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Galectin-3 Expression is a Potent Prognostic Marker in Colorectal Cancer

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Abstract. *Background: Galectin-3 is a β-galactoside-binding protein whose expression has been correlated with progression and metastasis in colon cancer. It is expressed at elevated levels in a variety of neoplastic cells. The current study was designed to investigate, by clinicopathological analysis, the relationship between prognosis and galectin-3 expression, in colorectal cancer. Patients and Methods: Galectin-3 expression was evaluated using immunohistochemical staining in 121 consecutive patients with colorectal cancer. The relationship between galectin-3 expression and clinicopathological factors was analyzed. Results: Galectin-3-positive expression was detected in 79 patients (65%). The incidence of lymph node and distant metastasis in galectin 3-positive cancer was significantly higher than that in galectin-3-negative cases (p=0.0007 and p=0.014, respectively). Furthermore, cancers with galectin-3-positive expression revealed frequent venous invasion (p=0.005) and lymphatic permeation(p=0.041), larger size (p=0.016) and deeper invasion to wall(p=0.01) than in galectin-3-negative cases. While univariate analysis showed that survival in patients with galectin-3-positive expression was significantly poorer than in galectin-3-negative cases (p=0.0027), galectin-3 expression was a prognostic factor independent of Dukes' stage and lymph node metastasis by multivariate analysis. Conclusion: We propose that galectin-3 expression is an independent factor for prognosis in colorectal cancer.*

The endogenous β-galactoside-binding protein galectin-3 is a member of a gene family of widely distributed carbohydrate-binding proteins that have been implicated in cell growth, differentiation, adhesion, malignant transformation and apoptosis (1-4). Several reports have shown that galectin-3 is expressed at elevated levels in a variety of cancer cells originating from the head and neck (5), thyroid (6), stomach (7) and brain (8). It has been reported that colon cancer cells with high levels of galectin-3 also have high levels of MUC2 mucin, whereas those with low galectin-3 levels have low MUC2 levels, and that galectin-3 plays an important role in colon cancer metastasis and progression (9-11). MUC2 mucin, a high molecular weight carbohydrate-rich glycoprotein, is a major secreted product of the gastrointestinal tract (12) that is thought to be a major ligand for galectin-3 (13). Human colon cancers and cell lines derived from tumors can differ significantly in the amount of MUC2 mucin synthesized. These differences correlate with altered biochemical and biological properties, including those with relevance to the metastatic progression of colon cancer (14-16). The close relationship between galectin-3 and MUC2 mucin is thought to be one basis for the correlation of galectin-3 levels with metastasis and progression in colorectal cancer.

Furthermore, recent studies have shown that a cancer-associated glycoform of haptoglobin is a major circulating ligand for galectin-3 in the sera of patients with colon cancer (17). Haptoglobin is distinct from mucin and carcinoembryonic antigen (CEA). Galectin-3, a novel CD95-binding partner, modulates the CD95 apoptotic signal transduction pathway (4), which indicates that galectin-3 may contribute to the progression of colorectal cancer in mechanisms independent of MUC2 mucin.

The aim of the current study was to elucidate the biological significance of galectin-3 expression and to investigate its potential as a potent prognostic and biological marker.

Patients and Methods

Patients. A total of 121 consecutive patients with colorectal cancer, who had undergone surgery at our department and affiliated hospitals between April 1983 and December 1994, were used in this study. Specimens were collected from the above patients and were embedded in paraffin and stained with hematoxylin and eosin (H&E)

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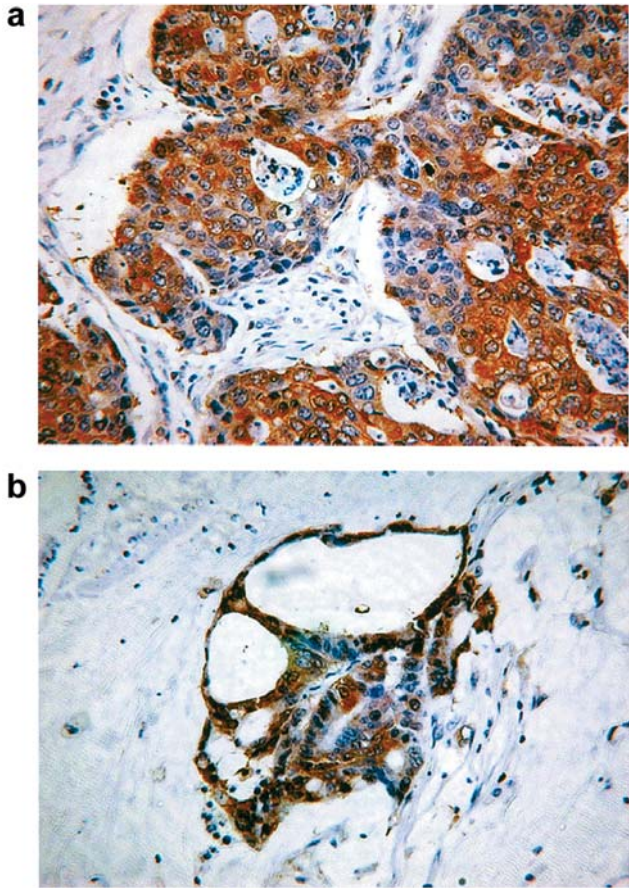

Figure 1. *a. Galectin-3 immunostaining of moderately-differentiated adenocarcinoma in the colon showing cytoplasmic expression of carcinoma cells (x200). b. Galectin-3 expression in mucinous carcinoma of the colon (x200)*

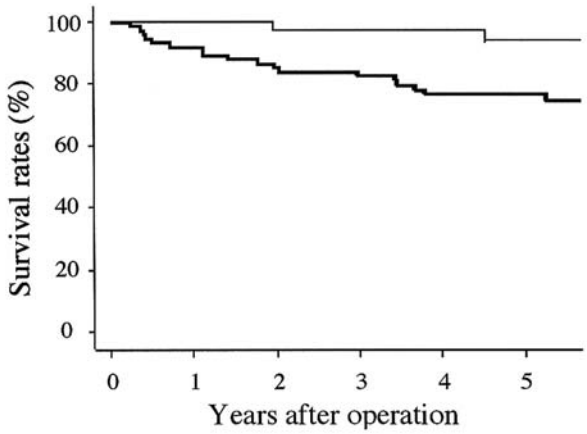

Figure 2. *Survival curve of colorectal cancer patients with positive and negative galectin-3 expression. The prognosis of patients was significantly worse in patients with galectin-3-positive expression (thick line) than in galectin-3-negative patients (thin line) (p=0.0027).*

to select representative sections of each specimen. The patients were followed-up, and only those who died of colorectal cancer were regarded as having died of tumor-related causes. The follow-up interval after surgery ranged from 54 days to 16 years and 11 months, with a mean of 6 years and 4 months. The clinicopathological results were assessed according to the general rules for clinical and pathological studies on cancers of the colon, rectum and anus outlined by the Japanese Research Society for Cancer of the Colon and Rectum (18).

Immunohistochemical staining. Immunohistochemical staining was performed using the peroxidase-labelled streptavidin-biotin technique with the Histone SAB-PO kit (Nichirei, Tokyo, Japan). Two consecutive sections of 4 μm thickness were prepared from each sample. One section was stained with H&E. The other was subjected to specific immunostaining with anti-galectin-3 antibody (clone 9C4, Neo Markers, Lab Vision Corporation, Fremont, CA, USA), a monoclonal antibody that is reactive with human galectin-3 (19, 20). Tissue sections were deparaffinized in xylene and rehydrated with a series of graded ethanols, and then were placed in phosphate-buffered saline (PBS) for 10 min. After heating the slides in citrate buffer solution (pH 6.0) for 6 min in a pressure cooker to retrieve the antigen, endogenous peroxidase activity was blocked for 30 min with methanol containing 0.3% hydrogen peroxidase. The sections were incubated with 10% non-immunized rabbit serum for 10 min to block non-specific binding of the immunoreagents, and were then reacted with the mouse anti-human monoclonal galectin-3 antibody at a 1:40 dilution for 30 min at room temperature. The sections were subsequently incubated with a second-stage biotinylated antibody for 20 min, followed by incubation with horseradish peroxidase-labelled streptavidin for 20 min at room temperature. After washing in PBS, galectin-3 localization was visualized with diaminobenzidine tetrahydrochloride.

All of the stained sections were analyzed by two observers (K.E., A.W.). In addition, results were assessed by a pathologist, who had not been given any clinical information about the sections. If the percentage of positive-staining cancer cells accounted for less than 20% of the total number of cancer cells, the staining was defined as negative and if more than 20%, staining was defined as positive.

Statistical analysis. The 121 patients were divided into two groups depending on positive or negative galectin-3 expression, and their clinicopathological characteristics were compared using the Chi-square and Mann-Whitney tests. The cumulative survival rates were calculated by the Kaplan-Meier method and the survival curves were tested by the Mantel-Cox method. Multivariate survival analysis was done according to Cox's proportional hazards model in the forward stepwise manner. A p value <0.05 was considered statistically significant.

Results

Galectin-3 was predominantly localized in the cytoplasm of cancer cells (Figure 1a, 1b). The relationship between galectin-3 expression and the clinicopathological features of the patients is shown in Table I. Based on the results of

Table I. *Galectin-3 expression and clinicopathological characteristics.*

Variables		Galectin-3-positive (n=79)	Galectin-3-negative (n=42)	*P* value
Gender	Male	42 (53.2%)	21 (50.0%)	N.S.
	Female	37 (46.8%)	21 (50.0%)	
Age	(years)	66.1±12.3	64.2±11.6	N.S.
Tumor size	(mm)	54.1±23.3	42.0±17.6	0.016
Location of tumors	Cecum and ascending colon	16 (20.3%)	5 (11.8%)	N.S.
	Transverse colon	7 (8.9%)	4 (9.6%)	
	Descending colon	3 (3.8%)	2(4.8%)	
	Sigmoid colon	27 (34.1%)	18 (42.8%)	
	Rectum	26 (32.9%)	13 (31.7%)	
Pathological type	Well	36 (45.5%)	33 (78.6%)	0.0037
	Moderately	29 (36.7%)	8 (19.0%)	
	Poorly	7 (8.9%)	0 (0.0%)	
	Mucinous	7 (8.9%)	1 (2.4%)	
Tumor depth	Mucosa	0 (0.0%)	4 (9.5%)	0.01
	Submucosa	4 (5.1%)	4 (9.5%)	
	Muscularis	8 (10.1%)	9 (21.4%)	
	Subserosa	21 (26.6%)	12 (28.6%)	
	Serosa	40 (50.6%)	11 (26.2%)	
	Invading surrounding organs	6 (7.6%)	2 (4.8%)	
Lymph node metastasis	Positive	30 (38.0%)	4 (9.5%)	0.0007
	Negative	49 (62.0%)	38 (90.5%)	
Lymphatic permeation	Positive	23 (29.1%)	5 (11.9%)	0.041
	Negative	56 (70.9%)	37 (88.1%)	
Venous invasion	Positive	28 (35.4%)	5 (11.9%)	0.005
	Negative	51 (64.6%)	37 (88.1%)	
Distant metastasis	Positive	10 (12.7%)	0 (0.0%)	0.015
	Negative	69 (87.3%)	41 (100%)	
Dukes' classification	A	9 (11.4%)	16 (38.1%)	0.0004
	B	37 (46.8%)	21 (50.0%)	
	C	23 (29.1%)	5 (9.8%)	
	D	10 (12.7%)	0 (0.0%)	

N.S.: not significant; Well: well differentiated adenocarcinoma; Moderately: moderately differentiated adenocarcinoma; Poorly: poorly differentiated adenocarcinoma; Mucinous: mucinous adenocarcinoma.

immunohistochemical staining, 79 out of 121 patients (65%) were positive for galectin-3 expression.

The clinicopathological factors for positive and negative galectin-3 expression groups were compared, and the positive group showed significantly larger tumor sizes ($p=0.016$), deeper invasion to the colonic wall ($p=0.01$) and poor differentiation in histology ($p=0.0037$). The frequency of poorly-differentiated adenocarcinoma and mucinous adenocarcinoma was 14 out of 79 (18%) in the positive group compared with 1 out of 42 (2%) in the negative group. The positive rate of poorly-differentiated adenocarcinoma and mucinous carcinoma was 7 out of 7 and 7 out of 8, respectively. As for lymph node metastasis, the frequency was 30/79 (38%) in the positive group, significantly higher than 4/42 (9.5%) in the negative group ($p=0.0007$).

Additionally, venous invasion, lymphatic permeation and distant metastases were frequent ($p=0.005$, $p=0.041$ and $p=0.014$, respectively). Distant metastases, including liver and

Table II. *Factors independently associated with prognosis.*

Variable	Coefficient	Standard error	Odds ratio	CI 95%	*P* value
Lymphatic invasion	0.072	0.508	0.142	0.316-2.365	0.887
Venous invasion	0.118	0.488	0.242	0.280-1.749	0.809
Galectin-3	2.069	0.824	2.512	0.025-0.635	0.012
Dukes' stage	3.485	0.809	4.309	0.006-0.150	<0.001
Lymph node metastasis	2.415	0.784	3.079	2.404-52.062	0.021

CI: Confidence interval

lung metastasis, were recognized in 10 patients, all of them belonging to the positive group. Tumor staging by Dukes' criteria was carried out. The proportion of patients classified as stage C and D in the positive group was significantly higher than in the negative group (33/79 (42%) and 5/42 (12%), respectively), while the proportion of patients classified as stage A and B was significantly lower than in the negative group (46/79 (58%) and 37/42 (88%); $p=0.0004$).

One-, 3- and 5-year survival rates of patients in the galectin-3-positive group were 89.6%, 82.7% and 76.8%, respectively, which were significantly lower than the survival rates of patients in the negative group (100%, 97.4% and 94.1%, respectively; $p=0.0027$) (Figure 2).

Multivariate analysis demonstrated that galectin-3 expression, Dukes' stage and lymph node metastasis were independent prognostic factors for patients with colorectal cancer (Table II).

Discussion

The data presented here indicate that galectin-3 expression is significantly related to various clinicopathological factors, specifically tumor size, pathological type, tumor depth, lymph node metastasis, lymphatic permeation, venous invasion, distant metastasis and Dukes' stage. Furthermore, galectin-3 expression is an independent prognostic factor ranking with lymph node metastasis and Dukes' stage. A valid explanation of the above result comes from the close relationship between galectin-3 expression and MUC2 mucin expression. Galectin-3 has pleiotropic biological functions and plays roles in cell growth (21, 22), adhesion (23, 24), RNA processing (21), regulation of apoptosis (4, 25-27) and metastasis (8, 9-11, 15) through interaction with its ligands. It has been reported that galectin-3 modulates the expression of its ligand MUC2 mucin, which plays an important role in colon cancer metastasis and progression through protein-carbohydrate interactions (9-11). Down-regulation of MUC2 expression significantly alters the ability of colon cancer cells to colonize the liver of experimental animals (9). Clinically, colorectal mucinous carcinomas are considered to have worse prognoses than typical adenocarcinomas (28, 29). MUC 2 mucin is thought to be a key modulator of progression and metastasis in colorectal cancer. In the current study, 7 out of 8 mucinous carcinomas (87.5%) had positive expression of galectin-3 (Figure 1b). This result shows that galectin-3 modulates malignant behavior through interaction with MUC 2 mucin.

Another important role of galectin-3 in cancer may be its ability to regulate apoptosis. Galectin-3 has been reported to protect epithelial cells from apoptosis induced by staurosporine, cisplatin, genistatin and anoikis. In other words, galectin functions as an antiapoptotic factor (25-27). Recent studies have demonstrated that galectin-3 is a novel CD95-binding partner. CD95 is a member of the death receptor family (30) with which galectin-3 interacts and determines which of the CD95 apoptotic signaling pathways the cell will select (4).

Galectin-3 may express its malignant potential through regulation of apoptotic signal transduction by interaction with its ligands. In addition to mucin, the ligands identified in colon cancer include Mac-2-binding protein, carcinoembryonic antigen, lamp 1 and lamp 2 glycoproteins, and haptoglobin-related protein (17). Of those ligands, haptoglobin-related protein has been reported to be a major circulating ligand for galectin-3, and is elevated in the sera of patients with colon cancer but not in healthy subjects. This indicates the possibility that detection of galectin-3 and its ligand in serum may serve as clinically useful tumor markers (17).

Obviously, mucin interactions, CD95 and haptoglobin-related protein cannot explain all of the clinical significance of elevated galectin-3. However, we hypothesize that various biological functions and the malignant behavior of galectin-3 are brought about as the result of an interaction between circulating galectin-3 and its ligands, and that galectin-3 plays a role as an important mediator of several ligands that directly modulate cancer progression from the early stages of colorectal cancer to metastasis.

In conclusion, immunohistochemical detection of elevated levels of galectin-3 is a potent prognostic marker in colorectal cancer, but its biological function is still

unclear. Elucidation of this function may contribute to our search for a new strategy against cancer progression and metastasis. Further studies are needed.

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