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Original

Positive Relationship between L-type Amino Acid Transporter 1 Expression and Liver Metastasis in T3 Colorectal Cancer

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Abstract : The aim of this retrospective study was to evaluate L-type amino acid transporter 1 (LAT1) expression in colorectal cancer with invasion to the subserosal layer (T3), its relationship with clinicopathological characteristics, and its potential metastatic significance. LAT1 expression was measured by immunohistochemistry in tumors from 65 patients with primary colorectal carcinomas. LAT1 expression was deemed positive when more than 10% of the tumor cells showed distinct membranous immunoreactivity. Positive LAT1 expression was demonstrated in 29.2% (19 of 65) of primary tumors. LAT1 expression showed no significant relationship with clinicopathological characteristics, such as age, gender, tumor location, tumor size, macroscopic/microscopic classification, or lymph node metastasis. However, LAT1 expression showed a positive relationship with liver metastasis ($P < 0.05$). LAT1 expression in cancer cells may be a good marker for predicting potential metastasis to the liver in colorectal cancer.

Key words : L-type amino acid transporter 1, colorectal cancer, liver metastasis

Introduction

Cancer-related death in colorectal cancer (CRC) is usually due to the development of distant metastases. Approximately 70% of all patients diagnosed with CRC undergo potentially curative surgery, but half of these patients present with, or develop, advanced local disease or metastases[1)]. Although several prognostic factors exist, including the clinical staging classification, a more specific recognition marker for CRC with high metastatic potential would provide useful information for evaluating adjuvant therapies. We have already reported a correlation between the immunohistochemical expression of CD44 and lymph node and liver metastases[2)], and more specific markers are required.

Amino acid transporters are essential for growth and proliferation in normal and trans-

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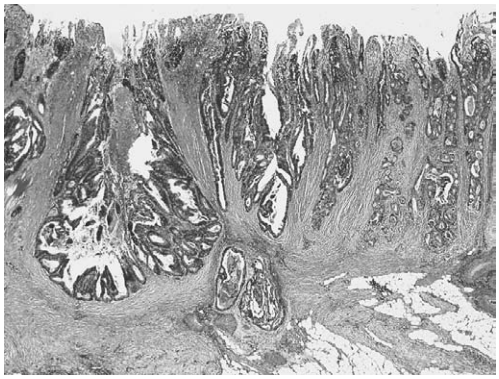

Fig. 1. Low power view of colorectal cancer (CRC).
Typical CRC with invasion to the subserosal layer (T3) is shown.
Hematoxylin and eosin staining; magnification ×1.25.

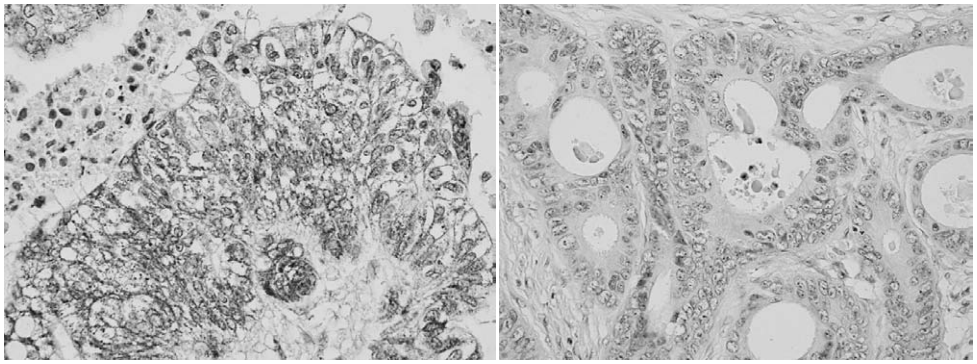

Fig. 2. Immunohistochemical staining for L-type amino acid transporter 1 (LAT1).
(A) More than 10% of the tumor cell membranes are stained and the tumor is graded as positive. Magnification ×400.
(B) Tumor cells are not stained and the tumor is graded as negative. Magnification ×400.

formed cells[3,4]. L-type amino acid transporter 1 (LAT1), one of the L-type amino acid transporters, transports large neutral amino acids such as leucine, isoleucine, valine, phenylalanine, tyrosine, tryptophan, methinine and histidine[5-7].

LAT1 expression is closely related to liver metastases in a rat tumor model[8], but little is known about this relationship in human primary digestive organ tumors[9]. The aim of the present study was to examine LAT1 expression in CRC with invasion to the subserosal layer (T3), and its relationship with potential liver metastases and associated clinicopathological characteristics. Because liver metastasis occurs more frequently in T3 than in T1 (invasion to the submucosal layer) or T2 (invasion to the muscular layer) tumors, it is important to clarify whether LAT1 expression in cancer cells would be a good marker for predicting potential liver metastasis in T3 CRC.

Materials and Methods

Tissue samples from 65 patients who had primary T3 CRC surgically resected at the Showa University Hospital between 2004 and 2007 were enrolled in this retrospective study. The non-necrotic portion of the tumor was processed using the standard paraffin wax technique after fixation in 10% formalin for 24 hours. Representative sections were stained with hematoxylin and eosin. T1, T2, and T4 (invasion to the serosa) tumors were not examined, because sufficient cases could not be collected for meaningful statistical analysis.

Immunohistochemistry

From representative blocks of each tissue sample, 3-μm thick sections were used for immunohistochemical staining with the monoclonal antibody clone 4D9 (anti-LAT1 ; Transgenic, Hyogo, Japan), at a dilution of 1 : 200. The streptavidin-biotin-peroxidase technique was used. Sections were deparaffinized in xylene and dehydrated in descending grades (100%-50%) of ethanol, and then subjected to antigen retrieval (microwave method using citrate buffer [pH 6.0], 25 minutes). After the slides had cooled to room temperature, they were incubated with 1% hydrogen peroxide in ethanol for 30 minutes to quench endogenous peroxidase activity. Nonspecific immunoreactivity was blocked in each section by incubation with normal donkey serum for 30 minutes. The sections were then incubated with primary antibody at 4° C overnight. After three, 5-minute washes with phosphate-buffered saline (PBS), the sections were incubated for 60 minutes with a multilink biotinylated anti-immunoglobulin (Dako, Kyoto, Japan). Sections were then washed with PBS three times for 5 minutes, before and after being treated with streptavidin-peroxidase reagent for 30 minutes. The reactions were visualized with diaminobenzidine (Dako) as the chromogen. All steps were followed by adequate washes in PBS. Finally, sections were counterstained with hematoxylin, dehydrated and mounted. LAT1 expression was considered positive only if distinct membranous immunoreactivity was present. The tumors in which immunoreactive tumor cells made up $>10\%$ of the tumor were graded as positive. This 10% cut-off value is widely used by many authors[9, 10]. All histological slides were examined by two experienced pathologists unaware of the clinical data or disease outcome.

Statistical analysis

The association of LAT1 expression with clinicopathological features, such as age, gender, tumor location, tumor size, macroscopic / microscopic classification, lymph node metastasis and liver metastasis[1], was examined using the χ^2 test as appropriate. $P<0.05$ was considered statistically significant.

Results

The characteristics of the study population are shown in Table 1. The subjects consisted

Table 1 LAT1 expression and clinicopathological parameters of CRC (n = 65)

		LAT1 expression, n (%)		
	n	−	+	P
Age (years)				
< 60	12	10 (83.3)	2 (16.7)	NS
≥ 60	53	36 (67.9)	17 (32.1)	
Gender				
Male	47	35 (74.5)	12 (25.5)	NS
Female	18	11 (61.1)	7 (38.9)	
Tumor location				
Colon	51	37 (72.5)	14 (27.5)	NS
Rectum	14	9 (64.3)	5 (35.7)	
Tumor size (cm)				
< 5.0	36	26 (72.2)	10 (27.8)	NS
≥ 5.0	29	20 (68.9)	9 (31.1)	
Macroscopic classification				
Type2	54	36 (66.7)	18 (33.3)	NS
Type3	11	10 (90.9)	1 (9.1)	
Microscopic classification				
Tub1	20	12 (60.0)	8 (40.0)	NS
Tub2 / por	45	34 (75.6)	11 (24.4)	
Lymph node metastasis (N)				
N0	30	19 (63.3)	11 (36.7)	NS
N1 / 2	35	27 (77.1)	8 (22.9)	
Liver metastasis (M)				
M0	49	38 (77.6)	11 (22.4)	$P < 0.05$
M1	16	8 (50.0)	8 (50.0)	

LAT1, L-type amino acid transporter 1; CRC, colorectal carcinoma; NS, not significant

of 47 men (72.3%) and 18 women (27.7%), with a mean age of 68.71 years (range, 29–90 years) and a mean tumor size of 4.5 cm (range, 1.2–9.5 cm).

LAT1 expression in the primary tumor was demonstrated in 29.3% (19 of 65) of patients. The relationships between tumor LAT1 expression and the clinicopathological parameters of CRC are also summarized in Table 1. LAT1 expression in the primary tumor was not significantly associated with age, gender, tumor location, tumor size, macroscopic classification, microscopic classification, or lymph node metastases. However there was a positive association between LAT1 expression and liver metastases, with 22.4% of M0 cases and 50.0% of M1 cases showing positive LAT1 expression in the primary tumor ($P < 0.05$; Table 1).

Discussion

LAT1 expression is associated with cancerous or proliferative cells, and LAT1 is suggested to be involved in the mechanism allowing continuous proliferation of tumor cells[6, 7].

Immunohistochemical studies have found LAT1 to be highly expressed in cultured cells and malignant tumors, such as oral squamous cell carcinomas[11], esophageal squamous cell carcinomas[12], gliomas[13], urothelial carcinoma of the upper urinary tract[14], and non-small cell lung cancers[15, 16]. The clinical significance of LAT1 expression has been fully investigated by Imai *et al*[10] and Kaira *et al*[15] in pulmonary cancers. They found a high lymphatic permeation and vascular invasion and a low five-year survival rate in LAT1-positive cases, and concluded that LAT1 expression is a significant factor in predicting a poor prognosis. Sakata and colleagues[17] also found a poor prognosis in LAT1-positive prostate cancer cases, and concluded that LAT1 was a novel biomarker for high-grade malignancy. However little is known about the clinical significance of LAT1 expression in digestive system cancers, especially CRC[9].

In rat colonic cancer models, LAT1 expression has been described as being associated with metastasis *in vivo*[8, 18]. When rat colon cancer cells (RCN-9) were injected into the spleen of rats, the size of the resultant metastatic liver tumors was directly correlated with LAT1 expression[8, 18]. These studies support our finding that LAT1 expression showed a significantly positive relationship with liver metastasis in patients with T3 CRC. There is only one previous report concerning LAT1 expression in human CRC. Kaira *et al*[9] examined LAT1, CD98 and Ki-67 expression in pulmonary metastases from colon cancer and other cancers, and reported that cancer cells in the metastatic foci showed higher LAT1 expression, indicating higher angiogenesis and cell proliferation, than those in the primary foci. In contrast to the report of Kaira *et al*[9], the present study showed a difference in LAT1 expression between cases with and without metastases, and revealed the clinical significance of LAT1 expression in CRC. However apart from liver metastasis, our results showed no relationship with any other clinical factor, such as lymph node metastasis. Although the reason is unknown, further studies are necessary to clarify the relationship between LAT1 expression and lymph node metastasis, because of its prognostic significance.

From a therapeutic point of view, reports about inhibitors of LAT1, such as L-leucine, 2-aminobicyclo-2(2,2,1)-heptane-2-carboxylic acid (BCH), melphalan and D-leucine, are increasing. L-leucine is reported to inhibit the growth of KB human oral cancer cells[19], while other studies conducted using a rat glioma model[20] and human neck cancer cell line[21] describe BCH as a specific inhibitor of LAT1, which inhibits the uptake of the essential amino acid, leucine. Furthermore, Shennan and Thomson[22] have described melphalan and D-leucine as inhibiting the growth of cultured breast cancer cells.

Thus, inhibitors of LAT1 may inhibit cancer cell growth, therefore a personalized selective therapy using specific inhibitors of LAT1 might have therapeutic potential for cancer patients with LAT1 expression[20-24].

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