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Close Correlation between Increased Sialyl-Lewis^x Expression and Metastasis in Human Gastric Carcinoma

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Abstract. Expression of sialyl-Lewis^x (sLe^x) antigen was studied immunohistochemically in 110 resected human gastric carcinomas using an anti-sLe^x monoclonal antibody. Lymph node, liver, and peritoneal metastases were clearly more prevalent in tumors expressing high levels of sLe^x than in those with no or low-level sLe^x expression. No correlation was found between sLe^x expression and histologic grade or histologic type of the Lauren classification. Among the tumors with lymph node metastasis, 44% expressed high levels of sLe^x in both the primary tumor and involved lymph nodes, and 14% of the metastatic lesions demonstrated increased sLe^x expression. The 5-year survival rate of the patients undergoing complete (R0) gastric resections was 60% in the sLe^x high-expression group, which was significantly lower than that of the sLe^x low-expression group (81%) and of the no-expression group (87%) ($p < 0.05$). These results suggest that high-level sLe^x expression is related to both an increased risk of metastasis and poor prognosis in gastric cancer patients.

The sialyl-Lewis^x (sLe^x) (Neu5Ac α2-3Gal β1-4 (Fuc α1-3) GlcNAc β1-R) carbohydrate antigen is expressed on the surface of human leukocytes [1, 2] and is involved in the attachment of leukocytes to cytokine-activated endothelial cells through its interaction with the E-selectin cell adhesion molecule [3–5]. The sLe^x antigen also is expressed by various human carcinomas [6–13]. It has been demonstrated that colorectal carcinoma cells that express high levels of sLe^x are more strongly adherent to activated endothelial cells and have a higher metastatic potential than tumors with low-level sLe^x expression [14], suggesting that sLe^x potentially mediates carcinoma cell–endothelial cell interactions in a manner similar to its involvement in leukocyte–endothelial cell interaction. Furthermore, it has been reported that increased sLe^x expression is related to poor prognosis in patients with colorectal and breast cancers [10, 15].

Gastric cancer remains the major cause of cancer death worldwide [16]. Currently, surgical treatment and adjuvant therapies cannot provide satisfactory outcomes for patients with distant metastases. The molecular mechanisms by which gastric carcinoma cells metastasize must be characterized to develop new therapeutic modalities. There have been no reports examining the clinical significance of sLe^x expression in gastric carcinomas. Therefore we immunohistochemically studied the correlation between sLe^x expression and clinicopathologic features in gastric carcinoma patients. We also characterized the influence of sLe^x on the prognosis of patients.

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Materials and Methods

Patients

A total of 110 patients with primary gastric cancer were studied. The 73 men and 37 women ranged in age from 31 to 81 years (57.3 ± 10.6, mean ± SD). All patients had undergone gastrectomy at the First Department of Surgery, Sapporo Medical University between January 1975 and December 1989. The surgical procedures for 80 patients were considered curative on clinical and pathologic grounds, whereas 30 procedures were regarded as noncurative because metastatic tumor remained in the liver, peritoneum, or both at surgery. All patients were followed until death or until the end of the observation period (December 31, 1995). Clinicopathologic features of gastric carcinomas were described in accordance with the TNM classification and the Lauren's category [17].

Immunohistochemical Staining

Surgically resected primary gastric carcinomas and their metastases were fixed in 10% buffered formalin, embedded in paraffin, and then cut into 4 μm sections. Sections were deparaffinized in xylene, washed with phosphate-buffered saline (PBS) three times for 5 minutes, and immersed in 1% hydrogen peroxide in methanol for 30 minutes to block endogenous peroxidase activity. Sections were then washed with PBS three times for 5 minutes and incubated with 30% normal bovine serum albumin (BSA) at room temperature for 60 minutes to minimize background staining. Slides from each tumor sample then were incubated overnight at room temperature with mouse anti-sialyl Lewis^x monoclonal antibody (sLe^x mAb) (Seikagaku, Tokyo, Japan). After rinsing in PBS, the slides were incubated with biotinylated antimouse immunoglobulin G (IgG) (Nichirei, Tokyo, Japan) for 60 minutes at room temperature, followed by incubation with a streptavidin–

biotin–peroxidase complex (Nichirei). Finally, immunoreactivity was visualized using 0.02% 3,3′-diaminobenzidine (Wako, Osaka, Japan). Negative controls prepared by substituting normal mouse serum for the primary antibody resulted in no detectable staining.

Evaluation of sLe^x mAb Immunoreactivity

Immunoreactivity was evaluated by complete examination of each section and was classified into three grades: −, no expression; +, expression less than 50% of all the carcinoma cells viewed under the microscope (low-level expression); ++, expression in more than 50% of the cells (high-level expression) (Fig. 1). Evaluations were performed independently by two investigators (H.U. and R.D.). In the event of disagreement, slides were additionally reviewed by a third observer (K.Y.), and a consensus was obtained.

Statistics

General group comparisons were made using the χ^2 test. Cumulative survival was calculated using the Kaplan-Meier approach. Additional comparisons were made by the generalized Wilcoxon test. Results were considered significant when the p value was less than 0.05.

Results

The incidences of lymph node, liver, and peritoneal metastases were clearly higher in tumors expressing high levels of sLe^x than in those with no or low-level sLe^x expression (Table 1). The proportion of tumors that penetrated the gastric wall or invaded adjacent structures was also higher in the high-level sLe^x expression group than in the low-level sLe^x expression group (Table 2). The incidence of lymph node metastasis according to the depth of invasion was still higher in the sLe^x high-expression group than in the sLe^x low-expression group (Table 3). A similar inclination was found with regard to liver and peritoneal metastases (data not shown). On the other hand, no correlation was found between sLe^x expression and histologic grade or the histologic type of the Lauren classification.

Of the tumors with node metastasis, 44% expressed high levels of sLe^x in both the primary tumor and the metastatic nodes, and 14% showed high-level sLe^x expression within the metastatic tumor despite no or low-level sLe^x expression in the primary tumor. The remaining metastatic tumors showed no or low-level sLe^x expression (Table 4). Samples of primary tumor and their corresponding metastases of 4 of 30 cases with liver metastases were available for study. All of the liver metastases expressed high levels of sLe^x, although one of four primary tumors showed low-level sLe^x expression (Fig. 2).

The 5-year survival of the patients undergoing complete (R0) gastric resections was 60% in the sLe^x high-expression group, 81% in the sLe^x low-expression group, and 87% in the no sLe^x expression group (Fig. 3). A significant difference was found between the former and latter two groups ($p < 0.05$).

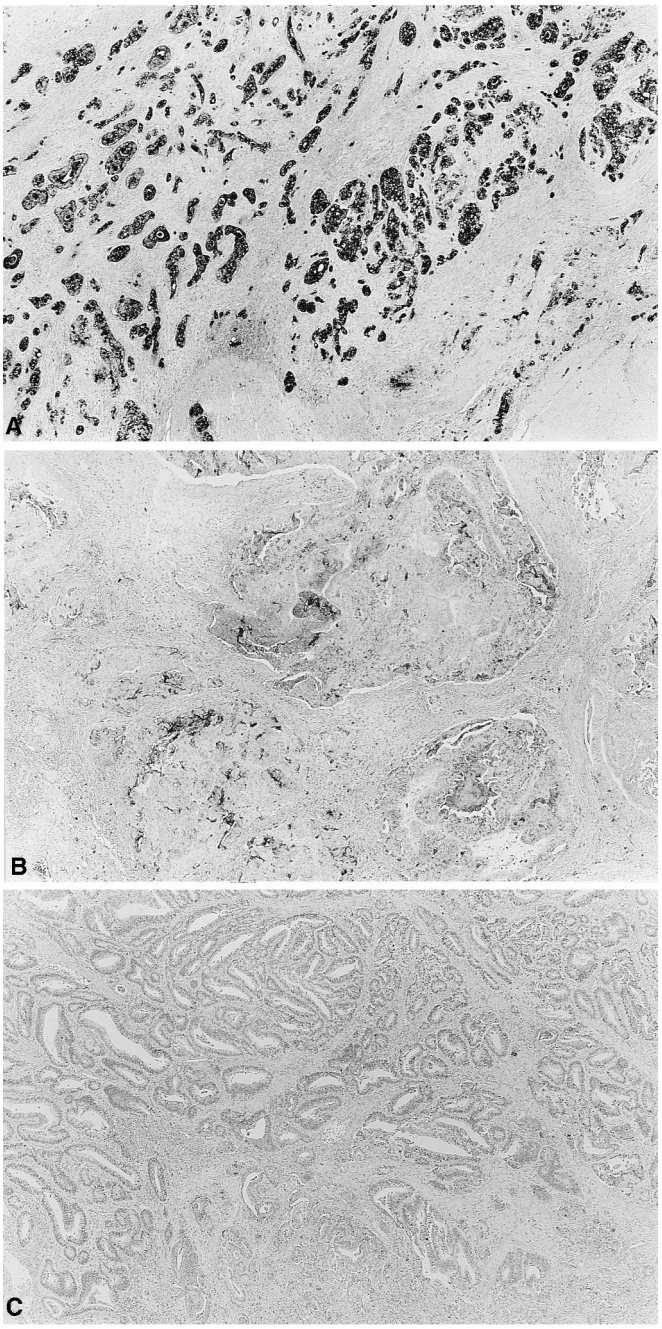

Fig. 1. Photomicrographs demonstrating the grades of sLe^x immunoreactivity: high-level expression (**A**), low-level expression (**B**), and no expression (**C**).

Discussion

Alteration of cell surface carbohydrate antigens during malignant transformation is a well known phenomenon in a variety of tumors [18, 19]. It has been reported that the expression of sLe^x by colorectal carcinoma cells increases during their progression from early disease to the metastatic stage [6, 15]. Our results support these findings. Specifically, sLe^x expression by gastric carcinoma cells correlated with advancing depth of invasion, and the incidence of tumor metastasis was significantly higher in tumors that expressed high levels of sLe^x than in those with no or low-level sLe^x expression. Furthermore, most of the tumors with high-level sLe^x expression also showed similar or greater levels of sLe^x

Table 1. Incidence of metastasis based on sLe^x expression in the primary tumor.

Grade of sLe^x expression	No.	Metastasis		
		Lymph node	Liver	Peritoneal
++	45	36 (80.0%)	18 (40.0%)	12 (26.7%)
+	46	21 (45.7%)	10 (21.7%)	4 (8.7%)
−	19	9 (47.4%)	2 (10.5%)	2 (10.5%)

$^*p < 0.05$.
$^{**}p < 0.005$.

Table 2. Relation between depth of invasion and sLe^x expression in the primary tumor.

Grade of sLe^x expression	No.	pT_1	pT_2	$pT_{3,4}$
++	45	15 (33.3%)	18 (40.0%)	12 (26.7%)
+	46	18 (39.1%)	24 (52.2%)	4 (8.7%)
−	19	8 (42.1%)	10 (52.6%)	1 (5.3%)

$^*p < 0.05$.

Table 3. Incidence of lymph node metastasis based on the depth of invasion and sLe^x expression in the primary tumor.

Grade of sLe^x expression	No.	pT_1	pT_2	$pT_{3,4}$
++	45	10/15 (66.7%)	16/18 (88.9%)	10/12 (83.3%)
+	46	8/18 (44.4%)	11/24 (45.8%)	2/4 (50.0%)
−	19	4/8 (50.0%)	5/10 (50.0%)	0/1 (0%)

$^*p < 0.05$.

Table 4. Comparison of sLe^x expression between primary tumor and metastatic lymph node.

Primary tumor	Metastatic lymph node	No. of patients (%)
++	++	29 (43.9)
	+	7 (10.6)
	−	
+	++	7 (10.6)
	+	10 (15.2)
	−	4 (6.1)
−	++	2 (3.0)
	+	2 (3.0)
	−	5 (7.6)
Total		66 (100)

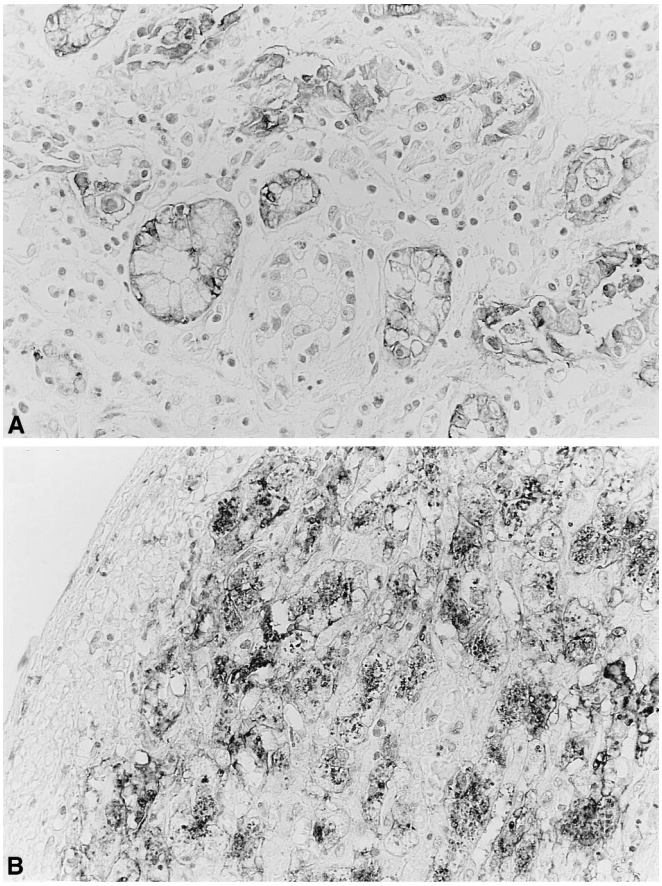

Fig. 2. Immunohistochemical staining of a moderately differentiated tubular adenocarcinoma using an anti-sLe^x antibody. **A**. Low-level expression of sLe^x in the primary tumor. **B**. Increased sLe^x expression in the liver metastasis. (×200)

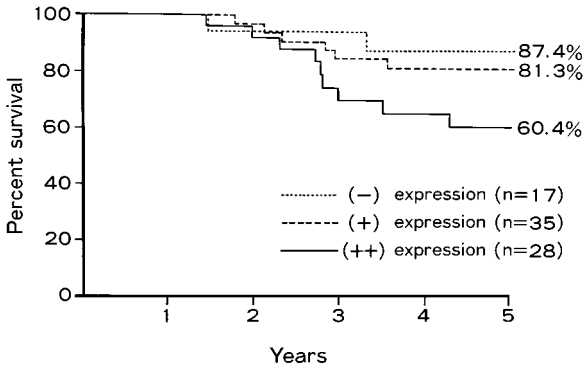

Fig. 3. Cumulative survival based on sLe^x expression by the primary tumor cells in 80 patients undergoing curative resection. There was a significant difference in survival between the high-level sLe^x expression group and the no or low-level sLe^x expression groups ($p < 0.05$).

expression in the metastatic foci. These results suggest that a portion of gastric cancer metastases may arise from a tumor cell subpopulation that expresses high levels of sLe^x. We found no correlation between sLe^x expression and the histologic grade or histologic type of the Lauren classification, which are generally related to the local invasiveness of tumors. These findings indicate that the aggressiveness of tumors with increased sLe^x expression may be correlated to alterations in the adhesion of tumor cells to endothelial cells of specific target organs, rather than in the process of local invasion.

It has been reported that the expression of sLe^x by carcinomas of the breast and colon are related to poor prognosis [10, 15]. In this study the prognosis of patients with gastric cancer was also poorer in the high-level sLe^x expression group than in the no or low-level sLe^x expression group. These results strongly suggest that increased sLe^x expression is related to poor prognosis through an increased risk of metastasis.

It is known that E-selectin is expressed on activated endothelial

cell surfaces and recognizes the sLe^x structure [3–5]. However, it is unclear if E-selectin is expressed by lymph vessel endothelium or peritoneal mesothelium. In this study we demonstrated that sLe^x expression was associated not only with vascular metastasis but also with lymphatic and peritoneal metastasis. If these metastases actually were promoted by the increased expression of sLe^x, the sLe^x antigen must have interacted with receptors present at these sites. We are currently studying the expression of E-selectin on lymphatic endothelial cells and peritoneal mesothelial cells to further clarify this hypothesis.

Résumé

On a étudié l'expression de l'antigène sialyl-Lewis^x (sLe^x) dans 110 pièces d'exérèse de cancer gastrique utilisant un anticorps anti-sLe^x monoclonal. On a noté plus de ganglions lymphatiques envahis, plus de métastases hépatiques et péritonéales en cas de tumeur exprimant des taux élevés de sLe^x qu 'en cas de tumeur ayant un taux d'expression bas. On n'a retrouvé aucune corrélation entre l'expression sLe^x et le grade ou le type histologique selon la classification de Lauren. Parmi les tumeurs accompagnées de métastases lymphatiques, 44% avaient une expression élevée de sLe^x à la fois dans la tumeur primitive et dans les ganglions alors que 14% des métastases avaient une expression sLe^x augmentée. La survie à 5 ans des patients ayant ou une résection gastrique complète (R0) a été de 60% dans le groupe à expression sLe^x élevée, significativement inférieure à celle du groupe à expression sLe^x basse (81%) ou celle du groupe sans aucune expression (87%) ($p < 0.05$). Ces résultats suggérent qu'en matière de cancer gastrique, l'expression élevée de sLe^x est en rapport avec un risque accru de métastases et un pronostic médiocre.

Resumen

Se hizo el estudio inmunohistoquímico de la expresión del antígeno de sialil-Lewisx (sLex) en 110 carcinomas gástricos resecados utilizando un anticuerpo monoclonal anti-sLex. Las metástasis ganglionares, hepáticas y peritoneales fueron claramente más prevalentes en los tumores con altos niveles de expresión de sLex, que en los tumores con ninguna o con baja expresión. No se halló correlación entre la expresión de sLex y el grado histológico, o el tipo histólico según la clasificación de Lauren. Entre los tumores con metástasis ganglionares, 44% exhibieron altos niveles de expresión de sLex tanto en el tumor primario como en los ganglios afectados, y 14% de las lesiones metastásicas demostraron una expresión de sLex aumentada. La tasa de sobrevida a cinco años de los pacientes sometidos a resección gástrica completa (R0) fue de 60% en el grupo con alta expresión de sLex, la cual es significativamente menor que la del grupo con baja expresión (81%) y que la del grupo sin expresión de sLex (87%) ($p < 0.05$). Estos resultados sugieren que en pacientes con cáncer gástrico, un alto nivel de expresión de sLex se relaciona tanto con un mayor riesgo de metástasis como con un mal pronóstico.

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