

Lumican expression in advanced colorectal cancer with nodal metastasis correlates with poor prognosis

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Abstract. Lumican is a member of a small leucine-rich proteoglycan family, and it is reportedly overexpressed in human breast cancer. The expression of lumican in the extracellular matrix in breast cancer is associated with a high tumor grade, low estrogen receptor levels and young age. Lumican expression has been previously reported in colorectal cancer, but the role of lumican in the tumor is not well understood. In this study, we examined the expression and role of lumican in advanced colorectal cancer. Immunohistochemical staining was performed on 158 patients who underwent curative surgery for advanced colorectal cancer with lymph node metastasis. In the normal colorectal tissues, lumican immunoreactivity was observed in the fibroblasts and neural cells, but not in the colorectal epithelial cells. Lumican was localized in the cytoplasm of the cancer cells and its overexpression was detected in 99 of the 158 (62.7%) colorectal cancer patients. Clinicopathologically, there was no association of lumican expression with age, sex, histological typing, or venous and lymphatic invasion. However, lumican expression tended to correlate with the spread of lymph node metastasis and the depth of tumor invasion ($p=0.136$ and 0.135 , respectively). Furthermore, the survival rate was significantly lower in patients with a high lumican expression level than in those with a low lumican expression level ($p=0.048$). These results indicate that lumican expression is a potential prognostic factor in patients with advanced colorectal cancer with nodal metastasis.

Introduction

Lumican belongs to a family of small leucine-rich proteoglycans (SLRPs), which have core proteins of ~40 kD and possess 6-10 leucine-rich repeats (between flanking cysteine-rich disulfide-bonded domains that are the N and C termini of the core protein) (1-4). In addition to lumican, SLRPs include keratocan, mimecan, decorin, biglycan, fibromodulin, epiphygan, osteoadherin (5). The human lumican protein contains 338 amino acids, including a putative 18-residue signal peptide, and its gene is located on chromosome 12q21.3-q22 (6,7). Lumican was first identified in the cornea as a keratan sulfate proteoglycan (KSPG) (4), and in noncorneal tissues, lumican exists as a low or non-sulfated glycoprotein (7-9). Lumican was then reported to be present in the extracellular matrix of many tissues, such as the skin, lung, intervertebral disk, skeletal muscle, kidney, pancreas, brain, placenta, heart, aorta, liver, and the intestine (7,9-14).

Mice, homozygous for null mutation in lumican, displayed corneal opacification and skin laxity (15). In the corneal tissue, lumican was reported to regulate the assembly and diameter of the collagen fibers (4). Lumican is considered a key regulator of collagen fibrillogenesis, a process critical to corneal transparency. Several studies have shown that SLRP proteins can modulate cellular behaviors, including cell migration and proliferation during embryonic development, tissue repair, and tumor growth, in addition to their extracellular matrix functions as regulators of tissue hydration and collagen fibrillogenesis (16).

In breast cancer tissues, lumican is overexpressed in the fibroblasts adjacent to the cancer cells. Its high expression level is further associated with a high tumor grade, low estrogen receptor levels and young patients (17). In addition, as we have previously reported, in human pancreatic, uterine cervical, and colorectal cancers, lumican is localized in the cancer cells, and the cancer cells as well as the fibroblasts adjacent to them, synthesize lumican mRNA and its protein (14,18,19). In a colorectal cancer study, lumican was expressed in five of the five colorectal cancer cell lines and in the cancer

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Table I. The clinicopathological characteristics of the 158 colorectal cancer patients.

Parameter	No. of patients
Sex	
Female/male	67/91
Age	
Range	26-86
Mean	63.1
Histological typing	
Well	81
Moderate	62
Mucinous	9
Poor	6
Location	
Colon	78
Rectum	80
Macroscopic findings	
1	13
2	121
3	24
Depth of tumor invasion	
mp	24
ss, a1	63
s, a2	71
Lymph vessel invasion	
ly (-)	17
ly (+)	141
Venous vessel invasion	
v (-)	60
v (+)	98
Status of nodal metastasis	
n1	91
n2	53
n3	14

cells in eight of the 12 colorectal cancer patients (18). However, the exact role of lumican in human colorectal cancer remains unclarified.

In this study, we examined lumican expression in advanced colorectal cancer with lymph node metastasis, and determined the correlations of its expression with the clinicopathological factors.

Patients and methods

Patients and tissue samples. One hundred and fifty-eight patients who underwent curative surgery for advanced colorectal cancer with lymph node metastasis from 1984 to 1997

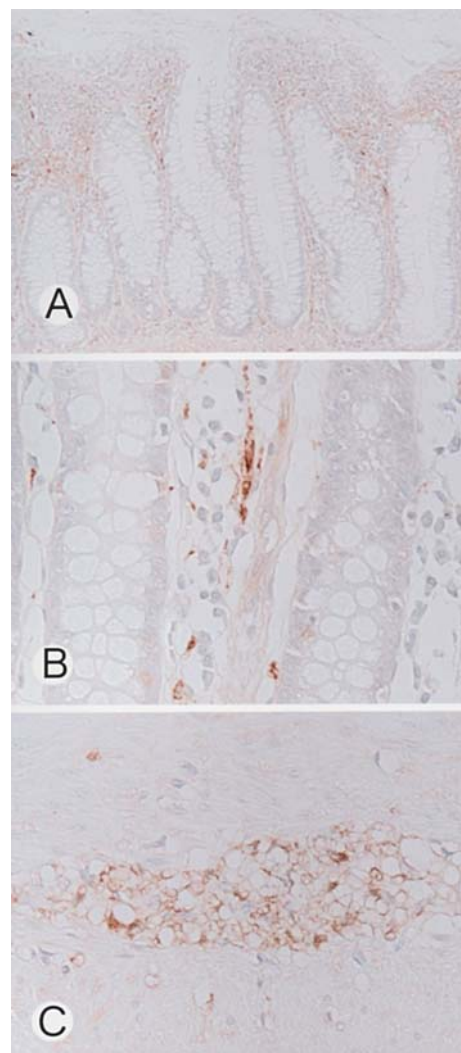


Figure 1. Immunohistochemical analysis of lumican in normal colorectal tissues. Lumican was not localized in the epithelial cells (A), but was weakly positively localized in the fibroblasts (B) and neural cells (C) in the normal colorectal tissues. Original magnification x200 (A); x400 (B and C).

at Nippon Medical School participated in this study. After being fixed in 10% neutral buffered formalin for 18-20 h, the colorectal cancer tissues were embedded in paraffin, serially sectioned at 3 μ m and stained with H&E for light microscopy. The clinicopathological characteristics of the patients (sex, age, histological typing, tumor location, macroscopic findings, depth of tumor invasion, lymphatic and venous invasions and the occurrence of lymph node metastasis) are summarized in Table I.

Immunohistochemistry. The polyclonal antibody against the human lumican protein used for immunohistochemistry was obtained as previously reported (12). Immunohistochemical analysis was performed using the Histofine Simple Stain PO[®] Max kit (Nichirei, Tokyo, Japan). After deparaffinization, endogenous peroxidase activity was blocked by incubation in 0.3% hydrogen peroxide in methanol for 30 min. The tissue sections were incubated with the anti-human lumican antibody (1:500) in phosphate-buffered saline (PBS) containing 1% bovine serum albumin (BSA) for 16 h at 4°C. Bound

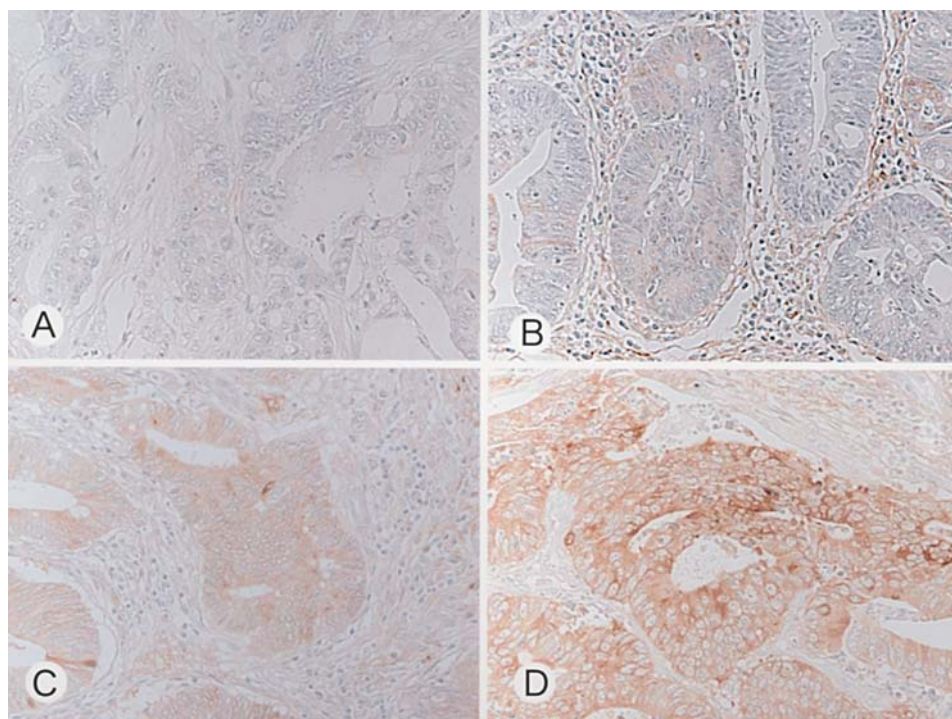


Figure 2. The characteristic staining pattern of lumican in the cytoplasm of colorectal cancer cells. The intensities of lumican immunoreactivity in the colorectal cancer cells were classified into four groups, namely, negative (A), weakly positive (B), positive (C) and strongly positive (D). Original magnification x200.

antibodies were detected with the Simple Stain PO[®] Max reagent using diaminobenzidine tetrahydrochloride as the substrate, and the sections were counterstained with Mayer's hematoxylin. To confirm the specificity of the primary antibodies, the anti-lumican antibodies were preincubated with a blocking peptide for lumican for 1 h and then applied to the sections, which showed no positive immunoreactivity.

Estimation of lumican expression level in colorectal cancer. The correlations of lumican expression with the clinicopathological factors were analyzed according to the Japanese classification of colorectal carcinoma (20). The immunoreactivity of the lumican protein was scored on the basis of the intensity of the predominant cytoplasmic staining area using the following classification system: 0 = negative; 1 = weakly positive; 2 = positive and 3 = strongly positive. To examine the associations of the lumican expression level with the clinicopathological features or the prognostic factors, the tumors were divided into two groups according to the intensity of staining, that is, scale 0 as the negative group and scales 1-3 as the positive groups. All the specimens were evaluated by two investigators (T.S. and N.T.) who were unaware of the clinical information of the patients.

Statistical analysis. Mann-Whitney tests were used to compare the quantitative and ordered variables, and the Student's t-test was used to analyze the differences in the normally distributed data between the two groups. The Chi-square test and Fisher's test were used to analyze the associations of lumican expression with the prognostic factors. The survival rate was calculated by the Kaplan-Meier method, and the significance of the difference in the survival rate

was analyzed by the log-rank test. A P-value of <0.05 was considered statistically significant.

Results

Localization of lumican in colorectal cancer. In the normal colorectal tissues, the epithelial cells showed no staining for lumican, but the fibroblasts and neural cells showed positive staining for lumican (Fig. 1A-C). In the colorectal cancer tissues, lumican was diffusely localized in the cytoplasm of the cancer cells and was detectable in 99 of the 158 (62.7%) colorectal cancer patients. The intensities of lumican immunoreactivity were classified into four groups, namely, negative, weakly positive, positive and strongly positive (Fig. 2A-D). In most lumican-positive tumors, lumican immunoreactivity was heterogeneous with a patchy distribution in the colorectal cancer cell nests. The cancer cells infiltrating the lymph follicle were intensely stained, and the staining was intense at the invasive front of the cancer cells (Fig. 3A and B).

Correlations of lumican expression with clinicopathological factors. Lumican expression did not correlate with sex, age, tumor location, or venous and lymphatic invasion (Table II). The associations of the lumican intensity score with the clinicopathological factors are summarized in Table III. Semiquantitative analysis showed that ulcerated tumors had more intensive staining for lumican than protuberant tumors (1.24 vs 0.79). Moreover, ulcerated tumors with infiltrative growth showed particularly higher staining intensity scores (1.42). Based on the extent of lymph node metastasis, the lumican expression level is elevated in advanced colorectal cancer cases (n1, 0.97; n2, 1.25; and n3, 1.29). As for the

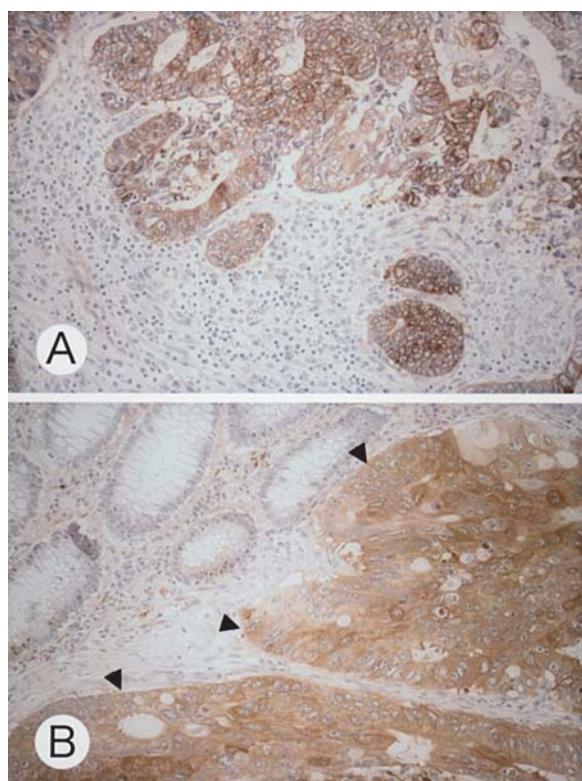


Figure 3. Immunohistochemical analysis of lumican in the colorectal cancer tissues adjacent to the lymph follicle. The cancer cells infiltrating the lymph follicle were intensely stained (A), and staining was intense at the invasive front of the cancer cells (B, arrowheads). Original magnification x200.

histological typing, moderately differentiated adenocarcinoma showed the most intensive lumican staining (1.31) compared with other tumors (well differentiated adenocarcinoma, 0.99; poorly differentiated adenocarcinoma, 0.67; and mucinous adenocarcinoma, 1.17). An elevated lumican expression level was positively associated with the depth of tumor invasion as shown in Table III. Tumors invading the *muscularis propria* had higher intensity scores than those restricted to the *muscularis propria* (1.16 vs 0.67). As a result, there was an association between lumican expression and the histological stage (stage IIIa, 0.98; and stage IIIb, 1.24).

Poor prognosis in patients with high lumican expression level. The prognosis between the patients with lumican-positive and lumican-negative tumors was compared (Fig. 4). The survival rates of the patients with lumican-positive tumors were significantly lower than those of the patients with lumican-negative tumors ($p=0.048$).

Discussion

The most intensive staining of lumican was shown in the cancer cells infiltrating the lymph follicles and at the invasive front of the cancer cells. Funderburgh *et al* demonstrated that the nonsulfated form of the lumican protein promoted macrophage attachment and spreading (21). This action is thought to be mediated by a lumican receptor expressed by macrophages (18). The finding that colorectal cancer cells infiltrating the lymph follicles showed the highest lumican expression

Table II. Lumican expression vs the clinicopathological parameters of the 158 colorectal cancer patients.

Parameter	Lumican (+)	Lumican (-)	P-value
Sex			
Female	42	25	0.99 ^a
Male	57	34	
Age			
≤40	2	1	0.77 ^b
41-50	14	4	
51-60	23	18	
61-70	32	21	
71-80	27	12	
>80	1	3	
Histological typing			
Well	45	36	0.12 ^a
Moderate	46	16	
Mucinous	5	4	
Poor	3	3	
Location			
Colon	48	30	0.77 ^a
Rectum	51	29	
Macroscopic findings			
1	9	4	0.952 ^b
2	74	47	
3	16	8	
Depth of tumor invasion			
mp	12	12	0.135 ^b
ss, al	40	23	
s, a2	47	24	
Lymph vessel invasion			
ly (-)	12	5	0.47 ^a
ly (+)	87	54	
Venous vessel invasion			
v (-)	41	19	0.25 ^a
v (+)	58	40	
Status of nodal metastasis			
n1	53	38	0.136 ^b
n2	35	18	
n3	11	3	

^aThe Chi-square, and ^bMann-Whitney tests were used.

level further indicates that lumican expression mediates the interactions between the colorectal cancer cells and the cells constituting the lymph follicle. This interaction may play

Score	Intensity score of lumican expression				Average score	Rank correlation coefficient
	0	1	2	3		
Type						
1	6	6	1	1	0.79	0.098
2	61	21	11	28	1.05	
3	10	2	4	8	1.42	
N						
n1	47	18	8	18	0.97	0.11
n2	24	8	5	16	1.25	
n3	5	3	3	3	1.29	
Grade						
Well	45	11	8	18	0.99	0.083
Moderate	23	15	6	18	1.31	
Mucinous	3	1	0	2	1.17	
Poor	5	2	2	0	0.67	
Depth						
mp	14	7	0	3	0.67	0.129
al, a2, ss, s	62	22	16	34	1.16	
Stage						
IIla	47	18	9	18	0.98	0.098
IIlb	29	11	7	19	1.24	

Spearman's rank correlation coefficients were used.

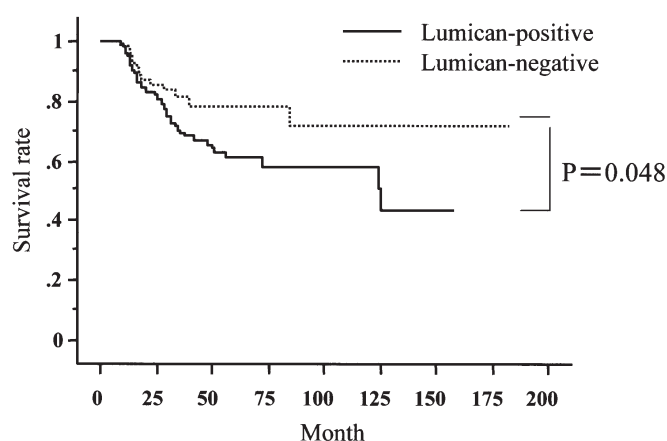


Figure 4. The prognosis of lumican-positive or lumican-negative advanced colorectal cancer patients with nodal metastasis. Kaplan-Meier survival curves for the patients with colorectal cancer showing a poorer survival in lumican-positive patients than in lumican-negative patients.

an important role in lymph node invasion. The role of macrophages in the lymph follicles must be examined more closely in the future.

In addition to lymph follicle infiltration, lumican showed intense staining at the invasive front of the cancer cells. Lumican expression at the invasive front of the cancer cells has been detected in other cancers. In uterine cervical cancer, the lumican protein was localized mainly at the periphery of the cancer cell nests (19). Furthermore, in breast cancer, an increased lumican deposition rate was observed in the collagenous stroma of the tumors, particularly at the margins of the invasive tumors (22). These accumulations of the lumican protein at the tumor invasive fronts may have important roles in cell growth and in the invasion of adjacent stromal tissues not only in uterine and breast cancers but also in colorectal cancer.

There are a few reports on lumican expression and the clinicopathological factors of cancer. In breast cancer, the lumican expression level correlates with cancer grade, the estrogen receptor level and patient age (17). In advanced colorectal cancer, lumican expression did not correlate with age, sex, tumor location or venous and lymphatic invasion. What are the differences between breast cancer and colorectal cancer concerning these factors? One possibility is that lumican in cancer tissues may play different roles in the stroma and cytoplasm. The side chains of lumican in the stromal tissues and the cytoplasm of cancer cells may be different.

Another possibility is that hormonal circumstances induced by breast cancer may influence the expression and role of lumican. Recent studies showed that lumican plays important roles in tumor growth inhibition and that it is an apoptotic inducer (23,24). To elucidate the role of lumican in cancer tissues, further molecular biological studies of colorectal cancer and clinicopathological studies of other cancers are needed.

In colorectal cancer, lumican expression tended to correlate with lymph node metastasis and the depth of tumor invasion. Furthermore, the survival rates in the patients with lumican-positive tumors were significantly lower than those in the patients with lumican-negative tumors. These results indicate that lumican expression could be a prognostic factor for advanced colorectal cancer with lymph node metastasis.

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