

Reduced Expression of Syndecan-1 Affects Metastatic Potential and Clinical Outcome in Patients with Colorectal Cancer

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Syndecan-1 is a transmembrane heparansulfate proteoglycan which regulates cell-to-cell or cell-to-extracellular matrix interactions and may influence malignant cell behavior. We investigated the alterations of syndecan-1 expressions in colorectal cancers and analyzed the relationship between histological and clinical characteristics. Syndecan-1 protein expression in colorectal cancer tissues was investigated with immunohistochemical staining of resected specimens. *In situ* hybridization was performed using syndecan-1 riboprobe to confirm the transcriptional signals. Syndecan-1 mRNA expression in cancer cell lines cultured with or without methylation inhibitor was also analyzed by quantitative PCR. Out of 105 specimens tested, less than 25% of tumor cells were stained with anti-syndecan-1 monoclonal antibody in 36 (34.3%). *In situ* hybridization showed a similar staining profile to that of immunohistochemistry. Syndecan-1 mRNA expression was increased by the methylation inhibitor 5-aza-2'-deoxycytidine, suggesting that the hypermethylation is involved in the suppression of syndecan-1 expression. Clinically, the incidence of metastasis to lymphnode or liver in patients with syndecan-1-negative tumors was significantly high. Among T1 colorectal cancers displaying a primary invasive phase, lymphnode metastasis, undifferentiated characters and 'budding' of cancer cells were more common in syndecan-1-negative tumors. The survival rate in patients with syndecan-1-negative tumors was decreased significantly in a stage-independent manner. These results suggest that the reduction of syndecan-1 expression in colorectal cancer cells, which is supposed to be regulated at the transcription level, is closely related to invasive character. The evaluation of syndecan-1 expression in colorectal cancer may allow prediction of patients' survival after surgery.

Key words: Syndecan-1 — Colorectal cancer — Metastatic potential — Prognosis

Gastrointestinal tract cancers disrupt the muscularis mucosa of the epithelia, with subsequent tumor outgrowth into the submucosal layer. When cancer cells are observed in the submucosal layer, invasion of lymphatic ducts or blood vessels is likely. Therefore, tumor cells in the submucosal layer have been considered to be an important pathological sign for predicting life-threatening metastasis. In order to disseminate into submucosal interstitial tissues, cancer cells must become detached from the tumor mass,¹ and this is closely associated with a reduction of cell-cell or cell-matrix adhesion.² The disruption of cadherin-dependent cell adhesion has been extensively studied in relation to the progression or metastasis of colorectal cancers, but involvement of other molecules has not been reported.

Recently, it has been suggested that syndecan, a family of transmembrane heparan sulfate proteoglycans, also plays an important role in the binding of tumor cells to extracellular matrix (ECM) molecules such as type I, III, V collagens, fibronectin, tenascin, and amphoterin on various cell lineages. Among syndecan family members, syn-

decn-1 (CD138) is mainly expressed in stratified squamous epithelia of epidermis, glandular epithelium of intestine,^{3, 4} hepatocytes,^{5, 6} and hematopoietic cell lineages.^{7, 8} In addition, syndecan-1 is expressed at sites of ECM-free structure in squamous epithelia and hepatocytes, suggesting that this molecule has an additional role in cell-cell interactions. From a functional aspect, syndecan-1 binds to basic fibroblast growth factor (bFGF)⁹ and augments bFGF-triggered signaling pathways as a co-receptor. Furthermore, syndecan-1 expression changes during the wound repair of skin and the morphogenesis of embryonic tissue interactions. Our recent report also revealed that the expression of syndecan-1 was augmented during epithelial cell regeneration and rearrangement in the stomach.¹⁰ Therefore, syndecan-1 is regarded as one of the key molecules participating in regulation of cell morphology, growth, and structural arrangement in various epithelial tissues.

Another important role of syndecan-1 in malignant cell transformation or cell differentiation has also been proposed. In squamous cell carcinoma, a reduced expression of syndecan-1 was noted.¹¹ In B lymphocyte development, syndecan-1 is expressed in the pre-B cell phase and disappears during the maturation process, but is expressed again in plasma and myeloma cells.¹² Day *et al.*¹³ reported that

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the expression of this molecule was reduced in some colon cancer tissues, while it was retained in benign adenoma and normal epithelia. We previously demonstrated that a reduced expression of syndecan-1 in hepatocellular carcinoma tissue is closely related to differentiation status and metastatic potential of tumor cells.⁶ These results indicate that reduced expression of syndecan-1 may alter the biological behavior of transformed epithelial cells. In addition, the CpG-rich genomic sequence within 600 bps upstream to the coding region of *syndecan-1* gene (GenBank accession No. NT 005394) raises the possibility that the altered expression of this molecule may also be regulated by hypermethylation of the promoter as well as by cancer suppressor genes.

We hypothesized that the altered expression of syndecan-1 in colorectal cancers may affect the invasive character and may serve as a novel biomarker predicting the metastatic potential of cancer cells. We investigated the correlation between the alteration of syndecan-1 expression in colorectal cancer and the histological manifestations of tumors or patients' survival. Moreover, we collected and studied tissue samples from T1 colorectal cancers to assess the primary changes in the expression of syndecan-1 during tumor progression. The results indicate that the loss of syndecan-1 expression is probably regulated epigenetically by DNA hypermethylation in colorectal cancer cells, and this is one of the critical events in tumor invasion.

MATERIALS AND METHODS

Tumor specimens Tumor specimens were obtained from 105 patients who underwent curative resection of a primary colorectal carcinoma at Asahikawa Medical College Hospital from 1990 to 1998. No patients received chemotherapy or radiation therapy prior to undergoing resection of the primary tumors. Tumor specimens and samples of normal colonic epithelium were collected at the time of resection and were fixed in 10% phosphate-buffered formalin solution. Clinico-pathological information and survival data were obtained from the hospital records or by contacting the patients themselves or their families.

Immunohistochemistry The avidin-biotin indirect immunoperoxidase method was used for immunohistochemical detection of syndecan-1 expression in cancer cells as described previously.^{6,10} Briefly, 4 μ m paraffin-embedded sections were cut and deparaffinized in xylene followed by a treatment with 1.2% hydrogen peroxide in methanol for 10 min to inactivate endogenous peroxidase. Then, the sections were heated in 0.01 M citrate buffer (pH 6.0) for 5 min, 3 times in a microwave oven for antigen retrieval. After blocking of the nonspecific binding of antibodies with normal horse serum, the sections were incubated with 1:50 diluted monoclonal anti human syndecan-1 antibody

(MCA681, Santa Cruz Biotechnologies, Santa Cruz, CA) clone B-B4 originally described by Wijidenes *et al.*¹⁴ for 1 h at room temperature. The sections were then incubated with biotinylated horse anti-mouse IgG for 30 min and with streptavidin-biotin-peroxidase complex (Vector, Burlingame, CA) for 30 min at room temperature. Immobilized peroxidase was visualized in diaminobenzidine solution. Sections were counterstained with hematoxylin. Normal colonic epithelium was used as an internal positive control. Parallel sections were incubated in normal mouse immunoglobulin G (IgG) as a primary antibody for the negative control.

Immunohistochemical reactions were classified for intensity as previously described.^{13,15} Briefly, -, no staining; \pm , weak staining or strong stains in less than 25% of tumor cells; +, moderate staining or strong stains in only 25-75% of tumor cells; ++, strong staining of more than 75% of tumor cells. All sections were scored independently by two authors who had no knowledge of clinical or histological information.

In situ hybridization (ISH) An antisense riboprobe was prepared from a reverse transcription-polymerase chain reaction-derived DNA template¹⁶ as we described elsewhere.¹⁰ In brief, RNA extracted from human gastric epithelium was reverse-transcribed to synthesize cDNA by extension of oligo-dT primers with MMLV reverse transcriptase (code RPN 1266, Amersham, Bucks, UK). PCR of the cDNA was performed with the 5'-primer, 5'-GGTG-CAGGTGCTTTGCAAGA-3' (Syn1), and the 3'-primer, 5'-CCCCGAGGTTTCAAAGGTGA-3' (Syn2), to amplify the sequence corresponding to human syndecan-1 nucleotides 347 to 856. Then, with this PCR product, DNAs containing the SP6 or T7 promoter sequence at the 5' ends were synthesized by second PCR reactions using primer pairs of 5'-ACGATTTAGGTGACACTATAGGTGCAGG-TGCTTTGCAAGA-3' (Syn1SP6) and Syn2 or Syn1 and 5'-TAATACGACTCACTATAGGGCCCCGAGGTTTCAAAGGTGA-3' (syn2T7). Digoxigenin-labeled riboprobes were synthesized with the two products from the second PCR by use of a DIG RNA labeling kit (Boehringer, Mannheim, Germany). DIG-labeled sense riboprobes and antisense riboprobes were produced by using SP6 and T7 RNA polymerase, respectively.

ISH to detect *syndecan-1* gene transcripts in colorectal cancer tissues was performed with those digoxigenin-labeled sense and antisense RNA probes. Serial sections neighboring the section used in immunohistochemistry were deparaffinized with xylene, rehydrated, and treated with 2 \times standard saline citrate (SSC) for 20 min at 60°C. Then the sections were treated with proteinase K (25 mg/ml) in 50 mM Tris-HCl (pH 7.6) for 1 h at 37°C, fixed with 4% paraformaldehyde and transferred to 2 N HCl. After having been washed with diethylpyrocarbonate (DEPC)-treated water, sections were hybridized with

1:100 diluted sense or anti-sense riboprobe for 18 h at 37°C. After further washing with 5× SSC, 2× SSC containing 50% formamide, and TNE buffer [0.5 M NaCl, 10 mM Tris-HCl (pH 8.0) and 5 mM EDTA] at 37°C, the sections were treated with 10 mg/ml RNase A diluted with TNE buffer for 20 min at 37°C. They were washed with TNE buffer and 2× SSC and 50% formamide at 37°C, then a DIG nucleic acid detection kit (Boehringer) was used for color development.

Cell lines and 5-aza-dC (5-aza-2'-deoxycytidine) treatment Human colon cancer cell lines SKCO-1, SW480, COLO320DM and HT-29 obtained from ATCC were used in analysis of syndecan-1 mRNA expression. SKCO-1, SW480 and HT-29 were cultured in Dulbecco's modified Eagle's medium supplemented with 10% FCS, nonessential amino acids and 5% penicillin/streptomycin. COLO-320DM was cultured in RPMI with the above supplements. Then 5×10⁵ cells were plated with 2 μM 5-aza-dC (Sigma, Saint Louis, MO) and incubated at 37°C in a 5% CO₂ atmosphere for 3 or 5 days based on their growth rate.

Quantification of syndecan-1 mRNA expression Syndecan-1 mRNA expression in colon cancer cells was quantified by quantitative PCR using the fluorescence monitoring method with a "Light Cycler" (Idaho Technology, Inc., Idaho Falls, ID). Briefly, total RNA from cancer cells cultured with or without aza-dC was extracted by the standard acid guanidinium-phenol-chloroform method. After treatment with DNAase I, 2 μg of total RNA was reverse-transcribed with oligo-dT primers, deoxynucleotide triphosphates and Superscript II reverse transcriptase (Life Technologies, Inc., Palo Alto, CA). Synthesized cDNA was amplified with specific primers for syndecan-1, and amounts of the PCR products were calculated by measuring the fluorescence of SYBR Green I dye, which binds to double-stranded DNA fragments in each cycle. Human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was also amplified from each cell as an internal standard. Primers for syndecan-1 were 5'-CCT GAA GAT CAA GAT GGC TCT-3' (sense) and 5'-AGT GTG AAG GTC AGT TTG GCT-3' (antisense) and those for GAPDH were 5'-TCA TCT CTG CCC CCT CTG CT-3' (sense) and 5'-CGA CGC CTG CTT CAC CAC CT-3' (antisense).¹⁷⁾ The program for syndecan-1 PCR amplification consisted of 60 s for the initial denaturation period at 95°C, and 50 cycles at 95°C for 0 s, 58°C for 5 s, and 72°C for 18 s. The program for GAPDH PCR consisted of 60 s for the initial denaturation period at 95°C, and 40 cycles of incubation at 95°C for 0 s, 55°C for 5 s, and 72°C for 18 s. Then, the amount of the template in each sample was calculated to the dilution-fold of the external standard and the actual values of syndecan-1 template was corrected based on the value of GAPDH template. The melting curve was analyzed after the amplification in order to confirm the similarity of *T_m* values.

Statistical analysis χ^2 test or Fisher's exact test where appropriate, was used to analyze the incidence of the standard clinico-pathological parameters in syndecan-1-negative and positive tumors. Overall patient survival rates were calculated by the Kaplan-Meier method, and the differences between survival curves were evaluated with the log-rank test. Variables were subjected to univariate analysis and significant variables were then subjected to multivariate analysis using Cox's proportional hazards model. A *P* value of <0.05 was accepted as statistically significant.

RESULTS

Detection of syndecan-1 immunoreactivity in normal colorectal epithelium and colorectal cancer cells Syndecan-1 was stained immunohistochemically in normal epithelium and colorectal cancer cells of surgically resected specimens which were obtained from 105 patients who underwent curative operation of colorectal carcinoma (Fig. 1). In normal epithelial portion, all the epithelial cells showed strong and evenly distributed staining for syndecan-1; syndecan-1 was localized mainly on the basolateral surface of normal epithelial cells. In contrast, syndecan-1 was not equally detected in cancer cells. In 26 of 105 colorectal cancer tissues, few cancer cells were stained by

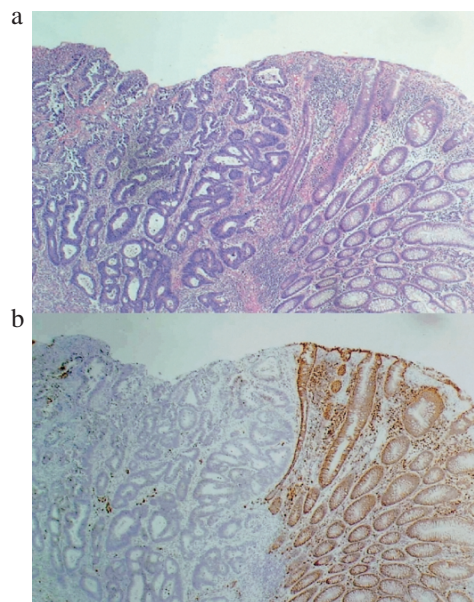


Fig. 1. Immunohistochemical staining with anti-syndecan-1 monoclonal antibody in colon cancer tissues. Normal epithelial cells show strong immunoreactivity of syndecan-1. In contrast, almost all cancer cells have no immunoreactivity of syndecan-1. This tissue is classified into the syndecan-1-negative group. a, Hematoxylin-eosin staining (×40); b, histochemical staining with anti-syndecan-1 antibody (×40).

anti-syndecan-1 antibody (classified as (-) group). In 10 colorectal cancer tissues, less than 25% tumor cells were stained ((±) group). In 51 tissues, 25 to 75% of cancer cells were stained in a randomly arranged manner ((+) group). More than 75% of cancer cells were stained strongly, as well as normal epithelium, in only 18 tissues out of 105 specimens examined ((++) group). In the majority of positively-stained cancer cells, syndecan-1 was found on the basolateral surface, which is a similar distribution to that seen in normal epithelial cells. However, in some undifferentiated cancer cells, syndecan-1 was distributed diffusely in the cytoplasm. Eventually, 69/105 (65.7%) specimens were classified into the syndecan-1 positive group ((++) and (+) groups), and 36/105 (34.3%) specimens were classified into the syndecan-1 negative group ((±) and (-) groups).

Expression of syndecan-1 gene transcript in colorectal cancer cells In ISH experiments, a digoxigenin-labeled antisense riboprobe hybridized to the cytoplasm was detected in normal colorectal epithelial cells. In cancer tissues, hybridization of antisense riboprobe was detected in the same cells that showed strong syndecan-1 immunoreactivity (Fig. 2). Hybridization of the probe to syndecan-1-

negative tumor cells was not seen. These results indicate that the decreased synthesis of syndecan-1 mRNA contributes to loss of syndecan-1 expression.

The results of the quantification of syndecan-1 mRNA in colon cancer cells are shown in Table I. In all 4 cell lines, syndecan-1 mRNA expression was increased by treatment with methylation inhibitor 5-aza-dC. These results raise the possibility that suppression of *syndecan-1* gene expression is caused by hypermethylation of the promoter.

Association of decreased syndecan-1 expression with clinical stage or histological features in colorectal cancer All colorectal cancers were staged according to the UICC TNM classification. Fifty tumors (50/105, 47.6%) were classified into T1, and 3/105 (2.9%), 32/105 (30.5%), and 20/105 (19.0%) tumors were classified into T2, T3 and T4, respectively. Thirty patients (30/105, 28.6%) had lymphnodal metastasis (21, N1; 7, N2; 2, N3) and 8 patients (8/105, 7.6%) had liver metastasis at the time of diagnosis. Differentiation of tumor cells was classified into three histological subclassifications according to the WHO criteria.¹⁸⁾ Forty-five (45/105, 42.9%) specimens were diagnosed as well-differentiated adenocarcinoma, and 53/105 (50.5%) and 7/105 (6.7%) specimens were classified into moderately differentiated and poorly differentiated adenocarcinoma, respectively.

As shown in Table II, the incidences of syndecan-1-negative tumors in T1–2 and T3–4 tumors were not different. The sizes of syndecan-1-positive and negative tumors were distributed equally. However, the incidence of well-differentiated adenocarcinoma was significantly lower in

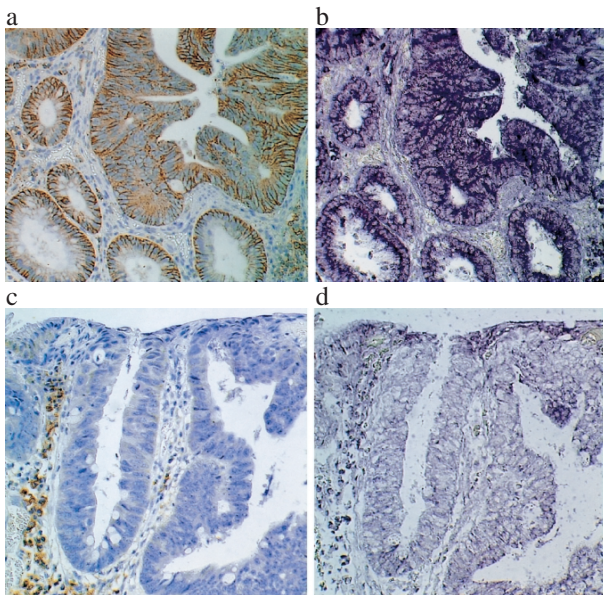


Fig. 2. *In situ* hybridization (ISH). a: Immunohistochemical staining with anti-syndecan-1 monoclonal antibody (×40). b: ISH with antisense riboprobe. Syndecan-1 mRNA is stained with purple substrate (×40). c: Immunohistochemical staining with anti-syndecan-1 monoclonal antibody (×100). Syndecan-1 immunoreactivity is not detectable in cancer cells in contrast to plasma cells. d: ISH with antisense riboprobe (×100). No cancer cells are hybridized with antisense riboprobes in ISH. Plasma cells which show strong immunoreactivity of syndecan-1 are positively hybridized.

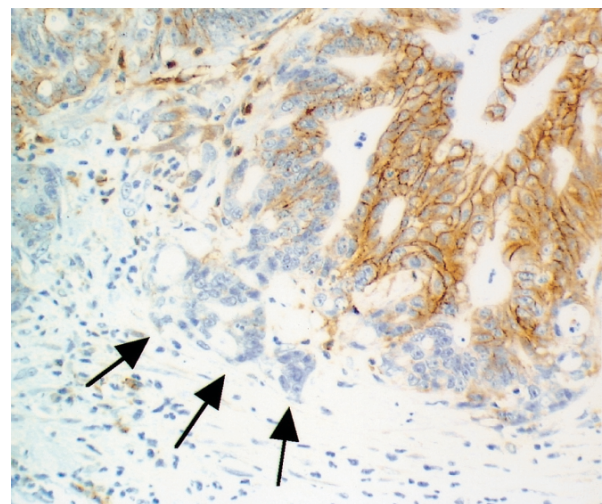


Fig. 3. Immunohistochemical staining of syndecan-1 at the site of tumor budding. Reduced syndecan-1 immunoreactivity is shown in the clusters of cancer cells which are detached from malignant glands at the invasion front of the tumor (arrows) (×100).

Table I. Syndecan-1 mRNA Expression in Colon Cancer Cells Treated with 5-Aza-2'-deoxycytidine (IU)

	Aza-dC (-)	Aza-dC 2 mM
SKCO-1	0.15	0.53
colo320DM	0.21	0.46
HT29	1.21	2.5
sw480	0.88	1.32

IU: The unit of PCR products is based on a volume of standard DNA.

syndecan-1-negative tumors. Moreover, the incidence of lymphnode metastasis or liver metastasis in syndecan-1-negative tumors was significantly higher. These results strongly suggest that the reduced or abolished syndecan-1 expression in colorectal cancer cells is closely related to cell differentiation status and metastatic potential.

To minimize the confounding factors, we analyzed the possibly involved characters in T1 colorectal cancers in both syndecan-1-positive and negative tumors. As indicated in Table III, the tumor size was distributed equally in both groups. The incidence of lymphatic metastasis and the undifferentiated character of cancer cells were significantly higher in syndecan-1-negative tumors. The 'bud-

Table II. Correlation of Syndecan-1 Expression with Seven Factors in Colorectal Cancers

	Syndecan-1 expression		P value
	Positive (++ or +)	Negative (± or -)	
Age (mean, range)	64 (35-81)	68 (40-82)	0.2154
Sex	M	45	0.3332
	F	24	
Tumor size (mean, range)	25 (5-120)	25 (5-90)	0.4576
T factor	T1-2	37	0.3719
	T3-4	32	
N factor	N0	54	0.0319
	N1-3	15	
Liver metastasis	-	67	0.0116
	+	2	
Histological grade Well differentiated adenocarcinoma		37	0.002
		8	
Moderately or poorly differentiated adenocarcinoma		32	0.002
		28	

ding' of cancer cells, defined as small solid clusters of undifferentiated cancer cells or microacinar structures found in the basal or lateral edge of a malignant gland structures,¹⁹⁾ was more frequently observed in syndecan-1-negative tumors ($P=0.034$). Even in some syndecan-1-positive tumors, syndecan-1 expression was often reduced in the clustered or detached cells from the neighboring malignant gland structures (Fig. 3). The incidences of invasion to small vessels such as lymphatic duct permeation and blood vessel permeation were not significantly different.

The results suggested that the loss of sufficient amount of syndecan-1 expression on the surface of cancer cells leads to detachment of these cells from the original tumor structure, breaking up the well-arranged glandular structures. The histological features of these detached cells or cell clusters often appeared as undifferentiated, so that syndecan-1-negative tumors are sometimes diagnosed as poorly differentiated cancers.

Survival analysis Among the 105 patients, 69 with syndecan-1-positive ((++) or (+)) colorectal cancer showed a better clinical outcome than those with no or a low level of syndecan-1 ($P=0.0036$) (Fig. 4). The 5-year survival rate of patients with syndecan-1-positive cancers was 85.4%, whereas that of patients with syndecan-1-negative

Table III. Correlation of Syndecan-1 Expression with Eight Factors in Colorectal T1 Cancers

	Syndecan-1 expression		P value
	Positive (++ or +)	Negative (± or -)	
Age (mean, range)	65 (35-81)	68 (56-73)	0.5604
Sex	M	22	0.8487
	F	13	
Tumor size (mean, range)	15 (5-32)	20 (5-40)	0.1857
N factor	N0	31	0.0479
	N1-3	4	
Histological grade Well differentiated adenocarcinoma		26	0.0063
	Moderately or poorly differentiated adenocarcinoma	9	
Lymphatic permeation	-	18	0.2394
	+	17	
Vessel permeation	-	21	0.6563
	+	14	
Budding	-	27	0.0343
	+	8	

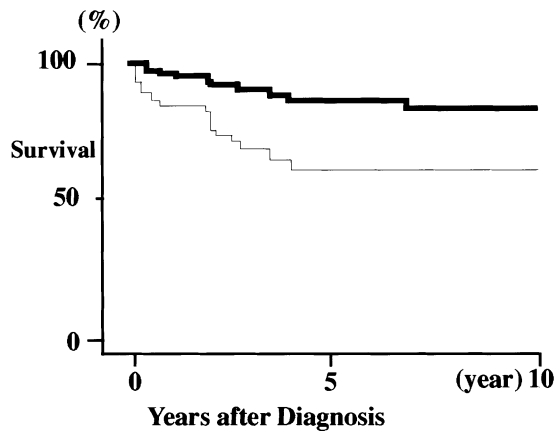


Fig. 4. Cumulative survival rate of patients with syndecan-1-positive or negative colorectal cancer (Kaplan-Meier method). Sixty-nine patients with syndecan-1-positive colorectal cancer show better clinical outcomes than those with cancer that expressed little or no syndecan-1 ($P=0.0036$). — syndecan-1-positive ($n=69$) 5-year survival rate, 85.4%; - - syndecan-1-negative ($n=36$) 5-year survival rate, 58.1%.

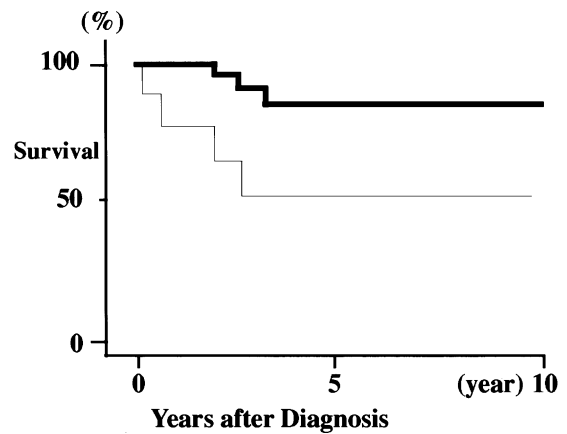


Fig. 5. Cumulative survival rate of patients with syndecan-1-positive or negative colorectal cancer in stage II (Kaplan-Meier method). Twenty-one patients with syndecan-1-positive colorectal cancer show better clinical outcomes than those with cancer that expressed little or no syndecan-1 ($P=0.034$). — syndecan-1-positive ($n=21$) 5-year survival rate, 85.0%; - - syndecan-1-negative ($n=9$) 5-year survival rate, 51.9%.

Table IV. Statistical Analysis for Detection of Factors That May Affect the Survival of Patients with Colorectal Cancer

	Hazard ratio	95%CI
T3 or 4 stage	25.98	3.49–193.64
Lymphnode metastasis	2.35	1.04–5.30
Liver metastasis	3.24	1.26–8.36
Syndecan-1 negative	2.91	1.28–6.64

((–) or (±)) cancers was only 58.1%. Next, we attempted to find the factors which affect the prognosis of these patients by using Cox’s proportional hazards model. We included the following factors: tumor size, invasion to subserosa or further, histological features of moderately or poorly differentiated adenocarcinoma, the presence of lymphnode or liver metastasis, and syndecan-1-negative character (Table IV). Syndecan-1-negative status had an independent prognostic value (hazard ratio (HR) 2.908, 95%CI 1.275–6.635). T3 or 4 stage (HR 25.981, CI 3.486–193.644), metastasis to lymphnode (HR 2.349, CI 1.041–5.299) and liver metastasis (HR 3.242, CI 1.258–8.356) were also independent prognostic factors. We further analyzed the contribution of syndecan-1 expression in the prognosis of stage II patients, to adjust the stage of the tumors in groups with or without syndecan-1 expression (Fig. 5). In 30 patients categorized into stage II, 21 patients with syndecan-1-positive cancer showed a better prognosis than those with syndecan-1-negative cancer. The 5-year survival rate of the patients with syndecan-1-positive tumors was 85.0%, whereas that of the patients with

syndecan-1-negative tumors was only 51.8%. These results also suggest that the loss of syndecan-1 expression in colorectal cancer cells is closely related to malignant character of the affected tumor, which strongly affects the patients’ outcome after surgery.

DISCUSSION

In the present study, we found that the loss of syndecan-1 expression was closely associated with poor prognosis of patients with colorectal cancer in a stage-independent manner. The loss of syndecan-1 expression substantially contributed to the prognosis of the patients with stage II colorectal cancers. These results imply that adjuvant treatment, such as the systemic chemotherapy, should be performed for patients with syndecan-1-negative tumors. Furthermore, our data on the loss of syndecan-1 in cancer cells with undifferentiated and more invasive characters suggest that syndecan-1 may play a suppressive role in scattering and in invasion or metastasis in colorectal cancer cells.

Reduced expression of syndecan-1 in squamous cell carcinoma of uterine cervix²⁰⁾ or of head and neck lesions,¹¹⁾ adenocarcinoma from alveolar epithelium,¹³⁾ and hepatocellular carcinoma⁶⁾ has been reported to be associated with de-differentiating cancer cells and increasing metastatic potential. These results clearly suggest that syndecan-1 is a common, important molecule in the regulation of cell proliferation and differentiation.

In glandular epithelium, especially in intestinal epithelium, syndecan-1 has a central role in morphogenesis of

normal intestinal mucosa.^{3,4)} It is also reported that syndecan-1 expression is lost during the progression of benign adenoma to adenocarcinoma in colon.¹⁵⁾ Our immunohistochemical results showed that syndecan-1 expression was actually decreased in one-third of the colon cancer tissue samples. The reduction of syndecan-1 expression was closely associated with loss of glandular structure of cancer tissue. Furthermore, the incidence of lymphnodal or liver metastasis of syndecan-1-negative tumors was significantly greater than that of syndecan-1-positive tumors. The higher incidence of metastasis strongly contributed to shortening the survival period of the patients after the resection of syndecan-1-negative colon cancer. These results raise the possibility that the expression of syndecan-1 around the surface of cancer cells may help to maintain epithelial structure, which depends on the tight attachment between the cells. It is also possible that syndecan-1-negative tumors may more readily invade the blood or lymph vessels.

However, the results of the immunohistochemical analysis of T1 colorectal cancers (Table II) did not show a significant difference among the incidences of lymphatic or blood vessel permeation in syndecan-1-negative and positive tumors. In contrast, the "budding" feature, which indicates the detachment of invading cancer cells from the nests, was more frequently observed in syndecan-1-negative tumors. Furthermore, the loss of syndecan-1 expression was observed in cells migrating from syndecan-1-expressing tumor structures (Fig. 3). We suggest that the remaining expression of syndecan-1 in colon cancer may limit the primary migration of the cancer cells into interstitial tissues.

As to the mechanisms of regulation of cell attachment, some reports have noted a function of syndecan-1 as a co-receptor of growth factors, such as bFGF.²¹⁻²³⁾ It was reported that bFGF type 1 receptor is present in benign adenoma, but the expression level was decreased in carcinoma cells, as was the syndecan-1 expression.^{13,24)} These results suggest that loss of syndecan-1 expression may weaken the signals which accelerate the differentiation of colon cancer cells through binding of bFGF, leading to dedifferentiation and detachment from ECM or neighboring cells.

As the cause of reduced or suppressed expression of syndecan-1 during transformation of normal epithelial cells, Levy *et al.* suggest the accelerated degradation of this molecule in a colon cancer cell line.²⁵⁾ Increased shed-

ding of the extracellular domain of syndecans was also predicted.¹³⁾ On the other hand, Nakanishi *et al.* reported a reduction of syndecan-1 mRNA due to alteration of the 3' untranslated region of mRNA in cervical-carcinoma cells.²⁶⁾ Thus, multiple or tissue-specific mechanisms may contribute to reduced expression of syndecan-1 in neoplastic tumor cells. Our ISH results revealed that mRNA production of syndecan-1-negative tumors was also suppressed in colon carcinoma cells. Furthermore, in our experiments with colon cancer cell lines, 5-aza-dC treatment, which inhibits the methylation of genomic DNA,²⁷⁾ augmented syndecan-1 mRNA synthesis. These results indicate that the transcriptional regulation of syndecan-1 expression may also exist in colon cancer cells.

The hazard model analysis of our patients showed that the reduced expression of syndecan-1 is an independent factor that affects the patients' survival. Poor prognosis of stage II colon cancer cases with syndecan-1-negative tumor (Fig. 5) suggests that undetectable micro-metastasis may frequently be present at surgery in these patients. Adjuvant chemotherapies are strongly recommended for these patients. However, these strategies are not always effective.²⁸⁾ If the metastatic potential of tumor cells could be decreased, it would help to prevent the further extension of these refractory tumors. Syndecans are known to be inducible in some cell lineages. PR-39, a natural antimicrobial peptide derived from leukocytes, can induce syndecan expression in fibroblasts.²⁹⁾ Our previous report indicates that syndecan-1 is also induced in gastric epithelium after mucosal injury.¹⁰⁾ These facts encouraged us to develop a novel molecular strategy to re-induce syndecan-1 expression in cancer cells. Our recent report showed that transfection of *PR-39* gene into hepatocellular carcinoma cell lines can augment the expression of syndecan-1, and reduce the invasive behavior of the cells.³⁰⁾ It is possible that inducers of syndecan-1 in colorectal cancer will suppress the invasive behavior of the cancer cells. Strategies utilizing substances that increase syndecan-1 expression, such as PR-39, may improve the prognosis of those patients.

In summary, decreased syndecan-1 expression of colon cancer cells was closely associated with metastatic potential. This is a possible biomarker for practical management of colon cancer patients, as well as a predictor for poor prognosis.

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