Expression of Lewis^a, sialyl Lewis^a, Lewis^x and sialyl Lewis^x antigens as prognostic factors in patients with colorectal cancer

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BACKGROUND: Altered expression of blood group-related carbohydrate antigens such as sialyl Lewis (Le)^x antigen in tumours is associated with tumour progression behaviour and subsequent prognosis. However, the prognostic value of the expression of Le-related antigens in colorectal tumours remains unclear.
PURPOSE: To clarify the prognostic value of Le^a, sialyl Le^a, Le^x and sialyl Le^x expression in colorectal carcinomas as prognostic factors after surgery.
PATIENTS AND METHODS: Colorectal carcinoma samples from 101 patients with primary colorectal carcinoma who underwent surgical resection were subject to immunohistochemical analyses for Le^a, sialyl Le^a, Le^x and sialyl Le^x expression with the respective monoclonal antibodies.
RESULTS: Le^a, sialyl Le^a, Le^x and sialyl Le^x were expressed in 69 (68.3%), 73 (72.3%), 66 (65.4%) and 76 (75.3%) carcinomas, respectively. The patients with sialyl Le^x-expressing tumours had more advanced cancer than those with nonsialyl Le^x-expressing tumours (P=0.0029). The survival time after surgery of patients with Le^x- or sialyl Le^x-expressing tumours was significantly shorter than the survival time of those with non-Le^x- or nonsialyl Le^x-expressing tumours, respectively (P=0.023 and P=0.0001, respectively). Cox's regression analysis revealed that Le^x and sialyl Le^x expression, separate from stage and histological type, were prognostic variables for patient survival (hazard ratio [HR] for sialyl Le^x-positive expression to sialyl Le^x-negative expression 2.90; HR for Le^x-positive expression to Le^x-negative expression 12.76 in stage I/IV, 0.63 in stage II and 1.69 in stage III).
CONCLUSIONS: Le^x expression and sialyl Le^x expression in colorectal carcinomas are each associated with poor prognosis. These variables should be considered in the design of future trials.

Key Words: *Colorectal cancer; Lewis antigen; Prognostic factor; Sialyl Lewis antigen*

Expression des antigènes Lewis^a, sialyl Lewis^a, Lewis^x, sialyl Lewis^x comme facteurs de pronostic chez les patients atteints d'un cancer du côlon et du rectum

CONTEXTE : Le changement d'expression des antigènes glucidiques sanguins comme l'antigène sialyl Lewis (Le)^x dans les tumeurs est associé à une progression de ces dernières et à un pronostic qui va de pair. Toutefois,

voir page suivante

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la valeur pronostique de l'expression des antigènes Le dans les tumeurs colo-rectales est incertaine.

BUT : Clarifier la valeur pronostique de l'expression des antigènes Le^a, sialyl Le^a, Le^x, sialyl Le^x dans les cancers du côlon et du rectum comme facteurs pronostiques après une intervention chirurgicale.

PATIENTS ET MÉTHODE : Des prélèvements de tumeurs cancéreuses obtenus chez 101 patients souffrant d'un carcinome colo-rectal primitif et ayant subi une résection chirurgicale ont été soumis à des analyses immunohistochimiques à la recherche des antigènes Le^a, sialyl Le^a, Le^x, sialyl Le^x à l'aide des anticorps monoclonaux respectifs.

RÉSULTATS : Les antigènes Le^a, sialyl Le^a, Le^x, sialyl Le^x ont été trouvés dans respectivement 69 (68,3 %), 73 (72,3 %) 66 (65,4 %) et 76 (75,3 %) des tumeurs cancéreuses. Les patients chez qui l'antigène sialyl Le^x a été détecté présentaient un cancer plus avancé que ceux chez qui il n'avait pas été détecté (p=0,0029). Le temps de survie après l'intervention chirurgicale chez les patients porteurs des antigènes Le^x et sialyl Le^x était sensiblement plus court que celui enregistré chez les patients qui n'en étaient pas porteurs (p=0,023 et p=0,0001 respectivement). L'analyse de régression de Cox a révélé que l'expression des antigènes Le^x et sialyl Le^x, stade et type histologique étudiés séparément, constituait des variables pronostiques quant à la survie des patients (rapport de risque [RR] sialyl Le^x positif sur sialyl Le^x négatif : 2,90; RR Le^x positif sur Le^x négatif : 12,76 au stade I/IV, 0,63 au stade II et 1,69 au stade III).

CONCLUSION : L'expression des antigènes Le^x et sialyl Le^x dans les adénocarcinomes colo-rectaux est associée à un sombre pronostic. Ce sont là des variables dont il faudrait tenir compte dans la conception de futurs essais.

Tumour development is usually associated with changes in cell surface carbohydrates (1). The changes in cell surface carbohydrates can be classified into changes in the terminal carbohydrate structure, which include incomplete synthesis and modification of normally existing carbohydrates, and changes in the carbohydrate core structure (1). The backbone regions of the terminal structures are classically divided into one of two categories – type 1 chains and type 2 chains (1,2). In some solid tumours, incomplete synthesis of cell surface carbohydrates results in increased expression of the precursor structures of blood group A, B and H antigen (ABH) such as Lewis (Le)^a, sialyl Le^a, Le^x and sialyl Le^x (1-3). The blood group-related Le^a and sialyl Le^a antigens have type 1 terminal structures, and Le^x and sialyl Le^x antigens have type 2 terminal structures (4,5). The epitopes of the Le^a and sialyl Le^a antigens are isomeric to that of the Le^x and sialyl Le^x antigens, respectively.

Determination of tumour-associated carbohydrate changes is used in the diagnosis of human cancer. Expression of particular carbohydrate structures is also associated with prognosis (1,3). Nakagoe et al (6) and Nakamori et al (7) reported that increased expression of sialyl Le^x in colorectal carcinomas is correlated with poor prognosis. That is, it has been established that the level of sialyl Le^x antigen expression in tumours is a useful prognostic variable in colorectal cancer patients (1). Recent studies indicate that sialyl Le^x, sialyl Le^a and related carbohydrate structures function as adhesion molecules and, theoretically, may be involved in tumour metastasis (1). Therefore, we expanded our study to elucidate whether the expression of their nonsialylated forms – Le^x and its isomer Le^a, and sialyl Le^a antigen – is correlated with prognosis after surgery in colorectal cancer patients. The relations between the expression of Le^a, sialyl Le^a or Le^x antigen in colorectal tumours and subsequent outcome remain unclear (1), although two studies demonstrated that sialyl Le^a antigen expression is a prognostic factor in patients with advanced colorectal carcinoma (8,9).

An immunohistochemical study of Le^a, sialyl Le^a, Le^x and sialyl Le^x antigen expression was performed using the respective monoclonal antibodies in colorectal carcinoma tissues, and the prognostic value of the expression of these antigens was clarified by univariate and multivariate analyses.

PATIENTS AND METHODS

One hundred and one patients with primary colorectal carcinoma who underwent surgical resection at Nagasaki University Hospital, Nagasaki, Japan, between January 1988 and December 1992 were studied retrospectively. Patients with more than one carcinoma of the colon and rectum, or polyposis coli were excluded. American Joint Committee on Cancer Classification and Stage grouping was used for tumour assessment (10). Two different tissue specimens were obtained from each patient. As a result, 101 colorectal carcinoma tissue specimens and 81 normal colonic mucosa specimens taken from parts of the colon distant from the carcinomas were examined. Each specimen was fixed in formalin, embedded in paraffin, and stained with hematoxylin and eosin or immunoperoxidase. The histological type of each tumour was determined according to the criteria of the World Health Organization International Histological Classification of Tumours (11), by two pathologists who examined the hematoxylin and eosin-stained tissue sections.

After surgery, each patient underwent standard follow-up examinations. The physicians were blind to the expression status of Le-related antigens in the patients. Laboratory testing was performed every three months, and chest roentgenogram, computed tomography, abdominal ultrasound scanning and colonoscopy were performed annually. The median duration of follow-up was 35.0 months (range 2.4 to 120.5 months). The outcomes of the 101 patients were confirmed at the time that this paper was written (July 1999): 39 patients were alive; 57 patients had recurrence of colorectal cancer and died; and five patients died of another disease. The data on patients who died of causes other than colorectal cancer were censored in the statistical analysis.

Antibodies: The monoclonal antibodies CLEA1, CSLEA1, CLEX1 and CSLEX1, which are directed toward the Le^a, sialyl Le^a, Le^x and sialyl Le^x structures, respectively, were used in the present study. These antibodies were provided by Professor PI Terasaki (UCLA Tissue Typing Laboratory, Los Angeles, California) (12-16). Peroxidase-conjugated goat $F(ab')_2$ antimouse immunoglobulin (Ig) G and IgM were purchased from Cappel Laboratory (USA).

Immunoperoxidase staining: Immunohistochemical staining was performed as previously described using an indirect

TABLE 1
Distribution of patients with colorectal cancer that did or did not express Lewis (Le)^a, sialyl Le^a, Le^x or sialyl Le^x antigen according to the stage of carcinoma

	Le^a, n (%)			Sialyl Le^a, n (%)			Le^x, n (%)			Sialyl Le^x, n (%)		
Stage	Negative (n=32)	Positive (n=69)	P	Negative (n=28)	Positive (n=73)	P	Negative (n=35)	Positive (n=66)	P	Negative (n=25)	Positive (n=76)	P
I	2 (6.3)	7 (10.1)	0.32	2 (7.1)	7 (9.6)	0.99	6 (17.1)	3 (4.6)	0.092	6 (24.0)	3 (4.0)	0.0029
II	7 (21.9)	23 (33.3)		8 (28.6)	22 (30.1)		7 (20.0)	23 (34.9)		10 (40.0)	20 (26.3)	
III	18 (56.3)	25 (36.2)		13 (46.3)	30 (41.1)		17 (48.6)	26 (39.4)		8 (32.0)	35 (46.1)	
IV	5 (15.6)	14 (20.3)		5 (17.9)	14 (19.2)		5 (14.3)	14 (21.2)		1 (4.0)	18 (23.7)	

P values were obtained using the Fisher's exact test

immunoperoxidase staining technique (13,16). All steps were performed at room temperature. The monoclonal antibodies were appropriately diluted in 0.05 M Tris-hydrochloride buffer, 2.5% bovine serum albumin, mouse liver acetone powder (100 mg/L; Sigma Chemical Co, USA) and 0.05% sodium azide. Negative controls were prepared by substituting Tris-buffered saline for the primary antibody. This resulted in no detectable staining.

Evaluation of monoclonal antibody reactivity: Immunohistochemical expression of an antigen was evaluated by examining all of the visual fields of each section under a light microscope. The result was expressed as a score based on the percentage of the total field that stained positive with the monoclonal antibody. A tumour or tissue in which over 5% of the cells were stained by a monoclonal antibody was defined as positive for the respective antigen, while a tumour or tissue in which 5% or less of the cells were stained was defined as negative for the respective antigen (13,16). Two of the authors independently judged the results of immunohistochemical staining of each antibody in each sample, and the final result was assigned by consensus.

Statistical analysis: Statistical analyses were performed using SAS statistical analysis software (SAS Institute, USA). Univariate analysis was performed as follows. Categorical data were analyzed by Fisher's exact test. Continuous data were analyzed using an unpaired *t* test. Survival analysis was performed using the Kaplan-Meier method (17), and differences between survival curves were tested for significance using the log-rank test (18).

Multivariate analysis was carried out using the Cox's proportional hazards regression model (19). Four variables (Le^a, sialyl Le^a, Le^x and sialyl Le^x antigen expression) were compared with other prognostic variables, including stage and histological type, that are generally used in colorectal cancer patient management and well-supported in the literature (10). All tests were two-tailed, and $P<0.05$ was considered significant.

RESULTS

Expression of Le-related antigens in normal colonic mucosa distant from the carcinoma: Of the 81 normal mucosa specimens distant from the carcinoma, Le^a, sialyl Le^a, Le^x and sialyl Le^x antigens were expressed in 74 (91.3%), 61 (75.3%), 48 (55.6%) and one specimen (1.2%), respectively.

Comparison of the clinicopathological features of patients with colorectal tumours that did or did not express particular Le-related antigens: Of the 101 carcinoma specimens, Le^a, sialyl Le^a, Le^x and sialyl Le^x antigens were expressed in 69 (68.3%), 73 (72.3%), 66 (65.4%) and 76 (75.3%) specimens, respectively (Table 1). There were no statistically significant relations between the expression of Le^a, sialyl Le^a or Le^x antigen, and clinicopathological variables including age, sex, tumour location, maximal tumour diameter, histological type, TNM factor and stage.

However, lymph node metastasis (N factor) was detected in 64.5% of the tumours that expressed sialyl Le^x and in 32.0% of the tumours that did not express sialyl Le^x – a significant difference (P=0.0056). Distant metastasis (M factor) was observed in 23.7% of the tumours that expressed sialyl Le^x and in 4.0% of the tumours that did not express sialyl Le^x – also a significant difference (P=0.037). The patients with tumours that expressed sialyl Le^x antigen were in a significantly more advanced stage than those with tumours that did not express sialyl Le^x (P=0.0029) (Table 1).

Comparison of survival after surgical resection between patients with tumours that did and those that did not express particular Le-related antigens: The survival time of patients with tumours that expressed Le^x or sialyl Le^x was significantly shorter than the survival time of patients with tumours that did not express Le^x or sialyl Le^x, respectively (P=0.023 and P=0.0001, respectively). The cumulative five-year survival rates were as follows: 61.4% in patients with Le^x-negative tumours, 41.1% in patients with Le^x-positive tumours, 83.8% in patients with sialyl Le^x-negative tumours and 36.3% in patients with sialyl Le^x-positive tumours. The survival time of patients did not differ based on Le^a or sialyl Le^a expression in the colorectal tumours (Figure 1).

Figure 2 shows the survival curves of patients with stage II or stage III colorectal tumours according to Le^x or sialyl Le^x antigen expression. Reduced survival was noted in patients with stage III tumours that were positive for Le^x expression relative to those with stage III tumours that were negative for Le^x expression (P=0.018), as well as in patients with stage II tumours that were positive for sialyl Le^x expression relative to those with stage II tumours that were negative for sialyl Le^x expression (P=0.045). The cumulative five-year survival rates were as follows: 58.2% in patients with stage III, Le^x-negative tumours; 26.2% in patients with stage III, Le^x-posi-

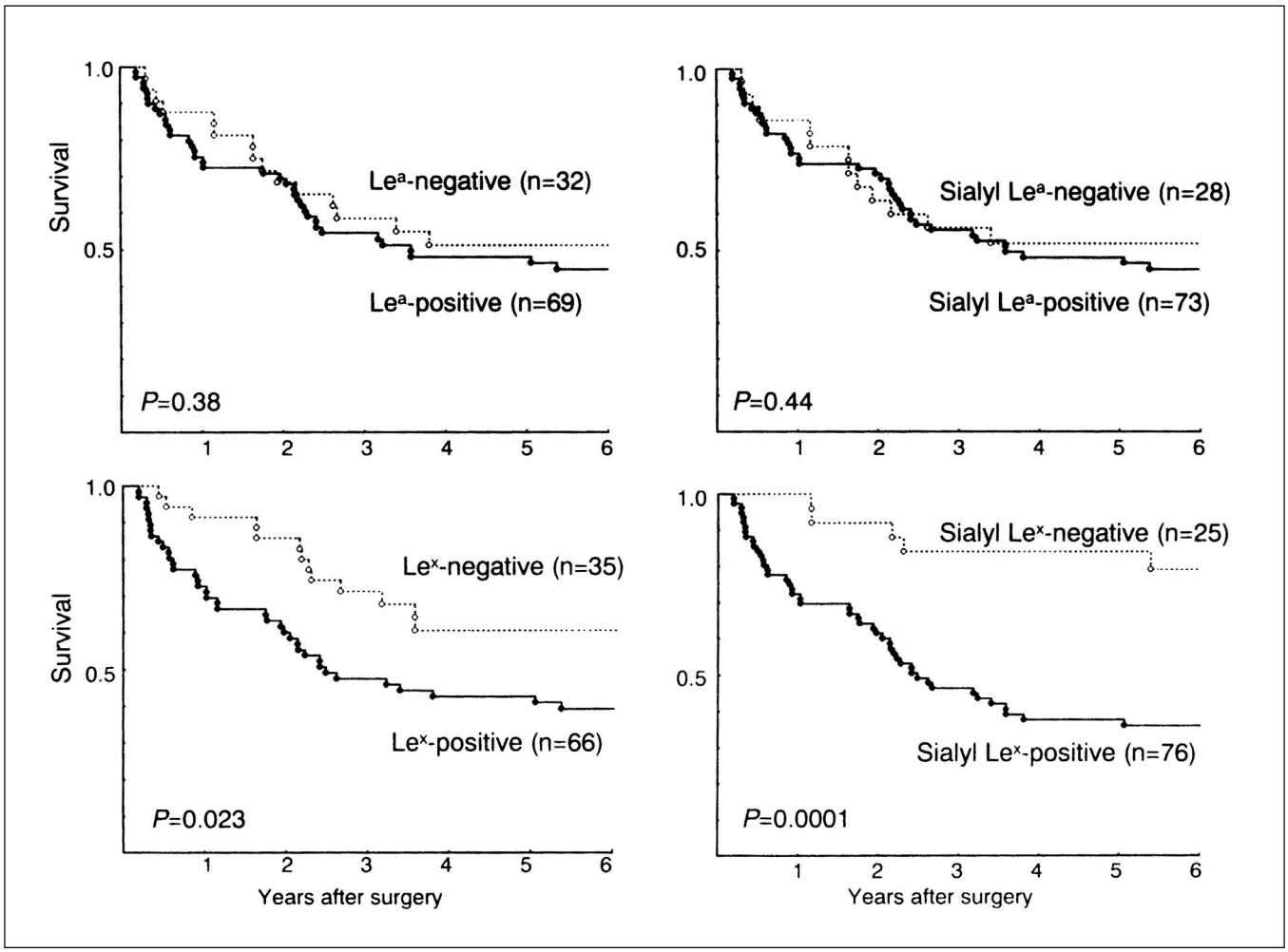

Figure 1) *Comparison of the survival of patients with colorectal carcinomas that did or did not express Lewis (Le)-related antigens. The survival time of patients with tumours that expressed Le^x or sialyl Le^x was significantly shorter than the survival time of patients with tumours that did not express Le^x or sialyl Le^x, respectively (P=0.023 and P=0.0001, respectively). The survival time of patients did not differ based on Le^a or sialyl Le^a expression in tumours*

tive tumours; 100% in patients with stage II, sialyl Le^x-negative tumours; and 66.7% in patients with stage II, sialyl Le^x-positive tumours.

Multivariate analysis for the prognostic value of the expression of Le-related antigens: The majority of Le^a-positive carcinomas were also positive for sialyl Le^a antigen (98.6%; 68 of 69), and the majority of Le^a-negative carcinomas were also negative for sialyl Le^a antigen (84.4%; 27 of 32). There were no other strong correlations between the expression of other pairs of antigens. To avoid the problem of collinearity, the variable 'expression of Le^a antigen' was excluded. Therefore, five variables (stage, histological type, sialyl Le^a, Le^x and sialyl Le^x antigen expression) were included in the Cox's regression analysis. Patients with Le^x- or sialyl Le^x-positive tumours had a worse survival outcome than patients with Le^x- or sialyl Le^x-negative tumours, respectively, separate from stage and histological type. The hazard ratio (HR) for Le^x-positive to Le^x-negative expression correlated with stage, that is, the HRs were 12.76 in stage I (95% CI 2.98 to 54.66), 0.63 in stage II (95%CI 0.14 to 2.88), 1.69 in stage III (95% CI 0.69 to 4.15) and 12.76 in stage IV (95% CI 2.98 to 54.66). In contrast to Le^x expression, the HR for sialyl Le^x-positive to sialyl Le^x-negative expression did not correlate with stage (HR 2.90; 95% CI 1.01 to 8.37). Survival did not differ based on sialyl Le^a expression in tumours (Table 2).

DISCUSSION

The present study revealed that the expression of Le^x or sialyl Le^x antigen (a type 2 chain carbohydrate antigen) in colorectal carcinomas is associated with poor prognosis. On the other hand, the expression of Le^a or sialyl Le^a antigen (a type 1 chain carbohydrate antigen) in colorectal carcinomas was not associated with patient survival.

Expression of Le^x and sialyl Le^x antigens in solid tumours as prognostic factors in cancer patients: In some solid tumours, incomplete synthesis of cell surface carbohydrates results in increased expression of ABH precursor structures such as Le^x and sialyl Le^x, and their isomers Le^a and sialyl Le^a, respectively (1-3). Because the syntheses of Le^x and sialyl Le^x are independent of the patient's ABH and secretor status, in contrast to ABH isoantigens, they are more suit-

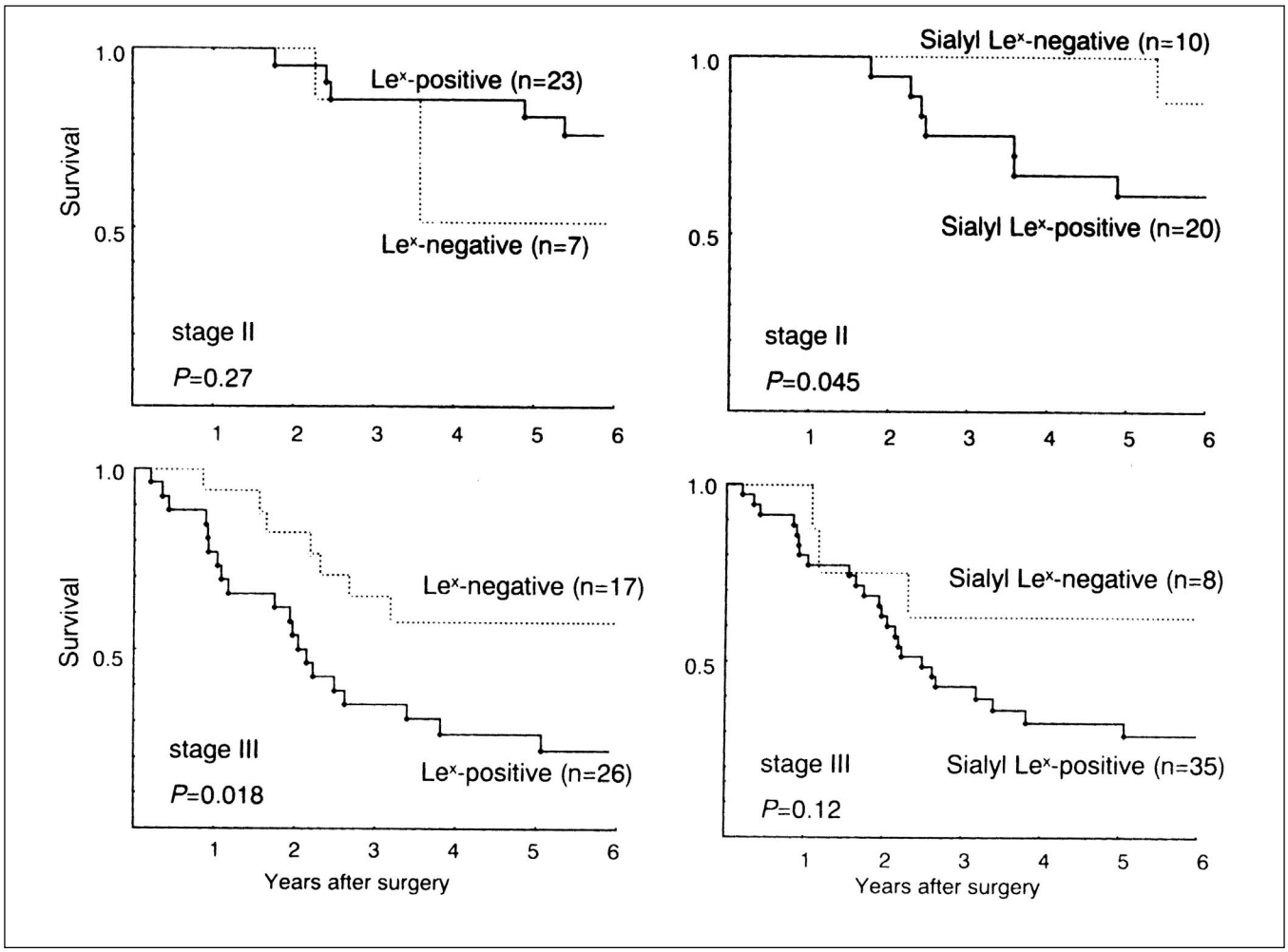

Figure 2) *Comparison of the survival of patients with stage II or stage III colorectal cancers that did or did not express Lewis (Le)^x or sialyl Le^x antigen. A reduced survival was noted in stage III patients positive for Le^x expression relative to those negative for Le^x expression (P=0.018), as well as in stage II patients positive for sialyl Le^x expression relative to those negative for sialyl Le^x expression (P=0.045)*

able for clinical studies (1). Sialyl Le^x and related carbohydrate structures function as adhesion molecules, and theoretically they may be involved in tumour metastasis (1,20). It is interesting that their expression in certain tumours is correlated with prognosis (1).

Sialyl Le^x was found in the extracts of most colon carcinomas but not in the extracts of normal colonic mucosa (21). Sialyl Le^x expression is stronger in liver metastatic lesions than in primary colorectal tumours (21). An immunohistochemical study demonstrated that metastatic lesions contain a higher percentage of sialyl Le^x-positive tumour cells than primary tumours (22). Ono et al (23) reported that focal dedifferentiation of cancer cells at the invasive front and sialyl Le^x antigen expression in colorectal carcinomas are each correlated with a high risk of developing liver metastasis. In addition, it was previously reported that increased sialyl Le^x antigen expression in colorectal carcinomas, detected immunohistochemically by monoclonal antibody CSLEX1, was correlated with poor prognosis (6). Nakamori et al (7) also reported that increased expression of sialyl Le^x in colorectal carcinomas is correlated with a poor prognosis. However, Nakamori et al (7) used monoclonal antibody FH-6 to detect sialyl Le^x and found that the specificity of FH-6, which is directed toward the dimeric version of sialyl Le^x antigen, and the specificity of CSLEX1 antibody differ slightly. Thus, it has been established that sialyl Le^x expression in colorectal tumours is a useful tumour-associated marker that indicates the degree of tumour aggressiveness and subsequent outcome (1).

The relation between Le^x antigen expression and tumour behaviour and subsequent outcome in breast, esophageal and lung carcinomas has been reported (24-26). However, the relation between Le^x antigen expression as shown by immunohistochemistry and patient prognosis among colorectal carcinoma patients has not been reported. Singhal et al (27) reported that the serum Le^x antigen level has prognostic value in Dukes C colon cancer patients after surgery and during postoperative follow-up. The present study revealed the prognostic value of Le^x antigen expression in colorectal carcinoma.

Le^x antigen, which is the precursor of type 2 ABH isoantigens, was initially discovered as a stage-specific embryonic

TABLE 2
Results of Cox's regression analysis

	Hazard ratio	95% CI
Stage		
I	1.00	
II	9.61	1.05–87.72
III	17.08	2.10–138.85
IV	16.34	3.36–79.56
Histological type		
Well differentiated	1.00	
Moderately differentiated	2.87	1.17–7.06
Poorly differentiated/mucinous	3.76	1.32–10.75
Sialyl Le^a antigen expression		
Negative	1.00	
Positive	0.67	0.32–1.37
Le^x antigen expression		
Negative	1.00	
Positive	12.76	2.98–54.66
In stage II*	0.050*	0.0070–0.34*
In stage III*	0.13*	0.024–0.72*
Sialyl Le^x antigen expression		
Negative	1.00	
Positive	2.90	1.01–8.37

**Correlation between stage and Lewis (Le)^x antigen expression*

antigen and is considered to be involved in cell differentiation during embryogenesis, and in the growth and differentiation of cells (28,29). A previous report showed that Le^x antigen is involved in cell-cell recognition at the beginning of cell differentiation (30). Loss of ABH isoantigens is correlated with tumour progression, metastatic potential and poor prognosis in various tumours (3). The loss of ABH isoantigens may result in the accumulation of precursor Le^x antigen during tumour development. Cancer patients have elevated sialyltransferase activity in both the cancer tissue and the sera (31,32). Consequently, the sialylated form of Le^x is more prevalent than its nonsialylated form in tumours (11,15).

The significance of increased sialyl Le^x expression in tumours may be related to the presence of receptors for sialyl Le^x-carrying ligands on activated endothelial cells (1,33,34). Cytokine-activated endothelial cells express endothelial leukocyte adhesion molecule-1 (ELAM-1) – an adhesion molecule belonging to the selectin family. ELAM-1 recognizes the sialylated form of Le^x and related structures (1). Several studies have shown that tumour cells expressing sialyl Le^x adhere to interleukin-activated, cultured endothelial cells (1,35). In addition, Tsumatori et al (36) reported that patients with sialyl Le^x antigen-positive, nonsmall cell lung cancer who had a high serum E-selectin level had poor prognosis after surgical resection.

Expression of Le^a and sialyl Le^a antigens in solid tumours as prognostic factors of cancer patients: Sialyl Le^a also serves as a ligand for ELAM-1. Tumour cells expressing sialyl Le^x and its positional isomer sialyl Le^a adhere to interleukin-activated cultured endothelial cells (1,35). Takada et al (35) investigated cancer cell lines expressing sialyl Le^x and/or sialyl Le^a, and found that the degree of adhesion of tumour cells to the endothelium correlated with the degree of sialyl Le^x and sialyl Le^a expression. This adhesion was significantly inhibited by pretreatment with antibodies to ELAM-1, sialyl Le^x or sialyl Le^a.

Although sialyl Le^a is expressed on many types of solid tumours, the relation between sialyl Le^a expression and metastatic behaviour remains unclear (1,36). Recently, Nakayama et al (8) and Asai et al (9) reported that sialyl Le^a antigen expression in colorectal tumours, detected immunohistochemically by monoclonal antibody NS19-9, is a useful marker for evaluating tumour aggressiveness and the prognosis of patients with advanced colorectal cancer. However, the present study failed to prove the prognostic value of sialyl Le^a antigen expression in colorectal carcinomas, and the existence of a relation between sialyl Le^a antigen expression and the degree of tumour aggressiveness (TNM factors). There are several differences among the study by Nakayama et al (8), the study by Asai et al (9) and the present study that may explain why different results were obtained. First, each of the three studies used different antibodies. Second, different methodologies of immunohistochemical staining were used. Third, different methods of evaluating monoclonal antibody reactivity were used. However, we believe that different results were obtained in the study by Nakayama et al (8) and the study by Asai et al (9) mainly because different antibodies were used. Monoclonal antibody CSLEA1 (15,37,38), which was used in the present study, recognizes the carbohydrate structure of sialyl Le^a, as does monoclonal antibody NS19-9 (39). Monoclonal antibody CSLEA1 shows a similar, but not identical, pattern of immunohistochemical staining as NS19-9 in colonic, gastric and pancreatic tumours and in normal tissues (14,37), ie, CSLEA1 shows a wider range of immunoreactivity (37).

The relation between sialyl Le^a expression, detected immunohistochemically using monoclonal antibody NS19-9, and metastatic behaviour and subsequent outcome has been reported by Arends et al (40) and Walker and Day (41). Arends et al (40) reported that the presence of sialyl Le^a in gastrointestinal cancers is not correlated with parameters known to be of prognostic significance. Walker and Day (41) found no correlation between the presence of sialyl Le^a in breast carcinomas and lymph node status. Thus, the relation between sialyl Le^a expression and metastatic behaviour remains unclear (1,36), although sialyl Le^a is expressed in many types of solid tumours.

Qualitative differences between type 1 and type 2 chain antigens: The reason for the difference between the significance of the presence of type 1 and that of type 2 chain carbohydrate structures, which was revealed in the present study, is not known. In general, most type 1 chain determinants such as sialyl Le^a antigen are closely related to Le blood group structures, which are substances (ie, carbohydrate human alloantigens) that are present in normal mature cells. On the other hand, type 2 chain antigens such as sialyl Le^x are usually present only in immature cells and frequently

appear during the course of malignant transformation (42). The present study also revealed that type 1 chain antigens (Le^a and sialyl Le^a) are more frequently expressed than type 2 chain antigens (Le^x and sialyl Le^x) in normal colonic mucosa. In the crypts of normal colonic mucosa, type 1 chain antigens are diffusely observed in cells located in the upper two-thirds of the crypts, whereas type 2 chain antigens are located in the lower one-third of the crypts, in particular, sialyl Le^x shows weak staining in very limited parts of some crypts (43). It has also been elucidated that circulating type 1 chain antigens are more frequently detected in the sera of patients with nonmalignant disorders than type 2 chain antigens, as determined by assay of the same sera (42). These fundamental qualitative differences between type 1 and type 2 chain antigens may explain why tumours expressing type 1 or type 2 chain antigens have different metastatic behaviour, and why type 1 and type 2 chain antigens have different prognostic value. Further studies are needed to confirm our theories and increase the level of understanding.

In summary, Le^x expression and sialyl Le^x expression are associated with poor prognosis in colorectal cancer patients. Currently, in the treatment of colorectal cancer, stratification into patient groups to select individuals for appropriate adjuvant therapy protocols is primarily based on clinicopathological criteria. Prospective clinical studies on Le^x and/or sialyl Le^x antigen expression may be helpful in planning additional adjuvant therapies after surgery. Furthermore, administration of sialyl Le^x, for example by using sialyl Le^x oligosaccharide in the form of liposomes, has been suggested as a therapy for cancers (1,33). Ongoing studies concerning the carbohydrate-protein interaction and its possible role in cell-cell adhesion, and the mechanism of metastasis will open new pathways of therapeutic intervention targeted at these molecules.

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