

Galectin-3 Expression in Colorectal Cancer: Relation to Invasion and Metastasis

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Abstract. *Background:* Galectin-3, a β -galactoside-binding protein, has been associated with various biological processes, such as cell adhesion, recognition, proliferation, differentiation and apoptosis. The aim of this study was to determine the relationship of galectin-3 expression to clinicopathological findings in patients with colorectal cancer. Furthermore, the correlation between the expression of galectin-3 and β -catenin, and the Ki-67 labeling index were investigated. *Materials and Methods:* Immunohistochemical assessment of galectin-3, β -catenin and Ki-67 expression was performed on samples from 108 patients with colorectal cancer. The expression of galectin-3 was classified at the tumor surface and the invasive front, and its relationship with clinicopathological factors was considered from a statistical viewpoint. *Results:* There was significant liver metastasis when the expression of galectin-3 was lower at the invasive front of a tumor compared to its surface ($p=0.04$). There were also significant correlations between β -catenin expression at the tumor surface and liver metastasis and tumor stage ($p=0.03$, $p=0.04$ respectively). *Conclusion:* The reduction of galectin-3 expression is associated with the invasion and metastasis of colorectal cancer. A possible involvement of galectin-3 expression in tumor invasion, metastasis and proliferation in patients with colorectal cancer is suggested.

The galectins are a family of carbohydrate-binding proteins characterized by domains and their affinity for β -galactoside-containing glycoconjugates. An important member of this family, galectin-3 is broadly expressed in normal and neoplastic cells and has been implicated in diverse biological

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functions including cell growth, differentiation, apoptosis, adhesion, malignant transformation and RNA processing (1, 2). β -Catenin plays critical roles in both intercellular adhesion and the Wnt signaling pathway (3). Wnt signaling can stabilize β -catenin and cause it to accumulate in the cytoplasm and nucleus, where it can act as a transcription coactivator by associating with the TCL/LEF family of transcription factors (4). Recent studies have revealed that alterations in the Wnt signaling pathway, including those resulting from mutations in APC, β -catenin, and axin, play important roles in the carcinogenesis of various types of malignant tumors (3, 5).

Recent findings have shown that the expression of galectin-3 is uniformly elevated with neoplastic progression in certain malignancies. Several studies have shown the potential diagnostic implication of galectin-3 expression in malignancies including thyroid (6-8), pulmonary (9), gastric (10) and colon cancer (11-13), and anaplastic large-cell lymphoma (14). Meanwhile, several studies have shown that decreased expression of galectin-3 is associated with malignancies including breast (15), cervical (16), melanoma (17) and colorectal cancer (18). This suggests that inconsistent and varying amounts of galectin-3 expression in tumors of the same origin reflect the heterogeneity of tumor cells; therefore, the existence of a correlation between galectin-3 and the malignancy is unlikely.

In this study, we considered the relationship between the expression of galectin-3 and β -catenin in colorectal cancer, and clinicopathological factors using immunohistochemical analysis. Ki-67 is a useful marker for evaluating the proliferative potential of normal and tumor cells; therefore, we studied the correlation between galectin-3 expression and the Ki-67 labeling index (19-22).

Materials and Methods

Patients. One hundred and eight patients with colorectal cancer were selected after curative surgery at the Department of Surgery I, Gunma University Faculty of Medicine (Maebashi, Japan)

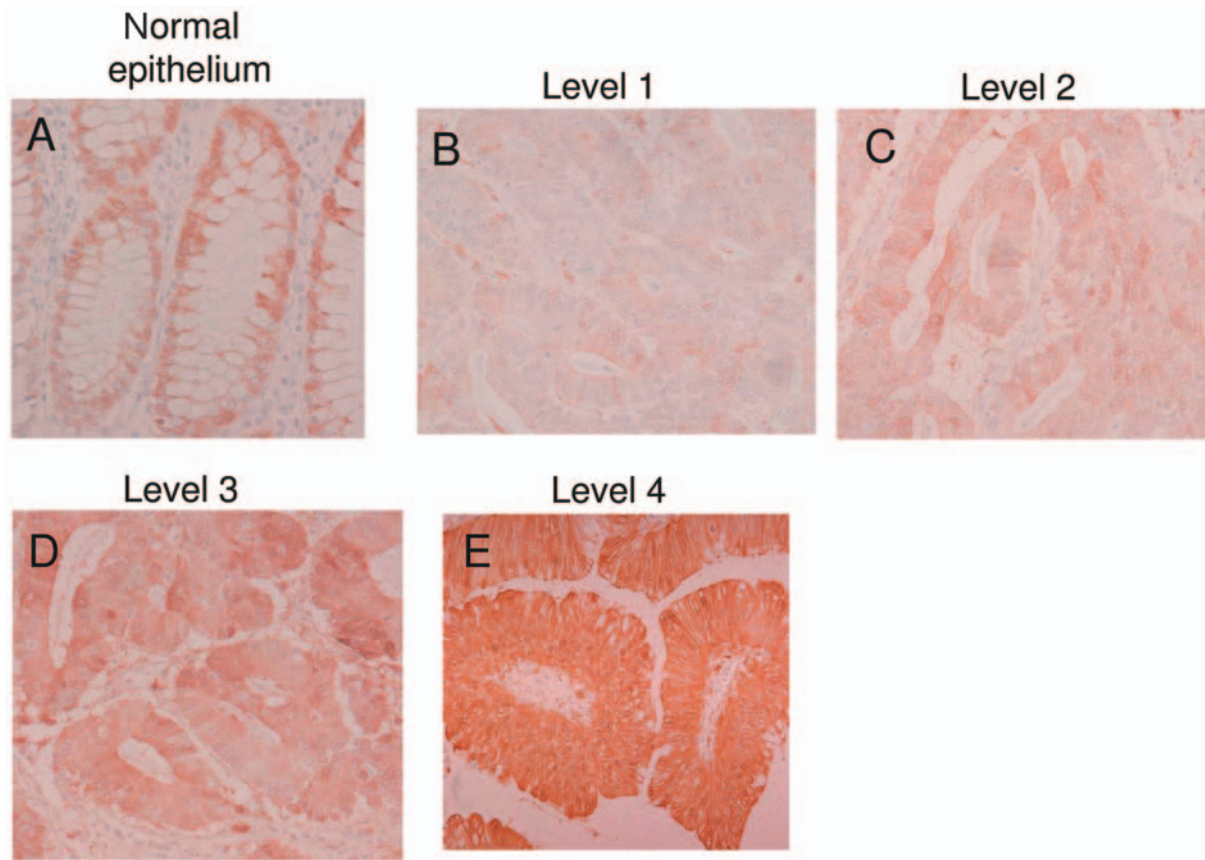


Figure 1. *Galectin-3* expression: the expression level of normal colorectal epithelium was set to 3 (A), and the expression level was classified into 1-4 levels. Expression level 1 (B) and level 2 (C) were defined as negative; levels 3 and 4 were defined as positive (D, E).

between 1998 and 2001. The group comprised 68 patients with non-liver metastases (46 men and 22 women; mean age, 61.1 years \pm 11.8 years) and patients with 40 liver metastases (28 men and 12 women; mean age, 59.6 years \pm 11.2 years). We classified patients as 40 liver metastasis cases and 68 non-liver metastasis cases more than five years after operation.

Tumor stage and disease grade were classified according to the fifth edition of the TNM classification of the International Union Against Cancer (UICC) (23). Written informed consent to participate in the study was obtained from each patient according to the ethical guidelines of our university.

Immunohistochemistry. Surgically resected colorectal cancer and liver metastasis tissues were fixed in 15% formalin and embedded in paraffin for routine pathological diagnosis. Paraffin blocks containing representative cancer tissue were selected and used for an immunohistochemical study, 4- μ m-thick paraffin sections were serially cut and mounted on silane-coated glass slides. The sections were deparaffinized in xylene, rehydrated and incubated with fresh 0.3% hydrogen peroxide in methanol for 30 min to inactivate endogenous peroxidase. After rehydration through a graded ethanol series, tissue sections were incubated with normal rabbit serum for 30 min and then overnight with primary rat anti-galectin-3 monoclonal antibody

(a gift from Prof. Avraham Raz, Wayne State University, Detroit, MI, USA) at a dilution of 1:500 in PBS containing 1% bovine serum albumin. After being rinsed with PBS, the sections were incubated with avidin-biotinylated horseradish peroxidase followed by the peroxidase substrate 3'-3'-diaminobenzidine. The sections were counterstained with hematoxylin. Negative controls were prepared by substituting normal rat serum for the primary antibody, and no detectable staining was evident. Immunohistochemical staining for β -catenin was performed according to a previously described method (3). The tissue sections were placed in 10 mM citrate buffer (pH 6.0) for 5 min. After incubation with normal rabbit serum, the sections were incubated with anti- β -catenin monoclonal antibody (Transduction Laboratories, Lexington, KY, USA) at a dilution of 1:1000. Immunohistochemical staining for Ki-67 was performed according to a previously described method (19). The tissue sections were placed in a 10 mM citrate buffer (pH 6.0) and autoclaved at 120°C for 5 min. After incubation with normal rabbit serum, the sections were incubated with anti-Ki-67 monoclonal antibody (DAKO, Glostrup, Denmark) at a dilution of 1:100.

Immunohistochemical evaluation.

Galectin-3: The percentage expression of colorectal cancer cells which stained positively for galectin-3 was evaluated over a x100

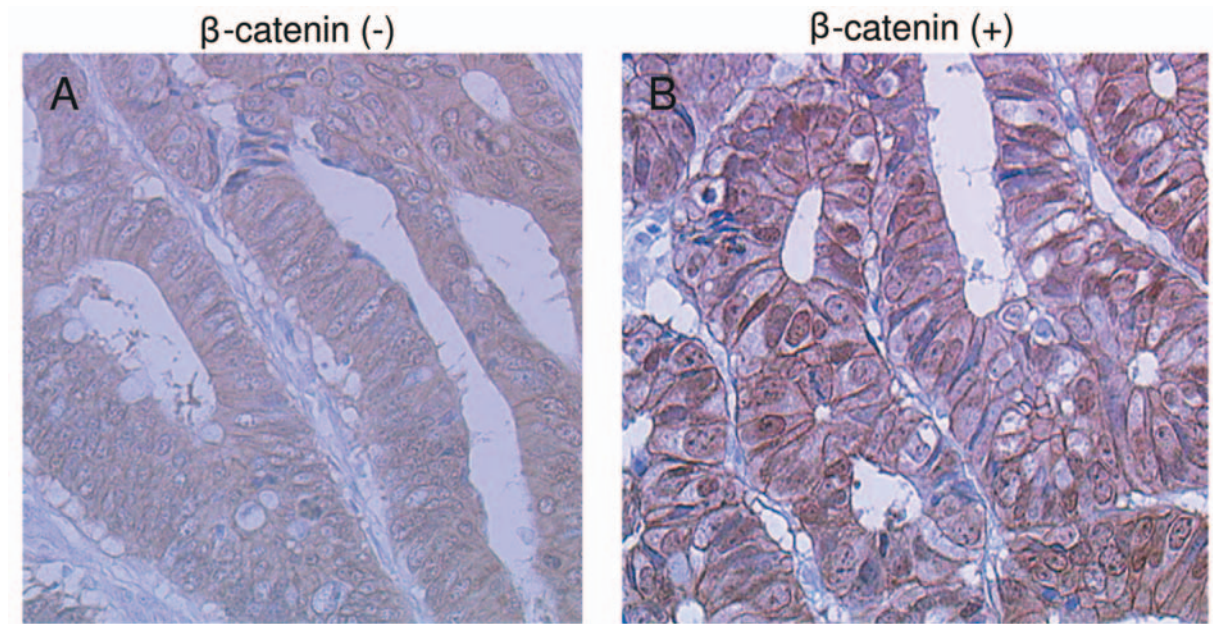


Figure 2. β -Catenin expression: the expression of membranous or cytoplasmic β -catenin in tumor cells was classified as negative (A). When β -catenin was located in the tumor nuclei, the sample was classified as β -catenin-positive (B).

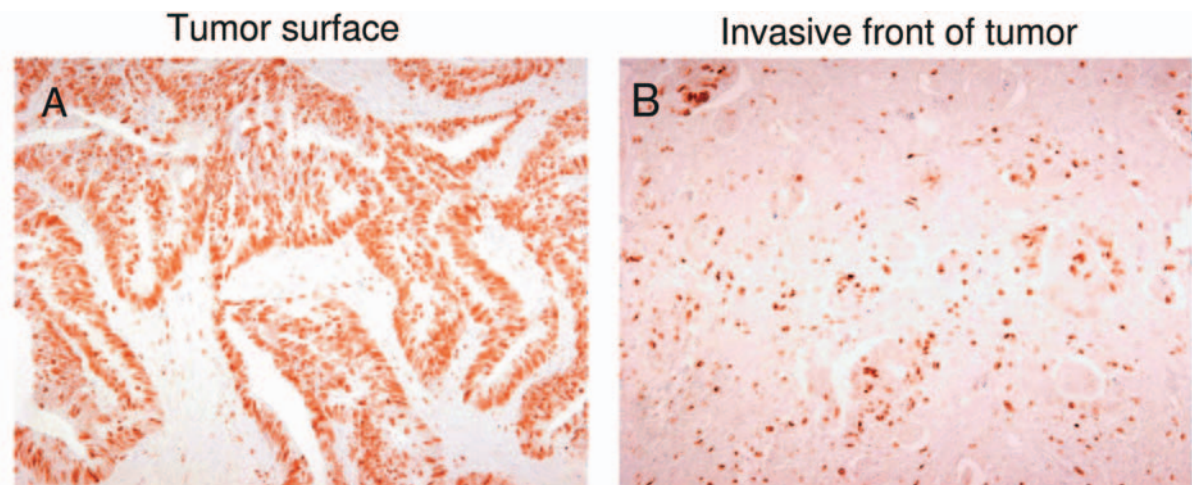


Figure 3. Ki-67 expression at two different tumors areas: at the surface of the tumor (A) and invasive front of the tumor (B).

field (x10 objective and x10 ocular) using a grid. The expression of normal colorectal epithelium was set to 3, and the expression was classified into 1-4 levels. Expression levels 1-2 were defined as negative, while 3-4 were defined as positive (Figure 1).

β -Catenin: Nuclear staining was defined as positive while cytoplasmic staining was considered negative (Figure 2).

Ki-67: The Ki-67 proliferative index (Ki-index) was defined as the percentage of nuclear-stained tumor cells among more than 1000 cells counted (Figure 3).

We immunohistochemically examined galectin-3, β -catenin, and Ki-67 expression of two different tumors areas (*i.e.* at the invasive

front and the surface of tumor tissue). All calculations were performed twice.

In each case, two cancerous areas, that is the superficial invasion site and the deep invasion site, were evaluated for each protein expression. Immunostaining was scored by two independent pathologists. Five high power fields were evaluated for each tumor sample at three different regions.

Statistical analysis. Continuous variables were compared using Student's *t*-test and Mann-Whitney *U*-test. Differences between the clinical parameters of two groups were determined with the Chi-

Table I. Clinicopathological parameters according to galectin-3 expression with colorectal cancer.

Clinicopathological factor	n	Galectin-3 tumor surface			Galectin-3 invasive front of tumor		
		-	+	p	-	+	p
Age (years mean±SD)		60.8±12.3	60.6±11.5	0.93	60.2±11.0	61.5±13.0	0.60
Gender							
Male	74	28	46	0.15	48	26	0.56
Female	34	8	26		24	10	
Histological differentiation							
G1	27	8	19	0.20	20	7	0.19
G2	68	23	45		43	25	
G3	2	2	0		2	0	
G4	6	3	3		4	2	
Depth of invasion							
pT2	3	3	0	0.02	2	1	0.19
pT3	97	32	65		67	30	
pT4	8	1	7		3	5	
Lymph node metastasis							
pN-	53	21	32	0.17	38	15	0.28
pN+	55	15	40		34	21	
Liver metastasis							
H-	68	25	43	0.32	42	26	0.16
H+	40	11	29		30	10	
Lymphatic invasion							
ly-	20	10	10	0.09	13	7	0.86
ly+	88	26	62		59	29	
Blood vessel invasion							
v-	41	22	19	<0.01	27	14	0.89
v+	67	14	53		45	22	
Clinical stage							
I,II	41	18	23	0.19	29	12	0.06
IIIa,IIIb	27	7	20		13	14	
IV	40	11	29		30	10	

squared test. Statistical significance was assumed for *p*-values <0.05. All analyses were performed using Statview statistical software (version 5.0; SAS Institute Inc., Cary, NC, USA).

Results

Relationship between galectin-3 expression and clinicopathological features. Galectin-3 expression in 108 patients with colorectal cancer was investigated using immunohistochemical analysis. The relationship between galectin-3 expression and the clinicopathological features of patients with colorectal cancer is summarized in Table I.

The study demonstrated that galectin-3 expression at the surface of the tumor was correlated with the depth of invasion (*p*=0.02) and blood vessel invasion (*p*<0.01). There was no significant correlation between galectin-3 expression at the invasive front of the tumor and clinicopathological features. On the other hand, when galectin-3 expression was low at the invasive front of the tumor in the primary lesion of colorectal cancer, galectin-3 expression at the site of liver

metastasis was high (Figure 4). Such a pattern was found in 62.5% of all cases (data not shown). With regard to the difference of expression at the tumor surface and the invasive front of the tumor, the expression of β-catenin at the invasive front of the tumor was also considered. When there was such a difference in the expression of galectin-3, staining for β-catenin at the invasive front of the tumor was positive (Figure 5); therefore, we examined the change in the expression of galectin-3 from the surface of the tumor to its invasive front. The galectin-3 expression of colorectal cancer with liver metastasis was weaker at the tip of the invasive front than at the surface of the colorectal cancer tissue (*p*=0.04) (Figure 6).

There was no significant correlation between galectin-3 expression and other factors, such as age, gender, histological type, lymph node metastasis, liver metastasis, or tumor stage (Table I). There was no significant correlation between β-catenin expression and a patient's age, gender, histological type, lymph node metastasis, lymphatic invasion, or blood vessel invasion; however, there

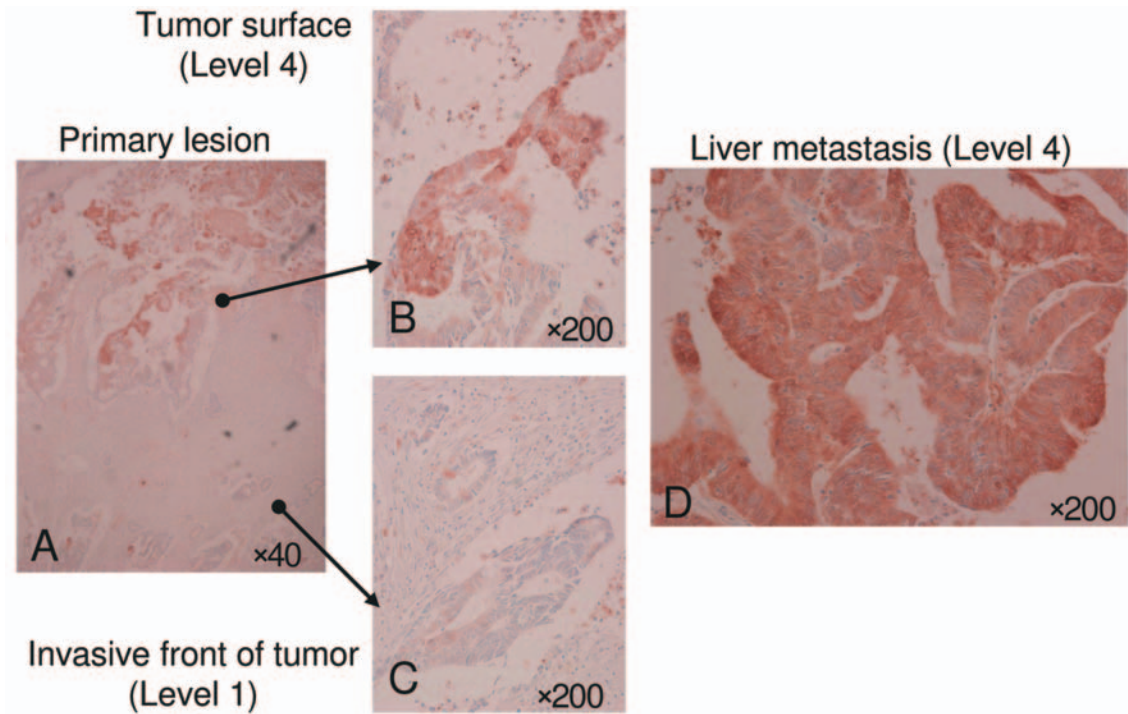


Figure 4. Relationship of the change of galectin-3 expression from the tumor surface to invasive front of tumor and liver metastasis: A case in which galectin-3 expression decreased towards the invasive front of the tumor in the primary lesion of colorectal cancer (A); tumor surface, level 4 (B); invasive front of tumor, level 1 (C); galectin-3 expression at the site of liver metastasis was high, level 4 (D).

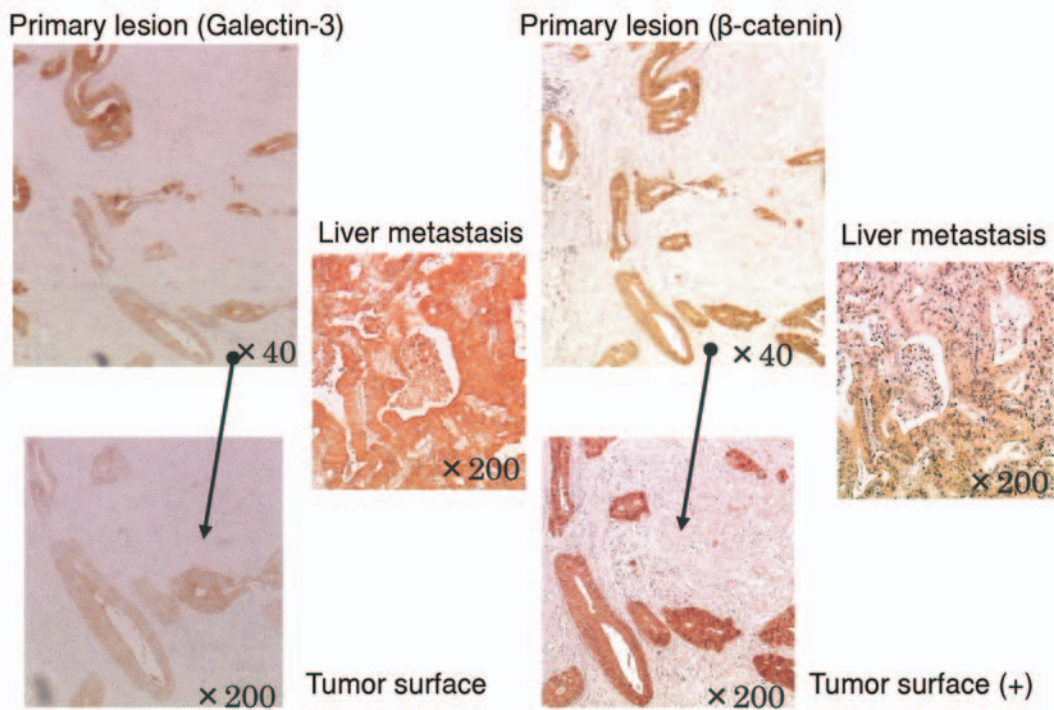


Figure 5. Relationship of the change of galectin-3 expression change and β -catenin: galectin-3 expression decreased towards the invasive front of tumor in the primary lesion of colorectal cancer, magnification x40 (A) and magnification x200 (B). Galectin-3 expression was higher at the site of liver metastasis (C). β -Catenin expression at the primary lesion of colorectal cancer, magnification x40 (D) and magnification x200 (E). β -Catenin expression at the site of liver metastasis (F).

Table II. Clinicopathological parameters according to β -catenin expression with colorectal cancer.

Clinicopathological factor	n	β -Catenin tumor surface			β -Catenin invasive front of tumor		
		-	+	p	-	+	p
Age (years mean \pm SD)		60.6 \pm 12.0	60.9 \pm 11.2	0.90	61.7 \pm 12.0	59.9 \pm 11.4	0.44
Gender							
Male	74	48	26	0.37	30	44	0.73
Female	34	25	9		15	19	
Histological differentiation							
G1	27	21	6	0.37	12	15	0.57
G2	68	45	23		26	42	
G3	2	2	0		1	1	
G4	6	3	3		4	2	
Depth of invasion							
pT2	3	3	0	0.44	1	2	0.30
pT3	97	66	31		40	57	
pT4	8	4	4		4	4	
Lymph node metastasis							
pN-	53	38	15	0.37	23	30	0.72
pN+	55	35	20		22	33	
Liver metastasis							
H-	68	51	17	0.03	32	36	0.14
H+	40	22	18		13	27	
Lymphatic invasion							
ly-	20	14	6	0.80	9	11	0.74
ly+	88	59	29		36	52	
Blood vessel invasion							
v-	41	27	14	0.76	17	24	0.97
v+	67	46	21		28	39	
Clinical stage							
I,II	41	33	8	0.04	22	19	0.13
IIIa,IIIb	27	18	9		10	17	
IV	40	22	18		13	27	

were significant correlations between β -catenin expression at the tumor surface and both liver metastasis and tumor stage ($p=0.03$, $p=0.04$, respectively) (Table II). There was no significant correlation between the expression of galectin-3 and β -catenin.

The mean Ki-67 labeling index at the surface of a tumor was 44.6 ± 21.4 , and at the invasive front of a tumor, it was 6.17 ± 8.33 ; however, these differences were not statistically significant.

Discussion

The β -galactoside-binding protein galectin-3 has pleiotropic biological functions and has been implicated in cell growth, differentiation, adhesion, RNA processing, apoptosis, and malignant transformation (1, 24), while the actual biological functions of galectin-3 remain largely unknown. Recent studies have revealed that galectin-3 overexpression is correlated with increased metastatic potential in some cancers (24, 25). Bresalier *et al.* reported that galectin-3

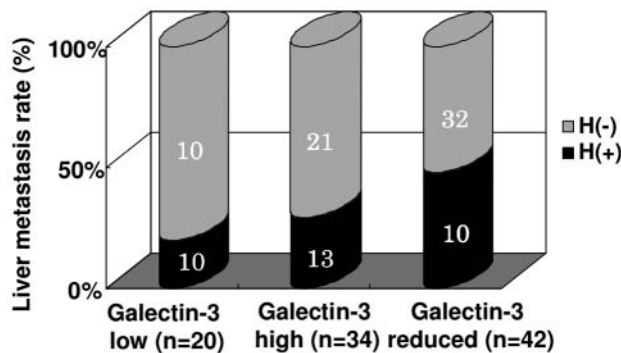


Figure 6. Relationship of the change of galectin-3 expression (tumor surface to invasive front of tumor) and liver metastasis: When the expression of galectin-3 decreased from the tumor surface to the invasive front of a tumor, many liver metastases were significantly noted ($p=0.04$). H (-), liver metastasis negative; H (+), liver metastasis positive.

expression was correlated with colon cancer metastasis (26). Galectin-3 provides tumor cells with anti-apoptotic and anti-anoikis activities, which are thought to be critical for

anchorage-independent cell survival in the circulation that takes place during dissemination (24, 27). Yoshii *et al.* observed that the anti-death activity of galectin-3 was regulated by its phosphorylation (28). Until recently, the pattern of immunohistochemical galectin-3 expression in human colorectal cancer was still a matter of debate because some investigators found decreasing galectin-3 levels in colorectal cancer progression, whereas others did not (29-35). In contrast, John *et al.* showed that galectin-3 is efficacious in reducing metastases and tumor volumes and weights in primary tumors in a nude mouse model of human breast cancer (27). However, inconsistent and varying amounts of galectin-3 expression in tumors of the same origin reflect the heterogeneity of tumor cells; therefore, the existence of a correlation between galectin-3 and malignancy is unlikely.

Sanjuan *et al.* observed that galectin-3 expression is down-regulated in the initial stages of neoplastic progression with a marked nuclear location, whereas cytoplasmic expression increases in the later phases of tumor progression (36).

To our knowledge, the staining pattern of galectin-3 in malignant tissue has been evaluated by many scientists; however, no study has focused on the change of galectin-3 expression in two different parts of a tumor until now.

Our findings demonstrated that, there was a relationship between galectin-3 expression and tumor progress.

When the expression of galectin-3 decreased from the surface of a tumor to the invasive front of a tumor, liver metastasis was considered to be significant; furthermore, the reduction in the expression of galectin-3 concurred with the expression of β -catenin at the invasive front of the tumor.

The following can be considered from these results. As galectin-3 expression decreased at the primary lesion, metastasis and invasion were stronger than tumor proliferation. On the other hand, in a metastasized lesion, adhesion and proliferation were more prevalent because β -catenin was expressed again. In other words, the reduction of galectin-3 expression in the primary lesion brings about an anti-anoikis phenomenon and is considered to be a preparatory stage for invasion and metastasis. Galectin-3 may inhibit anoikis through a mechanism involving increased adhesiveness of cancer cells which enter into blood vessels to the extracellular matrix. Re-expression of galectin-3 at the metastasis site might be responsible for leading to bulk tumor growth. Thus, the possibility that the expression of galectin-3 is involved in tumor adhesion and proliferation was suggested. As described in the Results section, galectin-3 immunoreactivity did not correlate with the expression of Ki-67, a useful marker for evaluating proliferation potential; therefore, no proliferative value of galectin-3 in patients with colorectal cancer could be established in this study.

Conclusion

A reduction of galectin-3 expression appears to be involved in invasion and metastasis. To our knowledge, no reports have evaluated the relationship between the reduction of galectin-3 expression and malignancy. The expression of galectin-3 might serve as a metastasis prediction factor for colorectal cancer.

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