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Prognostic impact of LAT1 and CD98 expression in cutaneous angiosarcoma

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L-type amino acid transporter 1 (LAT1) and CD98 are frequently expressed in various human cancers, and closely correlated with tumor aggressiveness and survival. However, little is known about the expression of LAT1 and CD98 in cutaneous angiosarcoma. The aim of this study is to investigate the clinicopathological significance of these markers in the dismal disease. A total of 52 patients with cutaneous angiosarcoma were retrospectively reviewed. Immunohistochemical staining of tumor specimens were evaluated using anti-LAT1, CD98 and Ki-67 antibodies. The rates of high expression for LAT1 and CD98 were 56% (29/52) and 79% (41/52), respectively. The frequency of high expression for CD98 was significantly higher than that for LAT1 (p=0.021). The low expression of CD98 was significantly associated with distant metastasis (p=0.044) and was identified as a significant prognostic predictor by multivariate analysis. The expression level of LAT1 was not significantly correlated with prognosis. The low expression of CD98 is a novel biomarker for predicting poor prognosis in patients with cutaneous angiosarcoma.

Key words: LAT1, CD98, skin cancer

Cutaneous angiosarcoma (CA) is an extremely rare disease, comprising less than 2% of all soft-tissue sarcomas [1]. This tumor originates from vascular endothelial cells anywhere in the body, with the head and neck being the most prevalent sites. The 5-year survival rate of CA is reported to be between 10 and 30% [2, 3]. In previous clinicopathological studies, there were fewer patients with CA than with other soft-tissue sarcomas. Chemotherapy, radiation therapy, or a combination of both comprises the treatment options for CA patients with distant metastasis or recurrent disease. However, the clinical benefit of these treatments is unsatisfactory. Therefore, it is necessary to discover appropriate biomarkers that can predict therapeutic efficacy and prognosis in CA.

Amino acid transporters are important for the proliferation and growth of neoplastic cells, as well as of normal cells [4, 5]. L-type amino acid transporter 1 (LAT1) is one of the L-type amino acid transporters, and transports large neutral amino acids such as leucine, isoleucine, valine, phenylalanine, tyrosine, tryptophan, methionine, and histidine [6, 7]. LAT1 requires a covalent association with the heavy chain of the 4F cell-surface antigen (CD98), for its functional expression in the plasma membrane [6, 7]. Previous studies have reported that LAT1 and CD98 are expressed in many human cancers, and are closely correlated with tumor cell proliferation, angiogenesis, and survival [8-12]. The clinicopathological significance of LAT1 and CD98 expression in human epithelial tumors has been highlighted, but little is known about their expression in soft tissue tumors. Recently, Koshi et al immunohistochemically examined LAT1 and CD98 expression in 226 surgically-resected tumor tissues of bone and soft tissue origin [13], and reported that LAT1 and CD98 were positively expressed in 35% and 42% of these, respectively. They investigated the clinicopathological significance of these markers, using 4 samples of angiosarcoma; however, they did not draw any clear results regarding the expression of LAT1 and CD98.

On the basis of this background, we conducted an immunohistochemical study to elucidate the clinical and prognostic significance of CD98 and LAT1 expression in CA.

Patients and methods

Patients. We retrospectively examined data from 52 consecutive patients who were definitively diagnosed with CA, at Gunma University Hospital and University of the Ryukyus Hospital, between October 1987 and September 2014. We obtained 52 paraffin-embedded tissue samples (resected as biopsy or as surgical resection), and medical records from Gunma University Hospital and University of the Ryukyus Hospital. This study was approved by the Institutional Review Board of Gunma University Hospital and University of the Ryukyus Hospital (ethics committee for clinical studies). The author's approach to the evaluation and resection of these tumors has been described previously [14]. To date, there has been no established clinical tumor staging for CA, related to prognosis. On the basis of a previous study [15], we classified CA patients into 3 stages: stage 1, for those with cutaneous local tumors; stage 2, for those with lymph node metastases; and, stage 3, for those with distant metastases.

Immunohistochemical staining. LAT1 expression was determined by immunohistochemical staining with an anti-human LAT1 antibody (1.6 mg/mL, anti-human monoclonal mouse IgG1, KM3149, provided by Kyowa Hakko Co., Ltd.; dilution of 1:800) (12). The anti-CD98 antibody used in this study is an affinity-purified, rabbit polyclonal antibody (Santa Cruz Biotechnology, Inc. 1:100 dilution), raised against a peptide mapped at the carboxy-terminus of human CD98. This antibody reacts with the antigen 4F2hc (SLC3A2/4F2hc/CD98hc). The detailed protocol for immunostaining was obtained from previously published reports [10, 16]. The expression of LAT1 and CD98 was assessed by the extent of staining as follows: score 1, ≤10% of tumor area stained; score 2, 11-25% stained; score 3, 26-50% stained; and score 4, ≥51% stained. The tumors scored as 1 or 2 were defined as low expression, and 3 or 4 as high expression.

For Ki-67, immunohistochemical staining was performed according to the procedures described in previous reports (10, 16). An anti-Ki-67 antibody (Dako, Glostrup, Denmark, 1:40 dilution) was used for staining. A highly cellular area of the immunostained sections was evaluated. Tumor cells with nuclear staining of any intensity were considered positive. 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-expressing cells.

The sections were assessed using light microscopy. This assessment was conducted as a blinded analysis, independently, by at least two of the authors. In case of any discrepancies, both investigators evaluated the slides simultaneously, until reaching a consensus on their final assessment. Neither of the investigators had any knowledge of the patient outcomes.

Statistical analysis. Probability values of <0.05 indicated a statistically significant difference. The significance of difference was determined by Fisher's exact test. The correlation between different variables was analyzed using the non-parametric Spearman's rank correlation 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 determined as the time from definite diagnosis to death from any cause. The progression-free survival (PFS) was defined as the time from definite diagnosis to the first sign of disease progression. Multivariate analyses were performed using the stepwise Cox proportional hazards model to identify independent prognostic factors. Statistical analysis was performed using GraphPad Prism 4 software (GraphPad Software, San Diego, CA, USA) and JMP 8 (SAS, Institute Inc., Cary, NC, USA) for Windows.

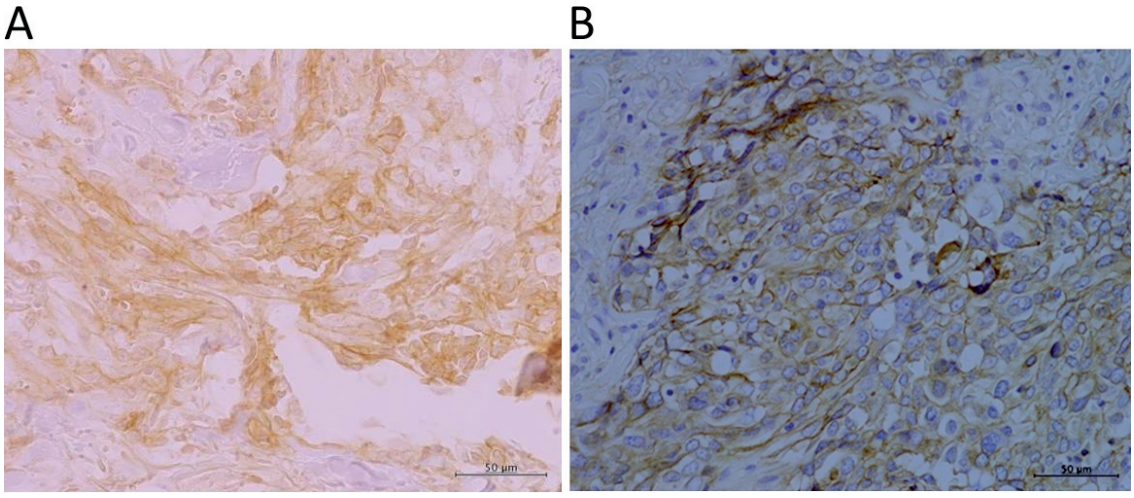

Figure 1. Immunohistochemical staining of LAT1 (A) and CD98 (B) in patients with CA. The scoring of LAT1 and CD98 were 4 and 4, respectively. The immunostaining pattern of these markers showed that they localized to the plasma membrane.

Results

Immunohistochemical evaluation. Immunohistochemical staining was performed on 52 primary site specimens of CA. Figure 1 shows the representative pictures of LAT1 and CD98 expression. LAT1 and CD98 localized predominantly to the plasma membrane in the CA cells. The rates of high expression and average scores for LAT1 and CD98 were 56% (29/52) and 79% (41/52), and 2.4 ± 1.1 and 2.9 ± 0.9, respectively. The frequency of high expression for CD98 was significantly greater than that for LAT1 (p=0.021). The percentages for scores of 1, 2, 3, and 4 for CD98 expression were 13% (7/52), 8% (4/52), 58% (30/52), and 21% (11/52), respectively; and those for scores of 1, 2, 3, and 4 for LAT1 expression were 29% (15/52), 15% (8/52), 41% (21/52), and 15% (8/52), respectively. Based on the results of analysis of tumor tissues, cut-off value for Ki-67 labeling was determined as follows: the median Ki-67 labeling index was 9% (range, 0-51), and this was chosen as the cut-off value. High Ki-67 expression was observed in 50% (26/52) of tumor tissues.

Table 1 shows the patient demographics according to the expression levels of LAT1 and CD98. The high expression of LAT1 was significantly associated with that of CD98, but there was no significant relationship between LAT1 expression and the other variables. The low expression of CD98 exhibited significant association with distant metastasis.

Correlation between LAT1 or CD98 expression and different markers. Spearman's rank correlation test revealed that the expression levels of LAT1 were significantly correlated with those of CD98; but CD98 exhibited no significant correlation with Ki-67 and with tumor size (Table 2).

Univariate and multivariate survival analysis. Forty-one patients died, and 40 developed recurrence of CA after initial treatment. In all patients, the median survival time (MST) and 1-year survival rate for OS and PFS, respectively, were 448 days and 55%; and 270 days and 37%. The results of survival analysis in all patients are listed in Table 3. Univariate analysis demonstrated that the low CD98 expression is a significant prognostic factor for OS. Significant prognostic factors for PFS were CD98 expression, gender, and clinical stage. Multivariate analysis confirmed that low CD98 expression was an independent factor for predicting poor OS and PFS. Clinical stage was also identified as an independent prognostic variable. Figure 2 shows the Kaplan-Meier curves

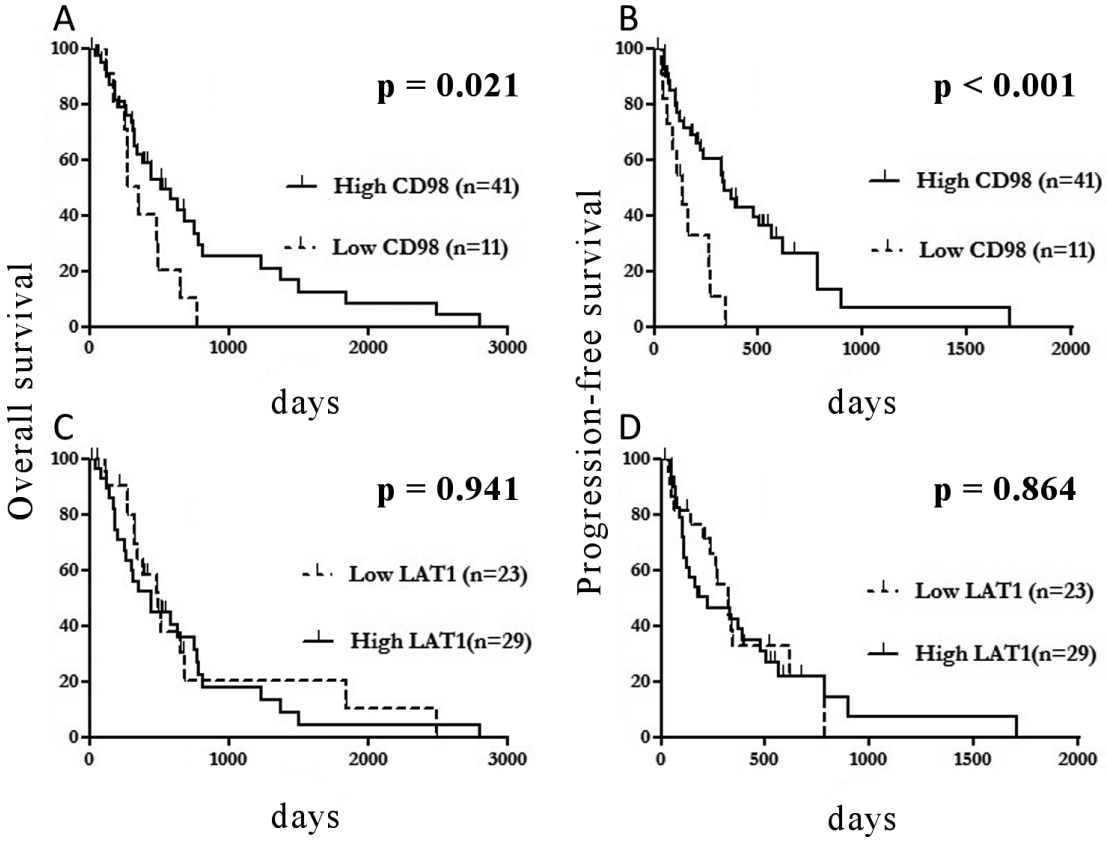

Figure 2. Kaplan Meier curves of overall survival (OS) and progression–free survival (PFS), according to the expression levels of LAT1 and CD98. A statistically significant difference in OS (A) and PFS (B) was recognized between patients with high and low CD98 expression, but not in patients with high and low LAT1 expression (C and D).

for the patients with high or low expression of LAT1 and CD98, respectively. Significant differences in the OS and PFS were observed with respect to the expression of CD98, but not of LAT1.

Discussion

There has been only one study investigating the expression of LAT1 and CD98 in sarcomas [13]. It reported that CD98 expression was higher than that of LAT1 in some bone tumors (67% vs. 47%, $p<0.006$); however, the percentage of CD98 expression was lower in soft tissue tumors than in bone tumors (28% vs. 67%). From 150 patients with soft tissue tumors, only 4 angiosarcoma specimens were included. LAT1 was positively expressed in all of them (4/4), but CD98 in none (0/4). This result is quite different from our results. As CA is not included in these soft tissue tumors, the clinicopathological roles of amino acid transporters may be different between angiosarcomas arising from skin and from other organs. The novelty of our study focus on the prognostic significance of CD98 expression as a heavy chain of LAT1 and our study indicates that not LAT1 but CD98 plays a crucial role in the tumor progression and survival of CA.

It is reported that LAT1 is highly expressed in various human cancers. Its expression levels are different in various tissues. The frequency of LAT1 expression is relatively low in adenocarcinomas: 29% in pulmonary adenocarcinoma [8)] 22% in prostate adenocarcinoma [12], 43% in breast adenocarcinoma [9], 43% in gastric adenocarcinoma [16], and 53% in pancreatic adenocarcinoma [17]. On the other hand, squamous cell carcinomas (SCC) exhibited high expression rates of LAT1: 90% in pulmonary SCC, 61% in head and neck SCC, and 86% in esophageal SCC [8, 18, 19]. However, the specific mechanisms of differential expression of LAT1 in various histological types have not been elucidated. There are a few clinicopathological studies on LAT1 expression in skin tumors [20]. Recently, we reported that LAT1 was highly expressed in 58% of 128 patients with malignant melanoma, and was closely correlated with tumor cell proliferation, angiogenesis, and survival [21]. In the present study, high LAT1 expression was observed in 56% patients with CA, corresponding to that of malignant melanoma. Although the enhanced expression of LAT1 has been reported to be a significant prognostic predictor in various human neoplasms(16-21), we found that the LAT1 was not correlated with OS and PFS in patients with CA.

CD98 is composed of a type II single-pass transmembrane heavy-chain (CD98hc) of about 80-85 kDa that is disulfide-linked to a multi-pass light chain of about 40 kDa [21]. CD98 is highly expressed in many tumors, and significantly correlates with tumor aggressiveness and survival. The biological roles of CD98 are recognized to be closely related to the functions of amino acid transport, and integrin signaling, which promote metastasis and tumor growth [22]. In in vitro studies, CD98 promotes malignant transformation and growth of tumor cells, and CD98-dependent integrin signaling enhances adhesion and cellular proliferation, through PI3K/AKT and MAPK pathway [23]. Recent studies indicated that CD98, but not LAT1, plays a crucial role in tumor aggressiveness in advanced-stage cancer patients [24, 25]. In early-stage lung cancer patients, the enhanced LAT1 expression was more important as a significant prognostic predictor than that of CD98, and the frequency of high CD98 expression increased as the disease progressed [26]. No information is available about the detailed mechanism for a sequential role of CD98 expression in patients at early and advanced stages. The roles of amino acid transporters, including LAT1 and CD98, might be different between skin tumors and non-skin tumors [27, 28]. The present study indicated that the high expression of CD98 was more frequent in CA than in other human neoplasms. Therefore, CD98 may play a crucial role in the development of this disease. On the contrary, we found a significant association between the decreased CD98 expression and distant metastasis. At present, we cannot explain this discrepancy.

There are several limitations of our present study. Firstly, our sample size is small, and this may bias the results of our study. Since CA is quite rare, it may be difficult to conduct a large-scale study. Therefore, other studies using validation cohorts are required. Secondly, we could not explain the relationship between the low CD98 expression and worse prognosis in CA. In previous studies, high CD98 expression was identified as a significant prognostic predictor of negative outcomes in various human cancers. The function of CD98 expression may be differently regulated in CA and in epithelial tumors. It remains unclear about the sequential roles of CD98 expression in CA, including the expression of CD98 in metastatic lesions and the clinicopathological significance of CD98 mRNA expression level. In future prospective study, the correlation of CD98 mRNA/protein expression of primary and metastatic lesions with cancer progression is worth investigating.

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