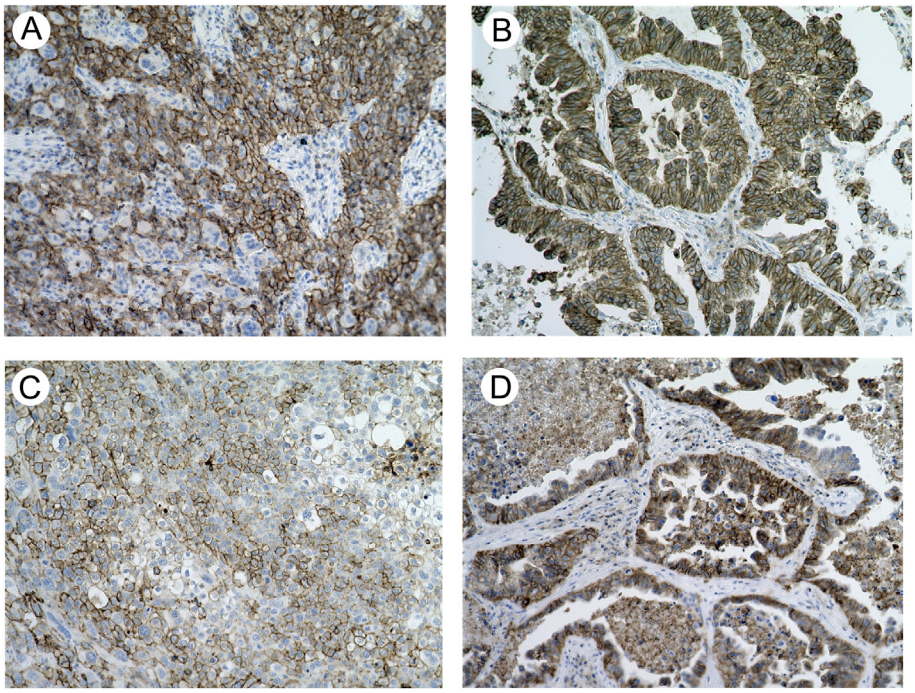

Fig. 1 A 53-year-old man with pleomorphic carcinoma consisting of giant cell component (p-T1N0M1). The score of LAT1 (A) and 4F2hc (B) immunostaining was grade 4 and grade 4, respectively, and their immunostaining pattern was membrane (original magnification ×200). An 88-year-old man with pleomorphic carcinoma consisting of spindle and AC component (p-T2N2M0). The score of LAT1 (C) and 4F2hc (D) immunostaining was grade 4 and grade 4, respectively (×200).

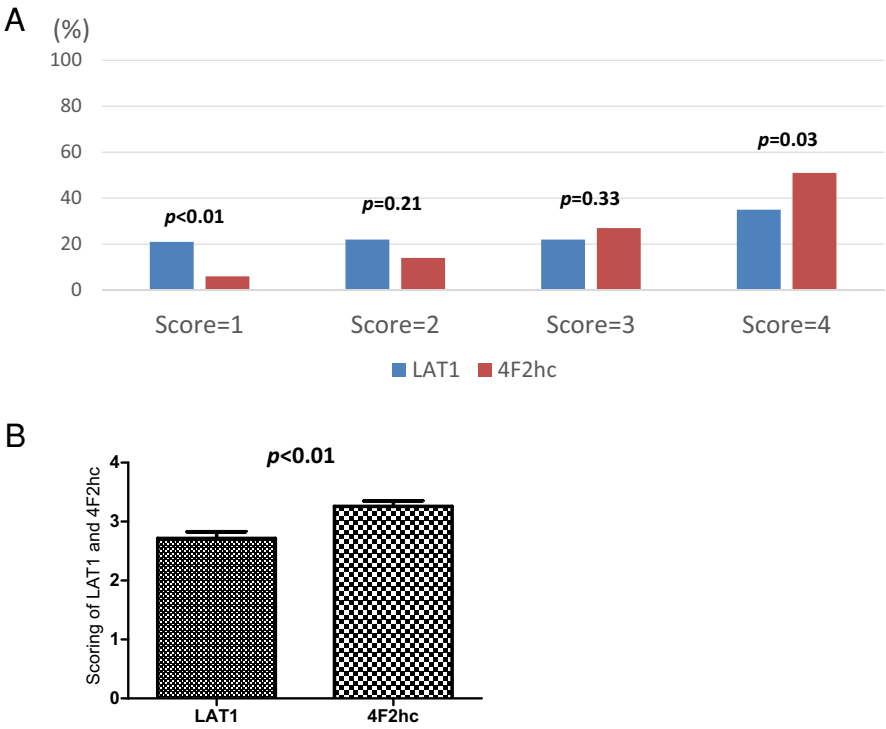

Fig. 2 A, Comparison of LAT1 and 4F2hc expression according to scores 1, 2, 3, and 4. The percentage of LAT1 expression was significantly higher in patients with a score of 1 than that of 4F2hc, whereas that of 4F2hc was significantly higher in those with a score of 4 than that of LAT1. No statistically significant difference in patients with a score of 2 or 3 was observed between the percentage of LAT1 and 4F2hc expression. B, In total, the score of 4F2hc was significantly higher than that of LAT1.

Table 2 Univariate and multivariate survival analyses

Variables	Univariate analysis		Multivariate analysis		
	1-y rate (%)	*P*	HR	95% CI	*P*
OS of total patients					
Age (≥69 y/<69 y)	47/71	.47			
Sex (female/male)	56/60	.55			
p-stage (I-II/III-IV)	74/28	<.01 *	1.75	1.32-2.33	<.01 *
Ly (present/absent)	53/71	.17			
v (present/absent)	58/61	.21			
Pl (present/absent)	53/65	.09			
Adjuvant CTx (present/absent)	66/56	.15			
LAT1 expression (high/low)	32/76	<.01 *	1.37	1.02-1.84	.03 *
4F2hc expression (high/low)	43/81	<.01 *	1.73	1.24-2.49	<.01 *
Ki-67 labeling index (high/low)	58/59	.85			
DFS of total patients					
Age (≥69 y/<69 y)	41/67	.15			
Sex (female/male)	40/58	.14			
p-stage (I-II/III-IV)	67/29	<.01 *	1.73	1.30-2.31	<.01 *
ly (present/absent)	46/68	.03 *	0.95	0.69-1.28	.74
v (present/absent)	53/58	.07			
pl (present/absent)	43/69	<.01 *	1.36	1.05-1.81	.02 *
Adjuvant CTx (present/absent)	48/56	.91			
LAT1 expression (high/low)	29/68	<.01 *	1.38	1.02-1.86	.03 *
4F2hc expression (high/low)	41/67	<.01 *	1.25	0.92-1.71	.14
Ki-67 labeling index (high/low)	51/58	.58			

Abbreviations: CI, confidence interval; ly, lymphatic permeation; pl, pleural invasion; HR, hazard ratio; v, vascular invasion.
* $P < .05$ is considered statistically significant, calculated with continuous variable.

26% to 50%; and 4, 51% to 100%. The staining intensity was not considered in assessing staining outcomes. High expression was defined when tumors contained cancer cells that were assigned with a staining score of 4.

For Ki-67, immunohistochemical staining was performed according to the procedures described in previous report [11]. The murine monoclonal antibody against Ki-67 (Dako, Glostrup, Denmark; 1:40 dilution) was used. For Ki-67, a highly cellular area of the immunostained sections was assessed. All epithelial cells with nuclear staining of any intensity were defined as 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 the tumor cells with greater than the median value were defined as high expression. The sections were evaluated using a light microscope in a blinded fashion by at least 2 of the authors. In case of discrepancies, both investigators simultaneously evaluated the slides until a consensus was reached. Both investigators were blinded to patient outcomes.

2.3. Statistical analysis

Statistical analyses were performed using Student *t* test and the χ^2 test for continuous and categorical variables, respectively. Correlations were analyzed using the nonparametric Spearman rank test. The Kaplan-Meier method was used to estimate survival as a function of time, and survival differences were analyzed using the log-rank test. Overall survival (OS) was the time from tumor resection to death from any cause. Disease-free survival (DFS) was the time between tumor resection and the first episode of disease progression or death. Univariate and multivariate survival analyses were performed using Cox proportional hazards model and a logistic regression model for radical surgery. Values of $P < .05$ were considered statistically significant. All statistical analyses were performed using GraphPad Prism version 4 software (GraphPad Software, San Diego, CA) and JMP Pro version 12.0 software (SAS Institute, Cary, NC).

3. Results

3.1. Patient characteristics and immunohistochemistry

Patient demographics based on LAT1 and 4F2hc expression are listed in Table 1. The median age was 69 years, ranging from 35 to 88 years. Seventy-nine patients (75%) were men, and 26 (25%) were women. Eighty-four patients (80%) were smokers, and 34 patients (32%) were diagnosed as having stage I cancer, 37 (35%) with stage II, 27 (26%) with stage

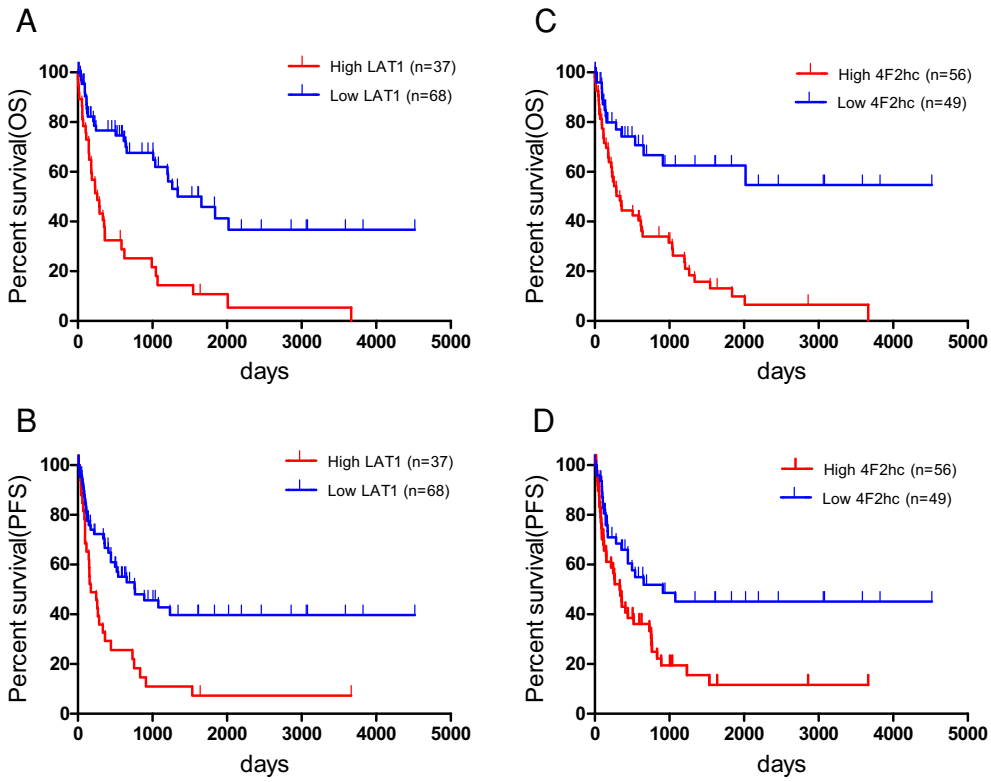

Fig. 3 Kaplan-Meier survival curves for patients with a high and low expression of LAT1 and 4F2hc. The 1-year OS and DFS rates of the LAT1-high and LAT1-low patients were 32% and 76%, respectively ($P < .01$; A), and 29% and 68%, respectively ($P < .01$; B). The 1-year OS and DFS rates of the 4F2hc-high and 4F2hc-low patients were 43% and 81%, respectively ($P < .01$; C), and 41% and 67%, respectively ($P < .01$; D).

III, and 7 (7%) with stage IV. All patients were diagnosed as having resected primary tumors. In the histologic analysis, 29 patients with PPC had a combination of carcinomatous and sarcomatous components. In the remaining 76 primary tumors, carcinomatous components were identified in 48 patients with adenocarcinoma (AC), 13 patients with squamous cell carcinoma, 8 patients with adenosquamous cell carcinoma, 2 patients with poorly differentiated carcinoma, and 5 patients with large cell carcinoma. Of the sarcomatous components, 69 patients exhibited spindle cell type; 10 patients, giant cell type; 13 patients, both spindle cell and giant cell types; and 13 patients, other types. The day of surgery was considered as the start day for measuring postoperative survival. The median follow-up period was 476 days (range, 30-4519 days).

Immunohistochemical examination was performed using 105 primary sites of PPC. The representative figures of LAT1 and 4F2hc are listed in Fig. 1. LAT1 immunostaining was detected in the PPC cells, localized predominantly on the plasma membrane. All positive cells displayed strong membranous immunostaining, whereas cytoplasmic staining was rare. The rates of high expression and average scores for LAT1 were 35% (37/105) and 53% (56/105; $P < .01$), and those for 4F2hc were 2.7 ± 1.2 and 3.3 ± 0.9 ($P < .01$), respectively, in all patients. Fig. 2 shows the comparison between LAT1 and 4F2hc expression according to score, demonstrating that 4F2hc expression was significantly higher than LAT1. In the analysis according to the epithelial components, the rates of higher expression of LAT1 and 4F2hc were 25% (12/48) and 48% (23/48; $P = .02$), respectively, in patients with AC, and 44% (25/57) and 58% (33/57; $P = .18$), respectively, in patients with non-AC. The frequency of high LAT1 expression was statistically higher in patients with a non-AC component than in those with an AC component ($P < .01$). In contrast, there was no significant difference in the rate of high 4F2hc expression in patients with non-AC and AC components ($P = .33$). The Ki-67 labeling index averaged 30% ± 21% (median, 31%), ranging from 1% to 89% in all patients. The Ki-67 labeling index was 28% ± 22% (median, 28%) in patients with AC component and 33% ± 21% (median, 31%) in those with non-AC component, without a statistical significance. The high expression of Ki-67 labeling index was observed in 47% (50/105).

High expression of LAT1 was significantly linked to the advanced stage, lymphatic permeation, tumor cell proliferation, and 4F2hc expression, and high expression of 4F2hc exhibited a significant relationship with T factor and advanced stage (Table 1). Moreover, in all patients, Spearman rank test revealed that LAT1 expression was significantly correlated

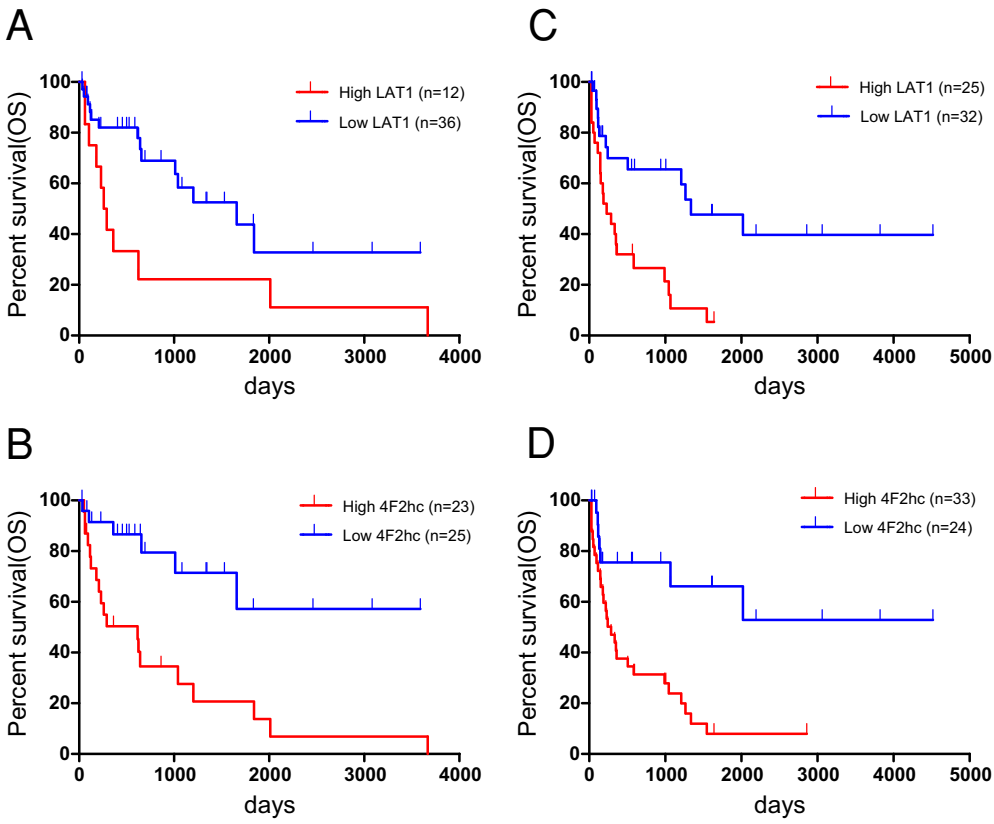

Fig. 4 Kaplan-Meier survival curves for patients with AC and non-AC component according to the expression of LAT1 and 4F2hc. The 1-year OS rates of the LAT1-high and LAT1-low patients with AC and non-AC component were 33% and 82%, respectively ($P < .01$; A), and 32% and 69%, respectively ($P < .01$; C). The 1-year OS rates of the 4F2hc-high and 4F2hc-low patients with AC and non-AC component were 50% and 86%, respectively ($P < .01$; B), and 41% and 67%, respectively ($P < .01$; D).

with 4F2hc ($r = 0.35$, $P < .01$) and Ki-67 labeling index ($r = 0.49$, $P < .01$). A significant correlation between LAT1 and 4F2hc was identified in patients with non-AC component ($r = 0.39$, $P < .01$) but not AC component ($r = 0.26$, $P = .07$). Moreover, the expression level of LAT1 significantly correlated with Ki-67 labeling index in patients with AC component ($r = 0.65$, $P < .01$) and in those with non-AC component ($r = 0.34$, $P < .01$).

3.2. Survival analysis

The median DFS and OS rates for all patients were 443 and 991 days, respectively. The median DFS and OS rates for patients with AC and non-AC components were 522 and 1038 days, and 336 and 507 days, respectively. Of the 105 patients, 60 died after initial surgery. Univariate and multivariate analyses were performed in all patients (Table 2). By univariate analysis, disease stage, LAT1, and 4F2hc were identified to be significant prognostic markers for OS; significant predictors for DFS were disease stage, pleural involvement, LAT1, and 4F2hc (Table 2). According to the results of the univariate log-rank test, we screened variables with a cutoff of $P < .05$. Multivariate analysis confirmed that disease stage, LAT1, and 4F2hc were independent prognostic factors to predict a worse OS; significant prognostic markers for DFS were disease stage, pleural involvement, and LAT1 expression (Table 2). Fig. 3 shows the Kaplan-Meier survival curves for patients with high and low expression of LAT1 and 4F2hc.

Next, the survival analyses according to histologic types were performed. We identified high expressions of LAT1 and 4F2hc to be significant prognostic markers for predicting a negative outcome in patients with AC and non-AC components (Fig. 4). Moreover, we analyzed the prognostic significance of LAT1 and 4F2hc expression according to each variable, including age, sex, and disease stage. We confirmed that the expressions of LAT1 and 4F2hc were significant prognostic predictors (Supplementary Table A1, online only).

4. Discussion

This is the first study to evaluate the clinicopathological significance of amino acid transporters such as LAT1 and 4F2hc in patients with surgically resected PPC. Our results indicated that high expression levels of LAT1 and 4F2hc can be used as independent prognostic variables for predicting a worse outcome after surgical resection. Regarding the relationship between other variables, LAT1 was closely associated with advanced stage, lymphatic invasion, tumor cell proliferation determined by Ki-67 labeling index, and the expression level of 4F2hc. Although LAT1 was expressed at significantly lower rates in patients with an AC component (25%) than in those with non-AC (44%), LAT1 seemed to play a crucial role in the survival of patients with PPC regardless of the histologic type. We confirmed that the expression level of 4F2hc was higher than that of LAT1, and it was identified as a significant prognostic predictor. PPC is recognized as a rare tumor with a dismal outcome, and little is known about any promising targets of this disease; however, LAT1 may be an attractive target for patients with PPC.

Recently, the frequency of LAT1 expression has been elucidated in thoracic neoplasms, such as NSCLC, pulmonary neuroendocrine tumor, malignant pleural mesothelioma, and thymic epithelial tumors [11,20-22]. Positive expression rates of LAT1 were identified in 51% of 321 NSCLCs, 46.2% of 13 small cell lung cancers, 52.4% of 21 large cell neuroendocrine carcinomas, 75% of 8 thymic carcinomas, and 50% of 21 malignant pleural mesotheliomas. In these previous studies, a score of 3 was assigned to indicate high expression of LAT1; however, in the present study, high expression of LAT1 was indicated by a score of 4, signifying that our study used a different scoring system. When we assessed LAT1 expression by using a score of 3 in PPC patients to compare our data with the results of previous studies, LAT1 was expressed in 57% of 105 PPCs, 46% of 48 PPCs with an AC component and 67% of 57 PPCs with a non-AC component. The rate of LAT1 expression according to histologic type of lung cancer has been elucidated; LAT1 was expressed in 91% of squamous cell carcinomas, 29% of ACs, and 67% of large cell carcinomas [11]. Moreover, Koshi et al [15] reported the expression of LAT1 in soft and tissue sarcoma. We also confirmed that LAT1 is positively expressed in sarcomatous tumor components.

4F2hc is required for the functional expression of LAT1 in tumor cells. Previous studies have shown the prognostic significance of 4F2hc expression in several human neoplasms [16,18,23]. Specifically, the expression of 4F2hc, rather than LAT1, seems to be necessary for tumor progression and metastasis of advanced human neoplasms [16,18]. A recent study documented that LAT1 and 4F2hc are positively expressed in 85.7% and 82.8% of advanced head and neck cancers, respectively, and that 4F2hc, but not LAT1, could a novel prognostic predictor [18]. Moreover, it has been reported that 4F2hc was identified as an independent predictor in advanced NSCLC with lymph node metastases [16]. Shimizu et al [24] described that the rates of high expression of LAT1 and 4F2hc were 56% and 79% in highly aggressive neoplasm such as cutaneous angiosarcoma, respectively, and that 4F2hc was closely associated with prognosis. In the present study, 4F2hc was expressed at higher rates than LAT1 in patients with PPC and was proven to be a significant prognostic predictor correlating with the expression of LAT1.

According to experimental studies, the inhibition of LAT1 was proven to suppress tumor progression and to contribute to apoptosis and G_1 arrest [25,26]. Recently, we described that the inhibition of LAT1 reduced the level of phosphorylation of the mammalian target of rapamycin [14]. In Japan, JPH203, a newly developed LAT1 inhibitor, has been administered to patients with any cancer as part of a phase I clinical trial. Further investigation is warranted to assess whether LAT1 inhibition is effective for the restriction of PPC tumor cell growth.

There are several limitations in this study. First, although our samples were collected from multiple institutions, the number of patients was too low to confirm our results statistically. Because of the rarity of PPC, we were unable to validate our results by using another cohort. A similar study should be performed using a validation cohort. Second, it continues to be unclear whether the inhibition of LAT1 within PPC cells could be a useful target for the decrease of tumor growth. From the results of the current study, it is clear that LAT1 is closely associated with the survival, metastasis, and tumor invasiveness of PPC, but the possibility of LAT1 inhibition as a chemotherapeutic agent is unclear. Further investigations should focus on the inhibition of LAT1 expression in PPC using in vitro and in vivo studies.

In conclusion, expression of the LAT1 amino acid transporter was proven to be an independent factor to predict poor outcome after surgical resection in patients with PPC. LAT1 was highly expressed in PPC and closely associated with 4F2hc expression, tumor cell proliferation, disease stage, and lymphatic invasion. The inhibition of LAT1 may provide a potentially promising target for the treatment of PPC in the future.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.humpath.2018.09.020.

Acknowledgments

We thank Dr Takayuki Kosaka, Dr Toshiki Yajima, and Dr Yoichi Ohtaki in Gunma University Hospital for their collection of materials and patient's treatment, and Ms Mai Yagame, Dr Bolag Altan, and Dr Erkhem-Ochir Bilguun in Gunma University Hospital for excellent technical assistant.

References

[1] Chang YL, Lee YC, Shih JY, et al. Pulmonary pleomorphic (spindle) cell carcinoma: peculiar clinicopathologic manifestations different from ordinary non–small cell carcinoma. Lung Cancer 2001;34:91-7.
[2] Kerr KM, Pelosi G, Austin JHM, et al. Pleomorphic, spindle cell, and giant cell carcinoma. In: Travis WD, Brambilla E, Burke AP, Marx A, Nicholson AG, editors. WHO Classification of Tumours of the Lung, Pleura, Thymus and Heart. Lyon: IARC Press; 2015. p. p88-90.
[3] Fishback NF, Travis WD, Moran CA, et al. Pleomorphic (spindle/giant cell) carcinoma of the lung. A clinicopathologic correlation of 78 cases. Cancer 1994;15:2936-45.
[4] Kaira K, Horie Y, Ayabe E, et al. Pulmonary pleomorphic carcinoma: a clinicopathological study including EGFR mutation analysis. J Thorac Oncol 2010;5:460-5.
[5] Kaira K, Endo M, Abe M, et al. Biologic correlates of ^{18}F-FDG uptake on PET in pulmonary pleomorphic carcinoma. Lung Cancer 2011;71:144-50.
[6] Bae HM, Min HS, Lee SH, et al. Palliative chemotherapy for pulmonary pleomorphic carcinoma. Lung Cancer 2007;58:112-5.
[7] McGivan JD, Pastor-Anglada M. Regulatory and molecular aspects of mammalian amino acid transport. Biochem J 1994;299:321-34.
[8] Oxender DL, Christensen HN. Evidence for two types of mediation of neutral amino acid transport in Ehrlich cells. Nature 1963;197:765-7.
[9] Kanai Y, Segawa H, Miyamoto K, et al. Expression cloning and characterization of a transporter for large neutral amino acids activated by the heavy chain of 4F2 antigen (CD98). J Biol Chem 1998;273:23629-32.
[10] Yanagida O, Kanai Y, Chairoungdua A, et al. Human L-type amino acid transporter 1 (LAT 1): characterization of function and expression in tumor cell lines. Biochim Biophys Acta 2001;1514:291-302.
[11] Kaira K, Oriuchi N, Imai H, et al. Prognostic significance of L-type amino acid transporter 1 expression in resectable stage I-III nonsmall cell lung cancer. Br J Cancer 2008;98:742-8.
[12] Kaira K, Oriuchi N, Otani Y, et al. Fluorine-18-alpha-methyltyrosine positron emission tomography for diagnosis and staging of lung cancer: a clinicopathologic study. Clin Cancer Res 2007;13:6369-78.
[13] Fuchs BC, Bode BP. Amino acid transporters ASCT2 and LAT1 in cancer: partners in crime? Semin Cancer Biol 2006;15:254-66.
[14] Imai H, Kaira K, Oriuchi N, et al. Inhibition of L-type amino acid transporter 1 has antitumor activity in non–small cell lung cancer. Anticancer Res 2010;30:4819-28.
[15] Koshi H, Sano T, Handa T, et al. L-type amino acid transporter-1 and CD98 expression in bone and soft tissue tumors. Pathol Int 2015;65:460-7.
[16] Kaira K, Oriuchi N, Imai H, et al. CD98 expression is associated with poor prognosis in resected non–small-cell lung cancer with lymph node metastases. Ann Surg Oncol 2009;16:3473-81.
[17] Kaira K, Takahashi T, Abe M, et al. CD98 expression is associated with the grade of malignancy in thymic epithelial tumors. Oncol Rep 2010;24:861-7.
[18] Toyoda M, Kaira K, Shino M, et al. CD98 as a novel prognostic indicator for patients with stage III/IVhypopharyngeal squamous cell carcinoma. Head Neck 2015;37:1569-74.
[19] Kaira K, Ohde Y, Endo M, et al. Expression of 4F2hc (CD98) in pulmonary neuroendocrine tumors. Oncol Rep 2011;26:931-7.
[20] Kaira K, Oriuchi N, Takahashi T, et al. L-type amino acid transporter 1 (LAT1) expression in malignant pleural mesothelioma. Anticancer Res 2011;31:4075-82.
[21] Kaira K, Oriuchi N, Imai H, et al. Expression of L-type amino acid transporter 1 (LAT1) in neuroendocrine tumors of the lung. Pathol Res Pract 2008;204:553-61.
[22] Kaira K, Oriuchi N, Imai H, et al. L-type amino acid transporter 1 (LAT1) is frequently expressed in thymic carcinomas but is absent in thymomas. J Surg Oncol 2009;99:433-8.
[23] Cantor JM, Ginsberg MH. CD98 at the crossroads of adaptive immunity and cancer. J Cell Sci 2012;125:1373-82.
[24] Shimizu K, Kaira K, Okubo Y, et al. Prognostic impact of LAT1 and CD98 expression in cutaneous angiosarcoma. Neoplasma 2017;64:283-8.
[25] Kim SG, Kim HH, Kim HK, et al. Differential expression and functional characterization of system L amino acid transporters in human normal osteoblast cells and osteogenic sarcoma cells. Anticancer Res 2006;26:1986-96.
[26] Kim CH, Park KJ, Park JR, et al. The RNA interference of amino acid transporter LAT1 inhibits the growth of KB human oral cancer cells. Anticancer Res 2006;26:2943-8.