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Original Article

L-type amino acid transporter 1 (LAT1) expression in lymph node metastasis of gastric carcinoma: Its correlation with size of metastatic lesion and Ki-67 labeling

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ABSTRACT

L-type amino acid transporter 1 (LAT1) is one of the major amino acid transporters. High levels of LAT1 expression have been reported in various tumors, which can act as a novel prognostic marker. Previously, we demonstrated that LAT1 is highly expressed in advanced gastric carcinoma with lymph node metastasis, and proposed that LAT1 is an independent prognostic factor in non-scirrhous gastric carcinoma. The aim of the present study was to investigate the relationship between LAT1 expression and the size of lymph node metastatic lesions in gastric carcinoma. LAT1 and Ki-67 expression was immunohistochemically analyzed in 64 cases of advanced gastric carcinoma with lymph node metastasis. LAT1 expression in the metastatic lymph nodes was correlated with that in the primary lesions. The high LAT1 expression group showed a larger size of metastatic lesion and a higher Ki-67 labeling index than the low LAT1 expression group. LAT1 expression had a weak association with Ki-67 labeling index and tumor diameter of lymph nodes. These results suggest that LAT1 expression is associated with disease progression in gastric carcinoma. We proposed that LAT1 could be a potential therapeutic target for gastric carcinoma cases with large lymph node metastasis.

Introduction

Amino acid transporters play important roles in cell survival and proliferation. L-type amino acid transporter 1 (LAT1) is one of the major Na^+-independent neutral amino acid transporters first identified by Kanai et al. [1]. In normal tissues, LAT1 is expressed in several organs such as kidney distal tubules, and mucosal epithelia of the colon, small intestine and esophagus [2]. High LAT1 expression is detected in various tumors, including lung cancer, glioblastoma, prostatic cancer, and pancreatic cancer [3–6]. LAT1 expression is correlated with cancer cell proliferation assessed by Ki-67 labeling index (LI). Therefore, LAT1 is considered as a tumor type amino acid transporter.

It has been reported that LAT1 is a novel biomarker for prognosis in prostatic, lung and pancreatic cancer [3,5,6]. In our previous study, we revealed that LAT1 is highly expressed in advanced gastric cancer [7]. LAT1 showed a significantly higher expression in cases with lymph node metastasis than in those without metastasis. In non-scirrhous gastric cancer, LAT1 expression in the primary lesion is an independent prognostic factor. These findings suggest that LAT1 expression is associated with progression of gastric cancer. However, its expression in metastatic lymph nodes has not yet been analyzed from the viewpoint of cancer cell proliferation.

The aim of this study was to examine LAT1 expression in lymph node metastatic lesions in gastric cancer, and to investigate the correlation between LAT1 expression and tumor progression and proliferation in the metastatic lymph nodes.

Materials and methods

Patients and samples

We enrolled 64 patients with advanced gastric cancer with lymph node metastasis surgically resected between 1993 and 2003

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at Kitasato University East Hospital, Japan. The lymph nodes classified as Group I regional lymph nodes according to the Japanese Classification of Gastric Carcinoma were evaluated [8], and the maximum diameter of the largest metastatic lesions in the lymph nodes was determined.

All of the resected stomach and metastatic lymph node tissues were fixed in 10% buffered formalin, routinely processed, and embedded in paraffin. Then, 4-μm-thick sections were cut and used for hematoxylin and eosin staining and immunohistochemistry.

Immunohistochemistry

Immunohistochemical staining was performed using 4-μm-thick sections of formalin-fixed, paraffin-embedded tissues as previously described [7]. Tissue sections were deparaffinized, and endogenous peroxidase was blocked with 1% hydrogen peroxide in methanol for 30 min. For LAT1 immunostaining, slides were placed in citrated buffer (pH 6.0) and heated for antigen retrieval using a microwave oven for 5 min. After retrieving antigenic reactivity, slides were incubated with primary antibodies, anti-LAT1 (2 μg/ml; J-Pharma, Yokohama, Japan) and anti-Ki-67 (1:100 dilution; Dako, Glostrup, Denmark) antibodies overnight at 4 °C. After incubation with peroxidase-labeled polymer (ChemMate EnVision Kit Dako) for 30 min, 3,3′-diaminobenzidine (DAB) was applied as the chromogen. Nuclei were counterstained with Mayer's hematoxylin.

In ten selected cases, double immunostaining (LAT1 and Ki-67) was performed to confirm each expression in the same carcinoma cells. Afterwards, LAT1 staining with DAB, sections were heated in a microwave oven for 15 min to facilitate antigen retrieval. Ki-67 antibody (1:50 dilution) was reacted with a mixture of $NiCl_2$–DAB as a different chromogen. Nuclei were counterstained with methyl green to facilitate histological assessment.

Evaluation of immunohistochemical staining

Evaluation of immunoreactivity for LAT1 was performed according to Sinicrope's method [9] with minor modifications [5]. Based on the immunointensity of the carcinoma cell membranes, four categories were defined as follows: intensity 0, no staining; 1, weakly or patchy positive; 2, moderate complete cell membrane staining; and 3, intense complete membrane staining. The highest LAT1 intensity was taken as the intensity score in each case. Representative intensity of LAT1 expression in carcinoma cells in the lymph nodes is shown in Fig. 1. LAT1-positive areas were also evaluated, expressed as a percentage of the whole carcinoma area, and classified as follows: 0, none; 1 (focal), 1–10%; 2 (partial), 11–30%; and 3 (diffuse), >30%. Immunoreactivity scores were calculated by multiplication of the values for the two parameters, intensity and area. Two pathologists (MI and TM) scored each case independently. Ki-67 positivity was assessed by counting at least 1000 cancer cells. In cases with <1000 metastatic cancer cells in a lymph node, all cancer cells were counted. Ki-67 LI was expressed as the percentage value of positive cells.

Statistical analysis

Comparisons between groups were conducted with the χ^2 test or Mann–Whitney *U* test, as appropriate. The statistical significance of differences between survival curves was tested by the log-rank test. Relations among LAT1 expression, Ki-67 LI, and maximum diameter of lymph node metastatic lesions were analyzed using the Spearman's rank correlation coefficient test. StatView software (Abacus Concepts, Berkeley, CA, USA) was used for all statistical analyses, and $P < 0.05$ was considered statistically significant.

Table 1
Clinicopathological features and distribution of LAT1 score in 64 gastric carcinoma cases with lymph node metastasis.

Age (years)	
Median (range)	60 (33–85)
Gender	
Male	37
Female	27
Primary histological grade	
Differentiated type	16
Undifferentiated type	48
Non-schirrhous carcinoma	25
Schirrhous carcinoma	23
Maximum diameter (metastatic lesions in the lymph node)	
Mean (range) (mm)	8 (1–23)
LAT1 score (0–9)	64
0	5
1	6
2	3
3	20
4	1
6	14
9	15

Results

Patients' characteristics

The patients' characteristics are shown in Table 1. The 64 patients with gastric carcinoma comprised 37 men and 27 women, aged 33–85 years, with a mean age of 60 years. The mean maximum tumor diameter in the lymph nodes was 8 mm. Regarding the histological type of the primary tumor, 16 cases were classified as differentiated and 48 were undifferentiated (including 23 scirrhous carcinomas).

Correlation of LAT1 expression and diameter of metastatic lesions

All cases were divided into high- and low-expression groups according to the LAT1 intensity and score of the lymph node metastatic lesions (intensity 0–2 as low and intensity 3 as high, score 0–4 as low and score 6–9 as high). The high LAT1 intensity group showed a significantly larger maximum diameter of metastatic tumor than the low LAT1 intensity group ($P = 0.0003$, Fig. 2A). The high LAT1 score group also showed a significantly larger maximum diameter of metastatic tumor than the low LAT1 score group ($P < 0.0001$, Fig. 2B). The distribution of LAT1 expression and the size of metastatic lesion was shown by scatter plots (Fig. 2C and D). These results indicate that there is a strong correlation between LAT1 expression and the maximum diameters of metastatic tumors (LAT1 intensity: $\rho = 0.449$, $P < 0.0001$, LAT1 score: $\rho = 0.647$, $P < 0.0001$). Further, we analyzed the relation between the LAT1 expression and number of metastatic lymph nodes (Fig. 2E and F), in which no significant correlation between LAT1 expression and number of metastatic lymph nodes was demonstrated. Therefore, it seems that the LAT1 expression in the metastatic lesion is associated with tumor growth in the metastatic lymph nodes, and less associated with tumor scattering to multiple lymph nodes.

Correlation between LAT1 and Ki-67 expression in the metastatic lesions

The high LAT1 score group had a higher Ki-67 LI than the low LAT1 score group (Fig. 2G). Weak correlation was observed between LAT1 score and Ki-67 LI ($\rho = 0.523$, $P < 0.0001$). In the double-stained sections, cancer cells did not always co-express LAT1 and Ki-67 (Fig. 1E).