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sLe^a and sLe^x expression in colorectal cancer: implications for tumourigenesis and disease prognosis

Susana Villar Portela[1], Cristina Vázquez Martín[1], Laura Muinelo Romay[1], Elisa Cuevas[2], Emilio Gil Martín[1]* and Almudena Fernández Briera[1]*

[1]Department of Biochemistry, Genetics and Immunology, Faculty of Biology, University of Vigo, Vigo, Spain and [2]Pathology Service, University Hospital Complex, Ourense, Spain

*These authors contributed equally to this work

Summary. The glycoconjugates expressed by cancer cells frequently contain sialylated oligosaccharide chains. Among these oligosaccharides the sialyl Lewis a (sLe^a) and sialyl Lewis x (sLe^x) antigens are found to be overexpressed in tumours of different origin. The current study assesses sLe^a and sLe^x expression in different colorectal specimens in order to establish the correlation of these biomarkers with both malignant transformation of colorectal mucosa and the progression of colorectal cancer (CRC). Healthy disease-free and inflammatory mucosa specimens showed no presence of the antigens. sLe^a was expressed in 6.7% of the healthy tissue from CRC patients, in 20.8% of the adenomas, and in 33.3% and 42.6% of the transitional tissue and tumour tissue, respectively. sLe^x expression was observed in 6.7% of the healthy tissue from CRC patients, in 27.0% of the adenomas, and in 25.6% and 74.8% of the transitional and the tumour tissue, respectively. The expression of the sLe^a and sLe^x antigens was correlated in adenomas, as well as in healthy and tumour tissue from CRC. Moreover, the high expression of sLe^x in adenomas was correlated with a high degree of dysplasia (p=0.042). Finally, the survival analysis suggested that sLe^a expression may be a prognostic factor for predicting disease-free survival in colorectal cancer (p=0.012).

Key words: Sialyl Lewis a, Sialyl Lewis x, Colorectal cancer, Prognosis

Offprint requests to: Almudena Fernández Briera, Department of Biochemistry, Genetics and Immunology, Faculty of Biology, University of Vigo, Campus As Lagoas-Marcosende, 36310 Vigo, Spain. e-mail: abriera@uvigo.es

Introduction

Alterations in cell surface glycosylation are a common phenotypic change observed during neoplasic transformation (Hakomori, 1989). The increase of sialylation is a common feature in the glycoconjugates of malignant cells. Well documented examples of tumour-associated sialylated glycoconjugates include those carrying the antigens sialyl Lewis a (sLe^a) and sialyl Lewis x (sLe^x) (Kim and Varki, 1997). Both antigens are positional isomers and can be found in terminal chains of glycolipids and N-/O-glycoproteins (Hakomori, 2002; Magnani, 2004). The biosynthesis of the sLe^a and sLe^x antigens is mediated by the sequential action of α(2-3)sialyltransferases and α(1,3/4)fucosyltransferases on type 1 and type 2 chain precursors, respectively (Magnani, 2004). sLe^a was originally discovered as a tumour marker in gastrointestinal and pancreatic cancers (Magnani et al., 1982), while sLe^x was first described in a ganglioside fraction from human kidney (Rauvala, 1976). sLe^x is present on leukocytes such as neutrophils and monocytes (Fukuda et al., 1984) where, as a ligand of E-selectin, it plays a role in the recruitment and extravasation of leukocytes at inflammatory sites (Lowe et al., 1990; Phillips et al., 1990; Walz et al., 1990). Likewise, different studies have revealed that sLe^a is also a ligand of E-selectin, and that both sLe^a and sLe^x antigens are ligands of P-selectin (Berg et al., 1991; Takada et al., 1991; Foxall et al., 1992). The increased binding of sLe^a and sLe^x overexpressing cancer cells to E-selectin expressed on vascular cells facilitates tumour angiogenesis and promotes hematogenous metastasis (Takada et al., 1991, 1993).

In the past decades, sLe^a and sLe^x have been widely

studied in relation to cancer development and progression. Several works have reported an overexpression of both antigens in different human carcinomas concomitant with a loss of mucosa homeostasis and the establishment of an immune status that favours tumour progression (Le Pendu et al., 2001; Kannagi et al., 2010). As sLe^a and sLe^x antigens can be released into the bloodstream, some authors have found that the high serum levels of sLe^a and sLe^x are correlated with a poor outcome, especially in the case of gastrointestinal tumours (Ørtonft and Bech, 1995).

The enhancement of α(2-3)sialyltransferase and α(1,3/4)fucosyltransferase activities has been proposed as one of the main mechanisms driving sLe^a and sLe^x overexpression in tumours, although alternative mechanisms have also been put forward (Kannagi et al., 2004, 2010). It is well known that an overexpression of sLe^a and sLe^x is tightly associated with neoplasic transformation and worse prognosis in different gastrointestinal tumours (Kannagi et al., 2004). This association derives from the binding of sLe^a and sLe^x, present on the surface of cancer cells, to the endothelial cell adhesion molecules E- and P-selectin during the extravasation step in the hematogenous metastasis of cancer cells (Kannagi et al., 2004).

Concerning CRC, different studies have shown an overexpression of the sLe^a and sLe^x antigens, although their expression has also been described in healthy colorectal tissue and in colorectal adenomas (Itzkowitz et al., 1986, 1988; Afdhal et al., 1987; Allen et al., 1987; Yuan et al., 1987; Hanski et al., 1990; Baldus et al., 1995, 2002; Tucci et al., 1999). The correlation between the expression of sLe^a and sLe^x antigens in CRC and an unfavorable prognosis has been well documented (Le Pendu et al., 2001; Kannagi et al., 2004). Moreover, the prognostic value of the sLe^a serum levels, known as CA19-9, has been extensively studied (Le Pendu et al., 2001; Yamashita and Watanabe, 2009). Nevertheless, it is worth bearing in mind that most of the studies focused on the impact of sLe^a and sLe^x tissue expression and serum levels in the prognosis of CRC patients have been carried out in the Japanese population. Some authors have reported differences in the prognostic value of some molecular markers in colorectal adenocarcinoma in relation with the ethnic origin of the patients (Manne et al., 1998, 2000).

The sLe^a and sLe^x antigens share structural similarities, and some glycosyltransferases are common to their respective biosynthetic pathways. However, to date the coexpression of both antigens in colorectal tissues has not been investigated. With this in mind, we have simultaneously examined the immunohistochemical expression of sLe^a and sLe^x in different colorectal tissue specimens with the aim of clarifying their role in the development and progression of CRC. We have also studied the prognostic value of sLe^a and sLe^x expression in CRC.

Materials and methods

Patients and specimens

A total of 158 patients of Caucasian ethnicity with primary CRC who underwent surgical resection at the University Hospital Complex (CHOU, Ourense, Spain) between 1995-1997 and 2003-2004 were included in the study. Specimens of healthy mucosa (at a distance at least 10 cm from the tumour) and transitional mucosa (immediately adjacent to the tumour but without microscopic features of malignancy) were obtained from 45 and 39 CRC patients, respectively. Tumour staging was carried out by means of the TNM classification system described by the International Union Against Cancer (UICC) (Sobin and Wittekind, 2002). In addition, 74 colorectal adenomas, 19 biopsies of colorectal inflammatory mucosa and 34 biopsies of healthy disease-free colorectal mucosa, obtained from CRC-free individuals of Caucasian ethnicity, were included.

A complete standarized follow-up was documented for at least 5 years for CRC patients. Patients who died within 30 days after surgery, or with distant metastasis at surgical resection, were excluded from the follow-up. All procedures involving human samples were performed according to the clinical practices of the "Xunta de Galicia" (Spain) and followed the tenets of the Helsinki Declaration.

Monoclonal antibodies

The monoclonal antibodies used were NS-19-9 (Sigma-Aldrich, MO, USA) (Koprowski et al., 1979), which is reactive with the sLe^a antigen, and KM93 (Chemicon, CA, USA) (Hanai et al., 1986), reactive with the sLe^x antigen. The NS-19-9 and KM93 antibodies do not display cross-reactivity (Shitara et al., 1987).

Immunohistochemical staining

All specimens were fixed in formalin and embedded in paraffin. Sections (3 μm) from selected paraffin embedded tissue blocks were deparaffinized in xylene, hydrated in a graded ethanol series, and heated in 0.1M citric acid buffer (pH 6.0) in a microwave oven for 15 min to unmask the epitopes. Endogenous peroxidase activity was blocked with 0.5% (v/v) hydrogen peroxide in methanol for 15 min, and non-specific binding was blocked with 2% bovine serum for 20 min (Sigma-Aldrich, MO, USA). Afterwards, the sections were incubated with monoclonal antibodies against sLe^a (dilution 1/200) and against sLe^x (dilution 1/100) for 30 min in a moist chamber. After a rinse with PBS, the sections were incubated for 30 min in a moist chamber with the secondary antibody bound to peroxidase (goat anti-mouse, labelled polymer, HRP, EnVision™ Detection System, Dako, CA, USA). All slides were

treated with DAB to develop colour. Finally, the sections were washed with water, counterstained with Papanicolau's hematoxilin, dehydrated in ethanol, washed with xylene and mounted in Dpex mounting medium. Negative controls were performed by adding PBS instead of primary antibody.

Evaluation of the sLe^a and sLe^x staining

The evaluation of the immunohistochemical staining, detected as a brown colour precipitate, was carried out independently by two pathologists (CHOU), who reached a final result by consensus. The staining scores were classified semi-quantitatively as follows: 0, tissue specimens without staining; 1 (weak), less than 10%; 2 (moderate), 10-50%; and 3 (strong), more than 50% of the tissue stained.

Statistical analysis

Statistical analysis was performed using PASW Statistics (18.0 version). The univariate analysis for categorical data was conducted by means of the χ^2 test or Fisher's exact probability test. For continuous data, we employed Wilcoxon's test and Mann-Whitney U test. Disease-free survival rates were calculated by the Kaplan-Meier method (Kaplan and Meier, 1958) and differences between the survival curves were analyzed by log-Rank test (Mantel, 1966). Multivariate Cox regression analysis (Cox, 1972), using the forward stepwise data selection method, was carried out to determine the factors with independent prognostic value. The results were considered significant when $p<0.05$.

Resuts

Expression of sLe^a and sLe^x antigens in colorectal tissues

sLe^a and sLe^x immunohistochemical expression was analyzed in 45 specimens of colorectal healthy tissue, 39 of transitional tissue and 155 specimens of tumour tissue from CRC patients. Moreover, 34 specimens of colorectal disease-free mucosa, 19 specimens of inflammatory mucosa, and 72 (for sLe^a) and 74 (for sLe^x) colorectal adenomas were also evaluated.

sLe^a expression was not found in healthy disease-free mucosa or inflammatory mucosa from CRC-free patients (Fig. 1A,B, Table 1). However, we observed the expression of sLe^a in 15 (20.8%) colorectal adenomas (Fig. 1C, Table 1). In the healthy tissue from CRC patients the antigen was detected in 3 (6.7%) specimens (Table 1), in the upper crypts of mucosa (Fig. 1D). In transitional tissue, sLe^a expression was found in 13 specimens (33.3%) (Fig. 1E, Table 1). Regarding the 155 CRC specimens analyzed, 66 (42.6%) showed positive expression for sLe^a (Fig. 1F, Table 1). In the colorectal specimens with positive staining, the antigen was located in the cytoplasm, apical cell membrane and luminal content. The expression of the sLe^a antigen in tumour tissue was statistically higher than its expression in healthy tissue from CRC ($p<0.001$, according to Wilcoxon's test), as well as with respect to the colorectal adenomas, inflammatory mucosa and healthy disease-free mucosa ($p<0.001$, according to Mann Whitney U test).

Regarding the sLe^x antigen, the colorectal specimens from healthy disease-free patients or inflammatory bowel disease did not show sLe^x expression (Fig. 2A,B, Table 1). However, the antigen was expressed in 20 (27.0%) colorectal adenomas (Fig. 2C, Table 1), in 3 (6.7%) healthy tissue specimens (Fig. 2D, Table 1), in 10 (25.6%) transitional tissue specimens (Fig. 2E, Table 1) and in 116 (74.8%) CRC specimens (Fig. 2F, Table 1). The sLe^x antigen was detected in the same tissue locations as sLe^a. Statistically significant differences were detected in sLe^x expression in tumour tissue in comparison with their healthy and transitional tissue counterparts from CRC ($p<0.001$, according to Wilcoxon's test), as well as with respect to the colorectal

Table 1. Immunoreactivity of sLe^a and sLe^x antigens in the colorectal specimens analyzed.

	0 (No expression)	1 (Weak)	2 (Moderate)	3 (Strong)
sLe^a staining score				
Healthy disease-free mucosa (*n*=34)	34 (100%)	0	0	0
Inflammatory mucosa (*n*=19)	19 (100%)	0	0	0
Adenomas (*n*=72)	57 (79.2%)	15 (20.8%)	0	0
Healthy tissue CRC (*n*=45)	42 (93.3%)	1 (2.2%)	2 (4.5%)	0
Transitional tissue CRC (*n*=39)	26 (66.7%)	7 (17.9%)	6 (15.4%)	0
Tumour tissue CRC (*n*=155)	89 (57.4%)	32 (20.7%)	12 (7.7%)	22 (14.2%)
sLe^x staining score				
Healthy disease-free mucosa (*n*=34)	34 (100%)	0	0	0
Inflammatory mucosa (*n*=19)	19 (100%)	0	0	0
Adenomas (*n*=74)	54 (73.0%)	14 (18.9%)	6 (8.1%)	0
Healthy tissue CRC (*n*=45)	42 (93.3%)	2 (4.5%)	1 (2.2%)	0
Transitional tissue CRC (*n*=39)	29 (74.4%)	8 (20.5%)	2 (5.1%)	0
Tumour tissue CRC (*n*=155)	39 (25.2%)	49 (31.6%)	34 (21.9%)	33 (21.3%)

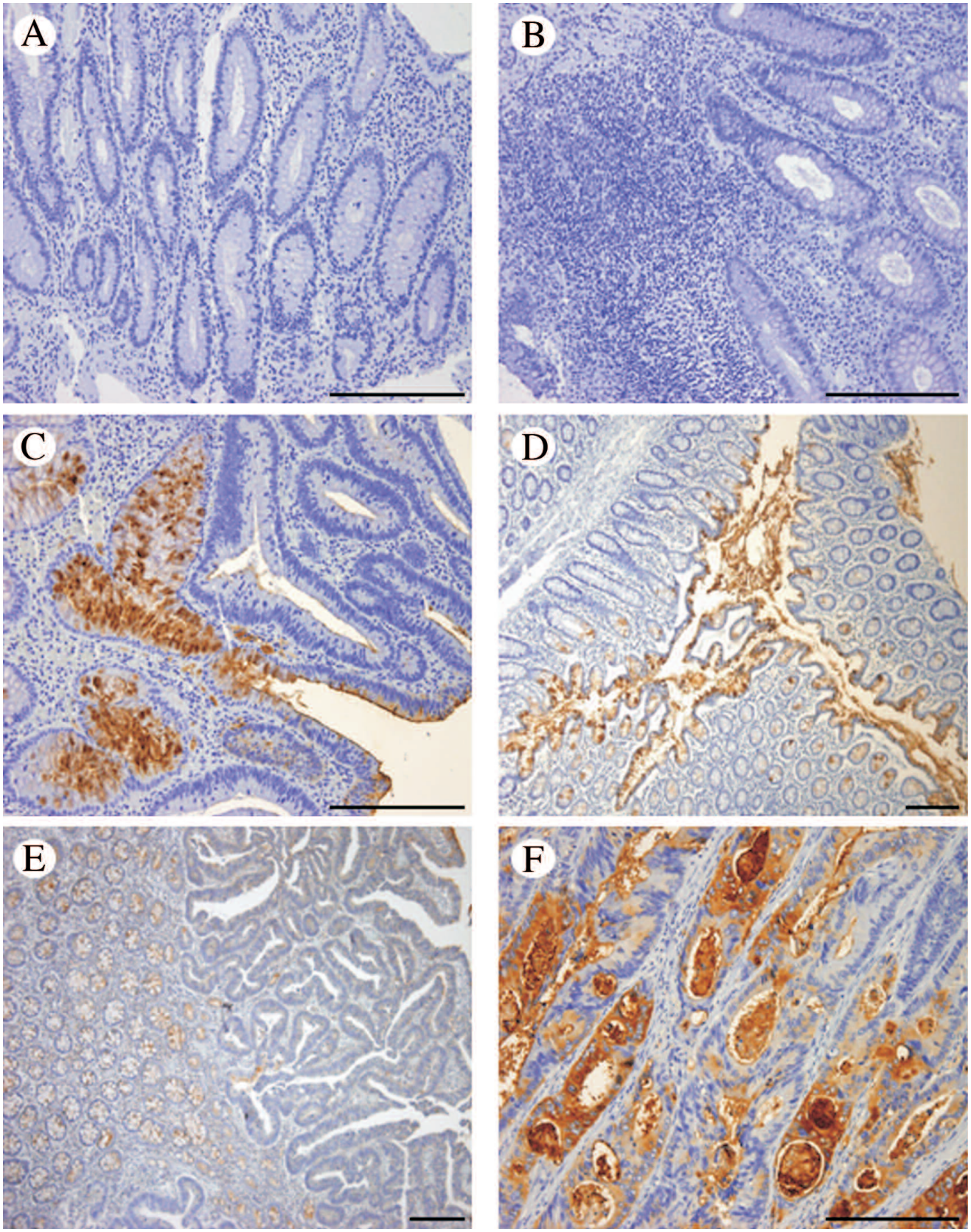

Fig. 1. Immunohistochemical expression of the sLe^a antigen. Sections from a healthy disease-free colorectal mucosa **(A)** and an inflammatory mucosa **(B)** showing no expression. Sections from a colorectal adenoma **(C),** a healthy colorectal specimen **(D)**, a transitional colorectal specimen **(E)** and a colorectal tumour specimen **(F)** with positive staining evidenced by the presence of a brown precipitate. Scale bar: 50 μm.

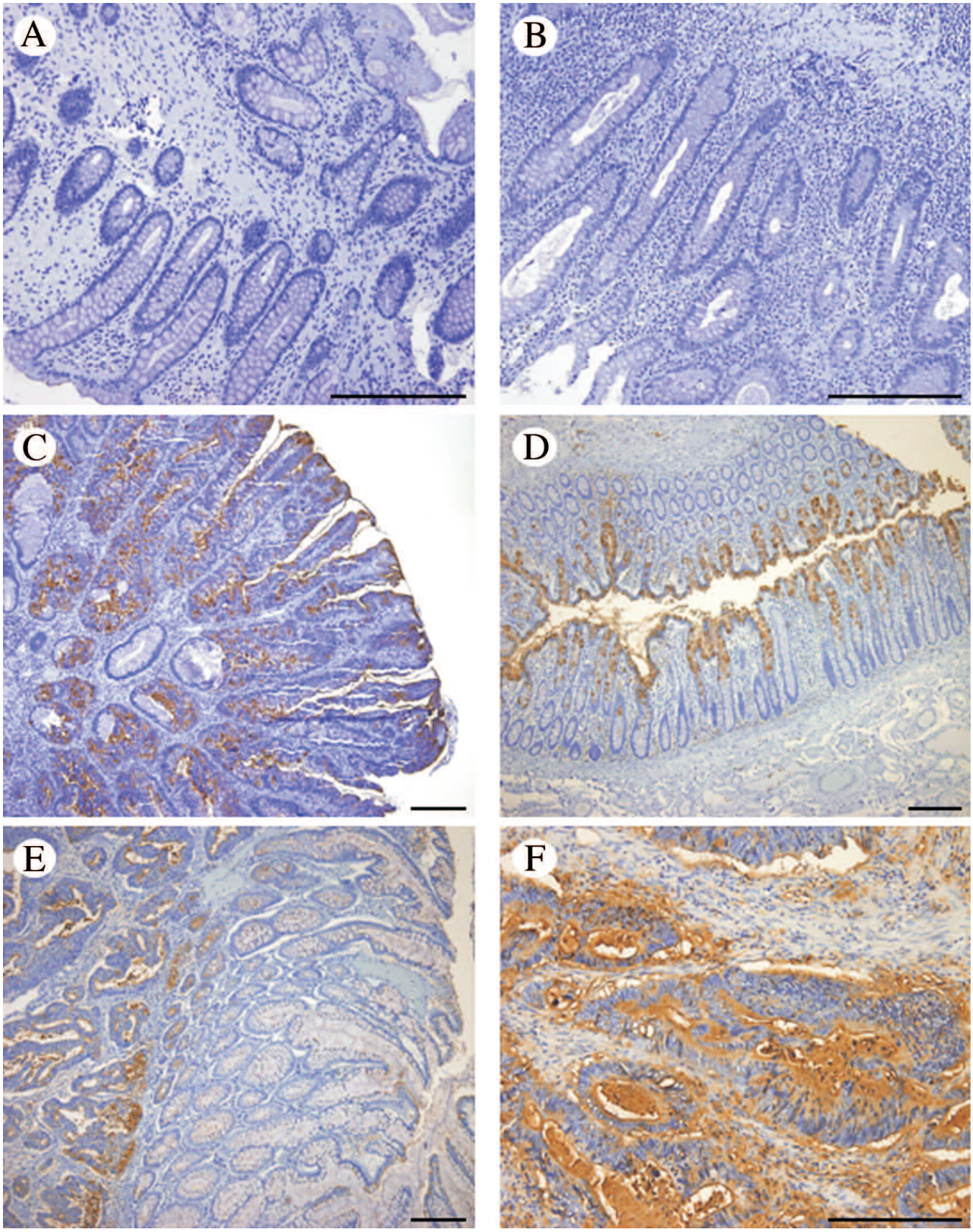

Fig. 2. Immunohistochemical expression of the sLe^x antigen. Healthy disease-free colorectal mucosa **(A)** and inflammatory mucosa **(B)** showing no expression. Positive expression, evidenced by the presence of a brown precipitate, in a colorectal adenoma **(C)**, a healthy colorectal specimen **(D)**, a transitional colorectal specimen **(E)** and a colorectal tumour specimen **(F)**. Scale bar: 50 μm.

adenomas, inflammatory and healthy disease-free mucosa (p<0.001, according to Mann Whitney U test). The differences in sLe^x expression between the transitional tissue and the healthy tissue from CRC were also statistically significant (p=0.025, according to Wilcoxon's test).

Correlation analysis between sLe^a and sLe^x expression in colorectal tissues

A statistical analysis based on χ^2 test was performed to establish a correlation between sLe^a and sLe^x antigen expression in colorectal tissues. The results obtained

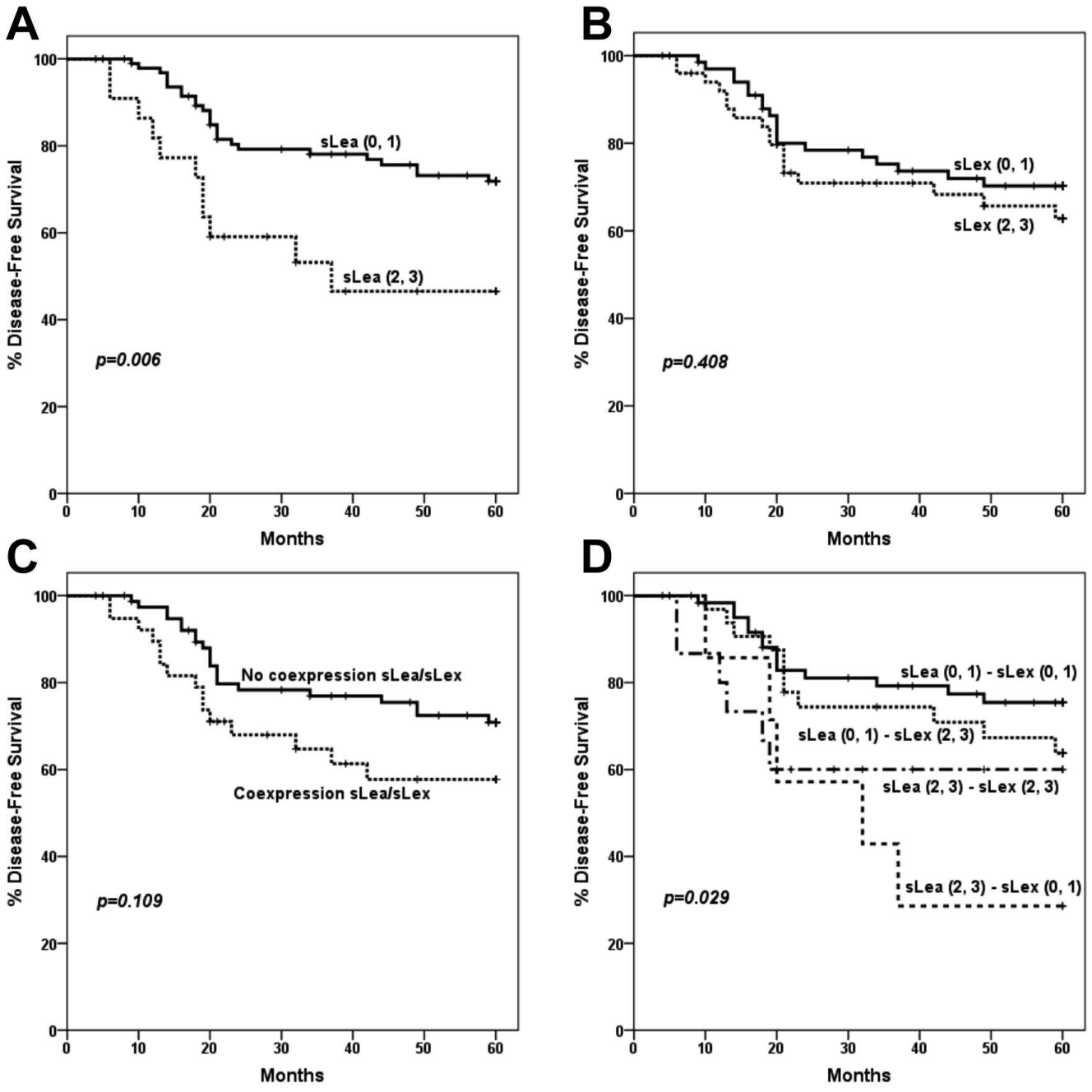

Fig. 3. Disease-free survival of CRC patients in relation to immunohistochemical sLe^a **(A)** and sLe^x **(B)** antigen expression according to staining results scored as low expression (0 or 1) and high expression (2 or 3). Disease-free survival curves corresponding to the immunohistochemical sLe^a and sLe^x coexpression **(C)** and expression groups **(D).**

showed a statistically significant correlation between sLe^a and sLe^x expression in colorectal adenomas (p<0.001), and in healthy (p<0.001) and tumour tissue (p=0.005) from CRC patients.

Relationship between sLe^a and sLe^x expression and clinicopathological features

In order to elucidate if sLe^a and sLe^x expression in CRC and colorectal adenomas was associated with the clinicopathological features, a correlation analysis was carried out. For this analysis we considered low expression cases (0 or 1) and high expression cases (2 or 3). The correlation between the coexpression of sLe^a and sLe^x and the clinicopathological features was also analyzed. For this purpose, we first compared the cases with no coexpression (where one of the antigens is not expressed) against the cases with coexpression (those where both antigens are expressed in any degree of intensity), and second, we made all the comparisons between low (0 or 1) vs. high (2 or 3) groups: sLe^a (0 or 1) – sLe^x (0 or 1), sLe^a (0 or 1) – sLe^x (2 or 3), sLe^a (2

Table 2. Comparison of sLe^a and sLe^x antigens expression and coexpression and the clinicopathological features.

Clinicopathological features	sLe^a			sLe^x			sLe^a - sLe^x Coexpression		
	Low (0 or 1)	High (2 or 3)	*p*	Low (0 or 1)	High (2 or 3)	*p*	Yes	No	*p*
Sex									
Male	71 (77.2)	21 (22.8)	0.844	53 (58.9)	37 (41.1)	0.622	59 (65.6)	31 (34.4)	0.862
Female	50 (79.4)	13 (20.6)		35 (53.8)	30 (46.2)		43 (68.3)	20 (31.7)	
Age (years)									
< 67	43 (81.1)	10 (18.9)	0.625	30 (56.6)	23 (43.4)	0.328	38 (71.7)	15 (28.3)	0.426
67 - 77	42 (79.2)	11 (20.8)		35 (63.6)	20 (36.4)		36 (67.9)	17 (32.1)	
> 77	36 (73.5)	13 (26.5)		23 (48.9)	24 (51.1)		28 (59.6)	19 (40.4)	
Tumour size (cm)									
< 4.1	54 (75.0)	18 (25.0)	0.694	38 (52.8)	34 (47.2)	0.303	46 (64.8)	25 (35.2)	0.887
4.1 - 5.0	32 (78.0)	9 (22.0)		22 (55.0)	18 (45.0)		27 (67.5)	13 (32.5)	
> 5.0	32 (82.1)	7 (17.9)		27 (67.5)	13 (32.5)		27 (69.2)	12 (30.8)	
Growth pattern									
Polypoid	63 (78.8)	17 (21.2)	0.848	44 (54.3)	37 (45.7)	0.626	53 (66.3)	27 (33.7)	1.000
Non-polypoid	58 (77.3)	17 (22.7)		44 (59.5)	30 (40.5)		49 (67.1)	24 (32.9)	
Tumour location									
Proximal colon	24 (75.0)	8 (25.0)	0.161	20 (58.8)	14 (41.2)	0.490	16 (50.0)	16 (50.0)	0.067
Distal colon	31 (70.5)	13 (29.5)		28 (63.6)	16 (36.4)		32 (72.7)	12 (27.3)	
Rectum	66 (84.6)	12 (15.4)		40 (52.6)	36 (47.4)		54 (71.1)	22 (28.9)	
Histological type									
Well differentiated	17 (89.5)	2 (10.5)	0.393	14 (73.7)	5 (26.3)	0.185	15 (78.9)	4 (21.1)	0.280
Moderately differentiated	91 (76.5)	28 (23.5)		63 (53.4)	55 (46.6)		76 (65.0)	41 (35.0)	
Poorly differentiated	6 (85.7)	1 (14.3)		5 (71.4)	2 (28.6)		6 (85.7)	1 (14.3)	
pTNM classification									
pT (Primary tumour extent)									
T1	5 (100)	0 (0)	0.127	5 (100)	0 (0)	0.252	5 (100)	0 (0)	0.252
T2	20 (90.9)	2 (9.1)		13 (59.1)	9 (40.9)		16 (72.7)	6 (27.3)	
T3	58 (71.6)	23 (28.4)		45 (54.9)	37 (45.1)		49 (61.2)	31 (38.8)	
T4	38 (80.9)	9 (19.1)		25 (54.3)	21 (45.7)		32 (69.6)	14 (30.4)	
pN (Lymph node metastasis)									
N0	77 (81.9)	17 (18.1)	0.168	59 (62.1)	36 (37.9)	0.099	62 (66.7)	31 (33.3)	1.000
N1/N2	44 (72.1)	17 (27.9)		29 (48.3)	31 (51.7)		40 (66.7)	20 (33.3)	
pM (Distant metastasis)									
M0	108 (80.6)	26 (19.4)	0.085	80 (59.7)	54 (40.3)	0.096	89 (67.4)	43 (32.6)	0.625
M1	13 (61.9)	8 (38.1)		8 (38.1)	13 (61.9)		13 (61.9)	8 (38.1)	
TNM stage									
I	23 (92.0)	2 (8.0)	0.333	17 (68.0)	8 (32.0)	0.425	19 (76.0)	6 (24.0)	0.895
IIa	37 (80.4)	9 (19.6)		29 (61.7)	18 (38.3)		28 (62.2)	17 (37.8)	
IIb	16 (76.2)	5 (23.8)		12 (57.1)	9 (42.9)		14 (66.7)	7 (33.3)	
IIIa	1 (100)	0 (0)		1 (100)	0 (0)		1 (100)	0 (0)	
IIIb	21 (77.8)	6 (22.2)		13 (50.0)	13 (50.0)		17 (65.4)	9 (34.6)	
IIIc	10 (71.4)	4 (28.6)		8 (57.1)	6 (42.9)		10 (71.4)	4 (28.6)	
IV	13 (61.9)	8 (38.1)		8 (38.1)	13 (61.9)		13 (61.9)	8 (38.1)	

Staining scores were grouped as low expression cases (0 or 1) and high expression cases (2 or 3).

or 3) – sLe^x (2 or 3), sLe^a (2 or 3) – sLe^x (0 or 1).

The results from both studies indicated no correlation between sLe^a and sLe^x expression or coexpression in CRC and the clinicopathological features (Table 2). However, a statistically significant correlation was found for high sLe^x expression in adenomas and high grade of dysplasia (p=0.042, Table 3).

Relationship between sLe^a and sLe^x expression and tumour recurrence

To study the influence of sLe^a and sLe^x expression and coexpression in the prognosis of CRC patients, a systematic 5-year survival analysis was carried out. The analysis was performed with 123 patients, 35 having been excluded from the initial 158-person cohort on the basis of metastasis at surgery (21 cases), postoperative mortality (9 incidences), or due to a lack of complete patient records (5 cases). During follow-up, recurrence was detected in 36 patients, local recurrence in 12 cases and distant metachronous metastasis in 24 cases. Cases affected by both local and distant disease recurrence (6) were considered according to the first event clinically reported.

Table 3. Relationship between sLe^x antigen expression and the clinicopathological features of colorectal adenomas.

Clinicopathological features	sLe^x Expression		
	Low (0 or 1)	High (2)	*p*
Histological type			
Tubular	38 (92.7)	3 (7.3)	0.601
Tubular-Villous	26 (92.9)	2 (7.1)	
Villous	4 (80.0)	1 (20.0)	
Grade of dysplasia			
Low grade (low)	52 (96.3)	2 (3.7)	0.042*
High grade (moderate or severe)	16 (80.0)	4 (20.0)	
Localization			
Colon	54 (91.5)	5 (8.5)	0.549
Rectum	7 (87.5)	1 (12.5)	

Staining scores were grouped as low expression cases (0 or 1) and high expression cases (2). *p<0.05 based on Fisher's exact probability test.

Table 5. Multivariate analysis of predictive factors for disease-free survival.

Clinicopathological features	Relative risk	95% CI	*p*
pN (Lymph node metastasis)			
pN0 (Absent) vs. pN1/N2 (Present)	2.414	1.161-5.019	0.018*
sLe^a expression			
Low (0 or 1) vs. High (2 or 3)	2.683	1.237-5.821	0.012*

CI, confidence interval. *p<0.05 based on the Cox regression analysis.

The disease-free survival rate of patients was significantly lower for sLe^a high expression cases (46.5%) than for sLe^a low expression ones (71.9%) (p=0.006, Fig. 3A). However, the difference in the

Table 4. Univariate analysis of factors affecting disease-free survival.

Clinicopathological features	n	Survival rate (%)	*p*
Sex			
Male	73	65.6	0.745
Female	50	70.8	
Age (years)			
< 67	41	67.7	0.776
67 - 77	44	72.4	
> 77	38	60.8	
Tumour location			
Proximal colon	25	63.2	0.726
Distal colon	32	71.5	
Rectum	65	69.3	
Tumour size (cm)			
< 4.1	62	70.4	0.887
4.1 - 5.0	27	71.6	
> 5.0	31	68.9	
Growth pattern			
Polypoid	66	73.1	0.290
Non-polypoid	57	62.6	
Tumour differentiation			
Well differentiated	16	85.1	0.458
Moderately differentiated	97	67.5	
Poorly differentiated	5	80.0	
pTNM classification			
pT (Primary tumour extent)			
pT1	5	100	0.298
pT2	22	75.2	
pT3	68	68.7	
pT4	28	52.2	
pN (Lymph node metastasis)			
pN0	84	75.9	0.004*
pN1/N2	39	51.5	
TNM stage			
I	25	79.2	0.012*
IIa	41	81.6	
IIb	18	58.7	
IIIa	1	100	
IIIb	26	59.9	
IIIc	12	33.3	
sLe^a expression			
Low (0 or 1)	97	71.9	0.006*
High (2 or 3)	23	46.5	
sLe^x expression			
Low (0 or 1)	70	70.2	0.408
High (2 or 3)	50	62.8	
Coexpression sLe^a-sLe^x			
No coexpression	79	70.8	0.109
Coexpression	39	57.7	
Expression groups sLe^a-sLe^x			
sLe^a (0 or 1) – sLe^x (0 or 1)	62	75.4	0.029*
sLe^a (0 or 1) – sLe^x (2 or 3)	33	63.8	
sLe^a (2 or 3) – sLe^x (2 or 3)	15	60.0	
sLe^a (2 or 3) – sLe^x (0 or 1)	8	28.6	

*p<0.05 based on the log-Rank test.

disease-free survival rate between the sLe^x high expression group (62.8%) and the sLe^x low expression group (70.2%), was not statistically significant (p=0.408, Fig. 3B). Similarly, the differences between the disease-free survival rate of patients with sLe^a and sLe^x coexpression (57.7%) and the patients with no coexpression (70.8%) was not statistically significant (p=0.109, Fig. 3C). However, the disease-free survival rate of the sLe^a (2 or 3) and sLe^x (0 or 1) group was significantly lower than the survival of the other expression groups (p=0.029, Fig. 3D). Univariate analysis showed that both the TNM stage (p=0.012) and the lymph node metastasis (p=0.004) were significantly associated with a lower disease-free survival rate (Table 4).

To compare the independent predictive value of the clinicopathological variables for disease-free survival, a multivariate Cox regression analysis was performed. This analysis revealed that sLe^a high expression (p=0.012) and lymph node metastasis (p=0.018) were independent prognostic factors for predicting 5-year disease-free survival (Table 5).

Discussion

Malignant transformation is frequently associated with alterations in the expression of cell surface glycoconjugates. The use of monoclonal antibodies now permits detailed histological studies of glycoconjugate distribution to be performed, as well as enabling the monitoring of changes in those expression patterns to occur in tumours (Alhadeff, 1989; Hakomori, 1989). Many tumour-associated antigens are sialylated structures, such as the sLe^a and sLe^x antigens, whose increased expression has been described in numerous carcinomas (Le Pendu et al., 2001).

In the present study we analyzed the immunohistochemical expression of the sLe^a and sLe^x antigens in different colorectal tissues, ranging from healthy disease-free mucosa to adenocarcinoma specimens, in an attempt to elucidate their implication in the malignant transformation of colorectal mucosa. The results indicated no expression of sLe^a and sLe^x in healthy disease-free mucosa. However, we detected expression of both antigens in 6.7% of the healthy tissue specimens from CRC. Such expression of sLe^a and sLe^x in healthy tissue from CRC has been described previously (Nakagoe et al., 1991; Ogata et al., 1995; Shimodaira et al., 1997). Since the antigens were not found in the colorectal mucosa from healthy patients, the expression detected in the healthy colorectal tissue from CRC may be a specific consequence of the tumour process. Likewise, the absence of sLe^a and sLe^x expression in the inflammatory mucosa suggests that their expression in the tumour process is specific and not a consequence of the tumour-associated inflammation.

Concerning the analysis of the colorectal adenomas, we observed the expression of sLe^a and sLe^x in 20.8% and 27.0% of the specimens, respectively. In addition, moderate sLe^x expression was correlated with high grade of dysplasia, a characteristic trait of adenomatous malignancy. These findings are in accordance with those of Hanski et al. (1990), but contradict other studies (Yuan et al., 1987; Baldus et al., 1995, 2002). In the case of sLe^a, a significant correlation between its expression and severe dysplasia had also been previously reported (Afdhal et al., 1987), although most of the studies, in agreement with our results, found no association with the clinicopathological features of adenomas (Baldus et al., 2002; Allen et al., 1987; Tucci et al., 1999). Either way the finding that sLe^a and sLe^x are expressed in the early stages of the adenoma-carcinoma pathway, together with the correlation between sLe^x expression and high grade dysplasia, could be indicative of their involvement in the malignant transformation of colorectal adenomas.

Immunohistochemical analysis of sLe^a and sLe^x in the transitional tissue located adjacent to the tumour revealed a statistically significant increase in the expression of both antigens when compared to their counterpart healthy tissue from CRC. Alterations in the transitional mucosa, such as an abnormal production of mucins, have led some authors to hypothesize the premalignant condition of this tissue (Mori et al., 1990; Tamai et al., 1998). We agree that the transitional mucosa represents a premalignant stage, exhibiting the molecular profile of the tumour and displaying overexpression of characteristic CRC-associated antigens.

The highest levels of sLe^a and sLe^x expression were observed in tumour tissue, with percentages of 42.6% and 74.8%, respectively. Likewise, the statistical analysis revealed a significant enhacement in sLe^a and sLe^x expression in tumourous tissue compared with the healthy tissue from CRC. In order to ascertain whether the tumour expression of sLe^a and sLe^x is correlated with the progression of CRC, a statistical analysis was carried out. Despite sLe^a and sLe^x expression being increased in cases with lymph node and distant metastasis, and therefore seeming to indicate involvement of these antigens in tumour progression, no statistically significant association was found.

Furthermore, taking into account the structure and biosynthetic similarities between sLe^a and sLe^x, we analyzed their hypothetical correlation in the different colorectal tissues studied. To our knowledge, there are no previous studies focusing on sLe^a and sLe^x coexpression in CRC. Our results reveal a correlation between the antigens' expression in the colorectal adenomas as well as in the healthy and tumour tissue from CRC. The correlation between sLe^a and sLe^x expression in tumour tissue is remarkable despite their different levels of expression. The presence of both antigens in tumourous colorectal specimens may be the result of a common mechanism acting upon both sLe^a and sLe^x expression, likely candidates being the activities of the glycosyltransferases involved in their biosynthesis. However, there is no conclusive evidence

to support the tenet that the correlation between overexpressed sLe^a and sLe^x in CRC arises from the enhanced activity of glycosyltranferases. Moreover, the correlation analysis did not reveal any association between clinicopathological features and sLe^a and sLe^x coexpression in tumour tissue.

The prognostic value of sLe^a and sLe^x expression in CRC has been widely studied in the Japanese population, and several reports have verified sLe^a and sLe^x expression during CRC as useful markers of patient prognosis (Shimono et al., 1994; Nakayama et al., 1995; Matsui et al., 2004). In this study we have shown that high levels of sLe^a expression are significantly associated with a reduced disease-free survival rate in patients. In addition, a multivariate analysis revealed that high sLe^a expression is an independent factor for predicting tumour recurrence. It is well known that the sLe^a antigen is a ligand for the adhesion molecule E-selectin (Berg et al., 1991; Takada et al., 1991), and that the interaction between sLe^a in the tumour cells and the vascular activated endothelium cells expressing E-selectin leads to the extravasation of the tumour cells through the endothelium, a key step in the hematogenous metastasis of cancer. Our results, showing that high sLe^a levels are associated with a shorter disease-free survival, agree with recent evidence which showed that CRC metastatic cells expressing high levels of sLe^a were more efficient at this extravasation than those expressing low levels of the antigen (Ben-David et al., 2008).

The prognostic value of the sLe^x antigen in colorectal cancer has been previously reported (Nakagoe et al., 1993; Nakamori et al., 1993, 1997; Grabowski et al., 2000); however we do not find this association. Nevertheless, Takada et al. (1993) suggested that sLe^a plays a more important role than sLe^x in the hematogenous metastasis of cancer from digestive organs, with the metastasis of lung, liver and ovarian carcinomas more likely to be mediated by sLe^x. While we did observe a decrease in the disease-free survival rate paralell to the concomitant increase in sLe^x expression (from none to moderate), this trend was not maintained, and the cases with high expression of sLe^x showed the highest disease-free survival rates. Interestingly, Ohyama et al. (2002) previously reported that those tumour cells which express high levels of sLe^x promote natural killer cell-mediated cytolysis, while those tumour cells expressing lower levels of sLe^x avoid such attacks and thus lead to the formation of metastasic lesions.

sLe^a serum levels have been extensively studied and validated as a biomarker for the diagnosis and prognosis of patients with gastrointestinal tumours. Nevertheless, with the aim of improving this diagnostic and prognostic potential, many different studies have analyzed sLe^a levels in combination with other serum markers that are used in the clinical practice of CRC (Carpelan-Holmström et al., 2002; Chen et al., 2005; Nozoe et al., 2006). Meanwhile, other studies have assayed the combination of sLe^a and sLe^x tissue and serum levels in CRC patient prognosis (Nakayama et al., 1997; Gebauer and Müller-Ruchholtz, 2001; Akamine et al., 2004). To our knowledge, the prognostic value of combined sLe^a and sLe^x expression has not been previously studied in CRC. We observed a statistically significant correlation between the sLe^a and sLe^x antigens' expression in CRC. This finding motivated us to study the influence sLe^a and sLe^x coexpression in patient tumour recurrence. Although patients who coexpressed both antigens showed a lower disease-free survival in comparison with those that did not, the differences were not statistically significant, with the exception of sLe^a (2 or 3) and sLe^x (0 or 1) group. This last result suggests that high sLe^a expression plays the more important role in the progression of CRC. Consequently, immunohistochemical analysis of the coexpression of sLe^a and sLe^x is not useful for predicting tumour recurrence in CRC patients.

In conclusion, we have demonstrated that the sLe^a and sLe^x antigens are detected in the early stages of colorectal tumourigenesis through the adenoma-carcinoma pathway. These antigens undergo a progressive increase in their tissue expression as the malignant transformation of colorectal mucosa progresses from the healthy disease-free mucosa to adenocarcinoma. Likewise, the high expression of sLe^a is an independent prognostic variable for predicting recurrence in CRC patients. Finally, we suggested that the clinical evaluation of sLe^a immunohistochemical expression in CRC could be useful in the planning of coadjuvant therapies after surgical resection, especially in those patients with high sLe^a expression in tumour tissue.

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References

Afdhal N.H., Long A., Tobbia I., Cullen A. and O'Donoghue D.P. (1987). Immunohistochemical Ca19-9 in primary colonic polyps and polyps synchronous with colorectal cancer. Gut 28, 594-600.

Akamine S., Nakagoe T., Sawai T., Tsuji T., Tanaka K., Hidaka S., Shibasaki S.I., Nanashima A., Yamaguchi H., Nagayasu T. and Yasutake T. (2004). Differences in prognosis of colorectal cancer patients based on the expression of sialyl Lewis^a, sialyl Lewis^x and sialyl Tn antigens in serum and tumor tissue. Anticancer Res. 24, 2541-2546.

Alhadeff J.A. (1989). Malignant cell glycoproteins and glycolipids. Crit. Rev. Oncol. Hematol. 9, 37-107.

Allen D.C., Foster H., Orchin J.C. and Biggart J.D. (1987). Immunohistochemical staining of colorectal tissues with monoclonal

antibodies to ras oncogene p21 product and carbohydrate determinant antigen 19-9. J. Clin. Pathol. 40, 157-162.

Baldus S.E., Hanisch F.G., Pütz C., Flucke U., Mönig S.P., Schneider P.M., Thiele J., Hölscher A.H. and Dienes H.P. (2002). Immunoreactivity of Lewis blood group and mucin peptide core antigens: correlations with grade of dysplasia and malignant transformation in the adenoma-carcinoma sequence. Histol. Histopathol. 17, 191-198.

Baldus S.E., Vierbuchen M., Hanisch F.G., Schwonzen M. and Fischer R. (1995). Expression of alpha-3/4-monofucosylated poly-lactosaminoglycan epitope, as defined by monoclonal antibody FW6, is a marker of the colorectal adenoma-carcinoma sequence. Cancer 76, 954-960.

Ben-David T., Sagi-Assif O., Meshel T., Lifshitz V., Yron I. and Witz I.P. (2008). The involvement of the sLe-a selectin ligand in the extravasation of human colorectal carcinoma cells. Immunol. Lett. 116, 218-224.

Berg E.L., Robinson M.K, Mansson O., Butcher E.C. and Magnani J.L. (1991). A carbohydrate domain common to both sialyl Le^a and sialyl Le^x is recognized by the endothelial cell leukocyte adhesion molecule ELAM-1. J. Biol. Chem. 266, 14869-14872.

Carpelan-Holmström M., Louhimo J., Stenman U.H., Alfthan H. and Haglund C. (2002). CEA, CA 19-9 and CA 72-4 improve the diagnostic accuracy in gastrointestinal cancers. Anticancer Res. 22, 2311-2316.

Chen C.C., Yang S.H., Lin J.K., Lin T.C., Chen W.S., Jiang J.K., Wang H.S. and Chang S.C. (2005). Is it reasonable to add preoperative serum level of CEA and CA19-9 to staging for colorectal cancer? J. Surg. Res. 124, 169-174.

Cox D.R. (1972). Regression models and life tables. J. R. Stat. Soc. Ser. B-Stat. Methodol. 34, 187-220.

Foxall C., Watson S.R., Dowbenko D., Fennie C., Lasky L.A., Kiso M., Hasegawa A., Asa D. and Brandley B.K. (1992). The three members of the selectin receptor family recognize a common carbohydrate epitope, the sialyl Lewis^x oliogosaccharide. J. Cell Biol. 117, 895-902.

Fukuda M., Spooncer E., Oates J.E., Dell A. and Klock J.C. (1984). Structure of sialylated fucosyl lactosaminoglycan isolated from human granulocytes. J. Biol. Chem. 259, 10925-10935.

Gebauer G. and Müller-Ruchholtz W. (2001). Carcinoembryonic antigen and CA19-9: implications of quantitative marker measurement in tissues for prognosis of colorectal cancer. Cancer Detect. Prev. 25, 344-351.

Grabowski P., Mann B., Mansmann U., Lövin N., Foss H.D., Berger G., Scherübl H., Riecken E.O., Buhr H.J. and Hanski C. (2000). Expression of sialyl-Le^x antigen defined by MAb AM-3 is an independent prognostic marker in colorectal carcinoma patients. Int. J. Cancer 88, 281-286.

Hakomori S. (1989). Aberrant glycosylation in tumors and tumor-associated carbohydrate antigens. Adv. Cancer Res. 52, 257-331.

Hakomori S. (2002). Glycosylation defining cancer malignancy: new wine in an old bottle. Proc. Natl. Acad. Sci. USA 99, 10231-10233.

Hanai N., Shitara K. and Yoshida H. (1986). Generation of monoclonal antibodies against human lung squamous cell carcinoma and adenocarcinoma using mice rendered tolerant to normal human lung. Cancer Res. 46, 4438-4443.

Hanski C., Bornhoeft G., Topf N., Hermann U., Stein H. and Riecken E.O. (1990). Detection of a mucin marker for the adenoma-carcinoma sequence in human colonic mucosa by monoclonal antibody AM-3. J. Clin. Pathol. 43, 379-384.

Itzkowitz S.H., Yuan M., Fukushi Y., Palekar A., Phelps P.C., Shamsuddin A.M., Trump B.F., Hakomori S. and Kim Y.S. (1986). Lewis^x- and sialylated Lewis^x-related antigen expression in human malignant and non malignant colonic tissues. Cancer Res. 46, 2627-2632.

Itzkowitz S.H., Yuan M., Fukushi Y., Lee H., Shi Z., Zurawski V., Hakomori S. and Kim Y.S. (1988). Immunohistochemical comparison of Le^a, monosialosyl Le^a (CA 19-9) and disialosyl Le^a antigens in human colorectal and pancreatic tissues. Cancer Res. 48, 3834-3842.

Kannagi R., Izawa M., Koike T., Miyazaki K. and Kimura N. (2004). Carbohydrate-mediated cell adhesion in cancer metastasis and angiogenesis. Cancer Sci. 95, 377-384.

Kannagi R., Sakuma K., Miyazaki K., Lim K.T., Yusa A., Yin J. and Izawa M. (2010). Altered expression of glycan genes in cancers induced by epigenetic silencing and tumor hypoxia: Clues in the ongoing search for new tumor markers. Cancer Sci. 101, 586-593.

Kaplan E.L. and Meier P. (1958). Nonparametric estimation from incomplete observations. J. Am. Stat. Assoc. 53, 457-481.

Kim Y.J. and Varki A. (1997). Perspectives of the significance of altered glycosylation of glycoproteins in cancer. Glycoconj. J. 14, 569- 576.

Koprowski H., Steplewski Z., Mitchell K., Herlyn M., Herlyn D. and Fuhrer P. (1979). Colorectal carcinoma antigens detected by hybridoma antibodies. Somatic. Cell. Genet. 5, 957-971.

Le Pendu J., Marionneau S., Cailleau Thomas A., Rocher J., Le Moullac Vaidye B. and Clément M. (2001). ABH and Lewis histo-blood group antigens in cancer. APMIS 109, 9-31.

Lowe J.B., Stoolman L.M., Nair R.P., Larsen R.D., Berhend T.L. and Marks R.M. (1990). ELAM-1-dependent cell adhesion to vascular endothelium determined by a transfected human fucosyltransferase cDNA. Cell 63, 475-484.

Magnani J.L. (2004). The discovery, biology, and drug development of sialyl Le^a and sialyl Le^x. Arch. Biochem. Biophys. 426, 122-131.

Magnani J.L., Nilsson B., Brockhaus M., Zopf D., Steplewski Z., Koprowski H. and Ginsburg V. (1982). A monoclonal antibody-defined antigen associated with gastrointestinal cancer is a ganglioside containing sialylated lacto-N-fucopentaose II. J. Biol. Chem. 257, 14365-14369.

Manne U., Weiss H.L., Myers R.B., Danner O.K., Moron C., Srivastava S. and Grizzle W.E. (1998). Nuclear accumulation of p53 in colorectal adenocarcinoma: prognostic importance differs with race and location of the tumor. Cancer 83, 2456-2467.

Manne U., Weiss H.L. and Grizzle W.E. (2000). Racial differences in the prognostic usefulness of MUC1 and MUC2 in colorectal adenocarcinomas. Clin. Cancer Res. 6, 4017-4025.

Mantel N. (1966). Evaluation of survival data and two new rank order statistics arising in its consideration. Cancer Chemother. Rep. 50, 163-170.

Matsui T., Kojima H., Suzuki H., Hamajima H., Nakazato H., Ito K., Nakao A. and Sakamoto J. (2004). Sialyl Lewis^a expression as a predictor of the prognosis of colon carcinoma patients in a prospective randomized clinical trial. Jpn. J. Clin. Oncol. 34, 588-593.

Mori M., Shimono R., Adachi Y., Matsuda H., Kuwano H., Sugimachi K., Ikeda M. and Saku M. (1990). Transitional mucosa in human colorectal lesions. Dis. Colon Rectum 33, 498-501.

Nakagoe T., Fukushima K., Hirota M., Kusano H., Ayabe H., Tomita M. and Kamihira S. (1993). Immunohistochemical expression of sialyl

Le^x antigen in relation to survival of patients with colorectal carcinoma. Cancer 72, 2323-2330.

Nakagoe T., Kusano H., Hirota M., Fukushima K., Hiratani K., Hara K. and Tomita M. (1991). Serological and immunohistochemical studies on sialylated carbohydrate antigens in colorectal carcinoma. Gastroenterol. Jpn. 26, 303-311.

Nakamori S., Kameyama M., Imaoka S., Furukawa H., Ishikawa O., Sasaki Y., Kabuto T., Iwanaga T., Matsushita Y. and Irimura T. (1993). Increased expression of sialyl Lewis^x antigen correlates with poor survival in patients with colorectal carcinoma: clinicopathological and immunohistochemical study. Cancer Res. 53, 3632-3637.

Nakamori S., Kameyama M., Imaoka S., Furukawa H., Ishikawa O., Sasaki Y., Izumi Y. and Irimura T. (1997). Involvement of carbohydrate antigen sialyl Lewis^x in colorectal cancer metastasis. Dis. Colon Rectum 40, 420-431.

Nakayama T., Watanabe M., Katsumata T., Teramoto T. and Kitajima M. (1995). Expression of sialyl Lewis^a as a new prognostic factor for patients with advanced colorectal carcinoma. Cancer 75, 2051-2056.

Nakayama T., Watanabe M., Teramoto T. and Kitajima M. (1997). CA19-9 as a predictor of recurrence in patients with colorectal cancer. J. Surg. Oncol. 66, 238-243.

Nozoe T., Rikimaru T., Mori E., Okuyama T. and Takahashi I. (2006). Increase in both CEA and CA19-9 in sera is an independent prognostic indicator in colorectal carcinoma. J. Surg. Oncol. 94, 132-137.

Ogata S., Ho I., Chen A., Dubois D., Maklansky J., Singhal A., Hakomori S. and Itzkowitz S.H. (1995). Tumor-associated sialylated antigens are constitutively expressed in normal colonic mucosa. Cancer Res. 55, 1869-1874.

Ohyama C., Kanto S., Kato K., Nakano O., Arai Y., Kato T., Chen S., Fukuda M.N. and Fukuda M. (2002). Natural killer cells attack tumor cells expressing high levels of sialyl Lewis x oligosaccharides. Proc. Natl. Acad. Sci. USA 99, 13789-13794.

Ørtonft T.F. and Bech E. (1995). Circulating blood group related carbohydrate antigens as tumour markers. Glycoconj. J. 12, 200-205.

Phillips M.L., Nudelman E., Gaeta F.C., Perez M., Singhal A.K., Hakomori S. and Paulson J.C. (1990). ELAM-1 mediates cell adhesion by recognition of a carbohydrate ligand, sialyl-Lex. Science 250, 1130-1132.

Rauvala H. (1976). Gangliosides of human kidney. J. Biol. Chem. 251, 7517-7520.

Shimodaira K., Nakayama J., Nakamura N., Hasebe O., Katsuyama T. and Fukuda M. (1997). Carcinoma-associated expression of core 2 ß-1,6-N-acetyl-glucosaminyltransferase gene in human colorectal cancer: role of O-glycans in tumor progression. Cancer Res. 57, 5201-5206.

Shimono R., Mori M., Akazawa K., Adachi Y. and Sgimachi K. (1994). Immunohistochemical expression of carbohydrate antigen 19-9 in colorectal carcinoma. Am. J. Gastroenterol. 89, 101-105.

Shitara K., Hanai N. and Yoshida H. (1987). Distribution of lung adenocarcinoma-associated antigens in human tissues and sera defined by monoclonal antibodies KM-52 and KM-93. Cancer Res. 47, 1267-1272.

Sobin L.H. and Wittekind C. (2002). International Union Against Cancer (UICC) TNM Classification Of Malignant Tumours. 6th ed. John Wiley & Sons. New York.

Takada A., Ohmori K., Takahashi N., Tsuyuoka K., Yago A., Zenita K., Hasegawa A. and Kannagi R. (1991). Adhesion of human cancer cells to vascular endothelium mediated by a carbohydrate antigen, sialyl Lewis A. Biochem. Biophys. Res. Commun. 179, 713-719.

Takada A., Ohmori K., Yoneda T., Tsuyuoka K., Hasegawa A., Kiso M. and Kannagi R. (1993). Contribution of carbohydrate antigens sialyl Lewis a and sialyl Lewis x to adhesion of human cancer cells to vascular endothelium. Cancer Res. 53, 354-361.

Tamai O., Miyazato H., Shiraishi M., Kusano T. and Muto Y. (1998). Morphologic and mucin histochemical analysis of transitional zones in advanced ulcerated colorectal carcinomas: potential prognostic indicators. J. Surg. Oncol. 67, 85-89.

Tucci G.F., Grande M., Stroppa I., Federico F. and Farinon A.M. (1999). Tissue expression of carbohydrate antigen 19-9 (CA 19-9) in adenomatous lesions of the colorectum. Chir. Ital. 51, 165-172.

Walz G., Aruffo A., Kolanus W., Bevilacqua M. and Seed B. (1990). Recognition by ELAM-1 of the sialyl-Lex determinant on myeloid and tumor cells. Science 250, 1132-1135.

Yamashita K. and Watanabe M. (2009). Clinical significance of tumor markers and an emerging perspective on colorectal cancer. Cancer Sci. 100, 195-199.

Yuan M., Itzkowitz S.H., Ferrell L.D., Fukushi Y., Palekar A., Hakomori S. and Kim Y.S. (1987). Expression of Lewis^x and sialylated Lewis^x antigens in human colorectal polyps. J. Natl. Cancer Inst. 78, 479-488.

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