Association of Syndecan-1 with Angiogenesis-related Markers, Extracellular Matrix Components, and Clinicopathological Features in Colorectal Carcinoma

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Abstract. *Syndecan-1 is a transmembrane heparansulfate proteoglycan, which regulates cell proliferation, migration, angiogenesis, cell-to-cell and cell-to-extracellular matrix adhesion and may influence malignant cell behavior. We investigated the alterations of syndecan-1 expression in colorectal cancer and analyzed the relationship between clinicopathological parameters, proliferation indices, angiogenic markers, and extracellular matrix components. Syndecan-1 protein expression observed in the tumorous epithelium was high in 52/97 (53.6%) of the studied cases, moderate in 20/97 (20.6%), and weak in 5/97 (5.22%) of the cases, and there was strong stromal expression in 34.02% of the tumors. Syndecan-1 expression was statistically correlated to VEGF expression in tumor (p=0.001) and vessels (p=0.007). In addition, there was a borderline correlation between syndecan-1 expression and tenascin (p=0.053). Patients with weak staining reaction had a more unfavorable prognosis (p=0.032) in univariate analysis. These results indicate the implication of syndecan-1 in the remodeling and angiogenesis of colorectal cancer tissue, through interaction with other extracellular matrix components and VEGF, probably influencing the tumor progression and aggressiveness.*

Colorectal carcinoma (CRC) is the third form of cancer in both men and women in the U.S.A and Europe (1). It remains a major cause of cancer mortality, with a 5-year survival rate of 60%, and its incidence is expected to increase in association with the ageing of Western populations (1, 2). Currently, lymph node metastasis and depth of the primary tumor are the most important prognostic factors predicting recurrence and disease-specific survival. The major therapeutic approach is surgical resection and there is an urgent need to identify new biomarkers to improve strategies for adjuvant therapies and postoperative monitoring (2, 3). The penetration of tumor cells into lymphoid vessels and blood vessels leads to tumor metastasis and ultimately the tumor becomes fatal (2, 3). Tumor invasion and metastasis are the result of highly coordinated processes that involve multiple intracellular and extracellular factors (4). In part, carcinoma cell migration is enabled by the altered differentiation status of the epithelial cells which includes changes in cell-cell and cell-matrix adhesion properties (5). During recent years, attention has been drawn to the role of cell adhesion in tumor development and progression. Cell-to-cell and cell-to-extracellular matrix (ECM) interactions are crucial with regard to tumor transformation and spreading.

Syndecans are type-I transmembrane proteins that belong to the family of heparin sulfate proteoglycans (HSPGs), with an *N*-terminal signal peptide, an ectodomain that contains several consensus sequences for glycosaminoglycan attachment, a single transmembrane domain and a short C-terminal cytoplasmic domain (6). There are four well-known syndecans, with homologous transmembrane and cytoplasmic domains, but extensively different extracellular domains. Syndecan-1 and -3 and syndecan-2 and -4 are considered to form subfamilies, based on sequence comparisons within their regions (7). Along with other cell adhesion molecules, such as integrins and cadherins, syndecans are implicated in the regulation of cell differentiation, proliferation, morphology, migration, and in the modulation of growth factors' activity (8). Syndecan-1 is the molecule most extensively studied of this family. In adult mouse tissues, it is expressed almost exclusively at the epithelial cell surface and is abundant in stratified squamous epithelium. Expression appears to be lost

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as the cells become mature or highly differentiated (9). Furthermore, syndecan-1 positivity has been demonstrated on both epithelial and stromal cells of the uterus, vagina, and in the embryonic kidney (10).

Syndecan-1 binds various ECM components, specifically collagens (11), fibronectin (12), thrombospondin (13), and tenascin (14), and also acts as a receptor for the heparin-binding growth factors, such as epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), and some of their receptors, such as (FGRF), suggesting a role in the generation of mitotic signals (15, 16). Its binding capacity to FGF, which exhibits angiogenic activities, can be crucial in the neovascularization of malignant neoplasms (16).

Several studies, specifically concerning tissue sections from squamous carcinomas and adeocarcinomas, have demonstrated that loss of syndecan-1 expression is implicated in malignant transformation of epithelial cells (17, 18). Despite the large number of studies, its role remains controversial. In fact while many types of cancer are associated with decreased syndecan-1 protein content, including squamous cell cancer and adenocarcinomas (19, 20), others, such as pancreatic (21) and breast cancer (22), have demonstrated increased levels. Its expression is also induced in the stroma adjacent to breast carcinoma cells, particularly in tumors exhibiting an aggressive phenotype (22), and in the stroma of gastric cancer tissue, where its presence has been correlated with a worse prognosis (18). These findings raise the suggestion that its presence is cell-type dependent.

Syndecan-1 has been reported to have a prognostic impact in certain malignancies, such as squamous cell (23), hepatocellular (24), laryngeal (25), gastric (18), CRC (26), prostatic (27) and lung carcinomas (28), and mesothelioma (29). In these studies, loss of syndecan-1 expression was associated with advanced tumor stage, increased metastatic potential and shorter overall survival. It has been reported that syndecan-1 expression is decreased in colorectal adenocarcinomas in comparison to adenomas and normal tissue (30, 31). Increased levels of syndecan-1 in the local stroma have also been described (32). However, the prognostic relevance of changes in syndecan-1 expression in CRC remains unclear, as indicated in current published studies.

The purpose of the present study is to investigate the relation between syndecan-1 and ECM components, as binding of cells to ECM involves a number of different adhesion receptors. Cancer cells are surrounded by a modified ECM composed of a complex mesh of collagens, fibrillar glycoproteins and proteoglycans which intercommunicate with the cell interior and thus modulate cell adhesion, proliferation and differentiation (33). Tensacin and fibronectin are glycoprotein components of the ECM, and seem to have competitive functions, while laminin and collagen type IV constitute the major intrinsic components of basement membranes and are involved in cellular adhesion to basement membranes and the ECM (34).

In the present study, we determined the syndecan-1 antigen content in a series of CRCs, in an attempt to clarify its potential clinical importance. Syndecan-1 expression was also correlated to the expression of ECM components, such as tenascin, fibronectin, type IV collagen, and laminin, proliferating indices and neovascularization markers.

Table I. *Patients' characteristics in the present study.*

Variable	Patients (n)	%
Gender		
Male	53	54.6
Female	44	45.4
Age, years		
<60	34	35.05
≥60	63	64.94
Grading		
G1	19	19.58
G2	67	69.07
G3	11	11.34
Dukes' stage		
B	56	57.73
C	41	42.26
Size, cm		
≤5	39	40.20
>5	58	59.79
Tumor type		
Mucinous	21	21.64
Non-mucinous	76	78.35

Materials and Methods

Patients. Surgical specimens of CRC (n=97), from patients with primary CRC, excluding those with multiple or metachronous cancer, were randomly selected from the archives of the Department of Pathology, University School of Ioannina, Ioannina, Greece. There were 53 (54.6%) men and 44 (45.4%) women. Sixty-three were >60 years old and 34 were <60 years old. The mean age at diagnosis was 64.92 years (range: 26-86). None of the patients had received chemotherapy or radiation therapy prior to surgery. The pathological features were classified using the UICC-TNM classification (35). Tumors were graded according to the WHO classification criteria as well-, moderately- or poorly-differentiated carcinomas (36). Fifty-six patients were classified as having Dukes' B, and 41 as having Dukes' C disease. There were 76 non-mucinous adenocarcinomas and 21 mucinous adenocarcinomas. Nineteen cases were well-differentiated, 67 were moderately-differentiated and 11 were poorly-differentiated. In 58 (59.79%) cases the tumor size was > (5 cm), and in 39 (40.2%) cases it was ≤5 cm. Main patients' characteristics are summarized in Table I. The observation time was 5-163 months (mean SD 56.03±36.32 months). From these patients 33.7% had recurrence or distant metastases and 31.7% were dead from the disease or other causes.

Table II. *Antibodies used in the present study.*

Antibody	Clone	Supplier	Country	Dilution	Incubation time
Syndecan-1	DL-101[a]	Santa Cruz	USA	1:50	Overnight
VEGF	JH121[a]	Neomarkers	USA	1:50	1 hour
CD34	QBEnd/10	Novocastra	UK	1:50	1 hour
Tenascin	TN2[b]	Dako	Denmark	1: 50	1 hour
Fibronectin	568[b]	Novocastra	UK	1: 100	1 hour
Collagen IV	CIV22[b]	Dako	Denmark	1: 50	1 hour
Laminin[b]	Lm	Menarin	UK	1;1000	1 hour
P53	DO-7[a]	Dako	Denmark	1: 50	1 hour
Ki-67 (MIB1)	MD722[a]	Dako	Denmark	1:10	1 hour
PCNA PC 10	MD879	Dako	Denmark	1:20	1 hour

[a]With microwave oven antigen retrieval; [b]incubation with pronase.

The specimens were fixed in 10% neutral-buffered formaldehyde and embedded in paraffin. Based on the quality of the morphological preservation of all available hematoxylin and eosin-stained slides of the surgical specimen sections, we selected one or two paraffin blocks for each case. Consecutive 4-μm sections were cut again from each study block: these sections were immunostained for syndecan-1, tenascin, fibronectin, collagen type IV, laminin, Ki-67, (PCNA), p53, VEGF, and CD34.

Immunohistochemical staining. Immunohistochemistry was performed on selected paraffin blocks, from each case on 4-μm tissue sections placed on poly-L-sysine-coated glass slides. In brief, tissue sections were de-paraffinized in xylene and dehydrated. For the detection of syndecan-1 (Santa Cruz, Biotechnology, USA) Ki-67 (MIB1, Dako Denmark), PCNA (Dako), p53 (Dako), VEGF (Neomarkers, USA) and CD34 (Novocastra, UK), slides were immersed in citrate buffer (0.1 mM, pH 0.6) in plastic Coplin jars and subjected to microwave irradiation twice for 15 min. For the detection of tenascin (Dako), fibronectin (Novocastra), type IV collagen (Dako), and laminin (Menarin, UK), slides were pre-treated with 1 μl/ml pronase (Dako) for 10 min at room temperature. Subsequently, all sections were treated for 30 min with 0.3% hydrogen peroxide in methanol to quench endogenous peroxidase activity and were incubated with primary antibodies. We used the method based on the streptavidin-biotin-peroxidase complex and developed the chromogen with immersion of the slides in a diaminobenzidine H_2O_2 substrate for 5 min. The slides were counterstained in Harris' hematoxylin, dehydrated and mounted. To determine the specificity of the reaction, control specimens were prepared from normal mucosa tissues. The antibodies used, sources and dilutions are shown in Table II.

Immunohistochemical evaluation. For syndecan-1 protein expression, slides were assessed for the proportion of cells stained as well as their intensity. Firstly the percentage of positive tumor cells was calculated from at least five representative high-power fields per slide, and then the mean percentage per field was calculated. The intensity of immunostaining was visually scored and classified into four groups: 0=negative, 1=weak, 2=moderate, and 3=strong staining. The syndecann-1 staining pattern of the stromal component was evaluated using a semi-quantitative score (0, 1+, 2+, 3+).

Ki-67 (MIB1), p53 and PCNA staining was evaluated by counting the number of positively stained cells in a total of at least 1000 tumors cells. Immunostaining of tumors with these antibodies reveals a primarily nuclear localization of these proteins. The Ki-67, p53 and PCNA reactivity was assessed as being positive only when tumors exhibited intense nuclear staining and reactivity was categorized into two groups: negative expression (fewer than 5% of positive tumor cells) and positive expression (at least 5% positive tumor cells). VEGF staining was considered positive if appropriate brown staining was seen in the tumor cell cytoplasm. The expression of VEGF was assessed according to the percentage and intensity of immunoreactive cells in a total of 1000 neoplastic cells. Furthermore, the qualitative intensity of staining for VEGF was assessed using a scale of 0-3+, with 0 representing no detectable staining, and 3+ representing the strongest staining.

Microvessel density (MVD) was assessed by light microscopy at the site of the tumor containing the highest number of capillaries and small vessels. Areas of invasive tumor containing the most capillaries and small venules were also examined. Areas of neovascularization were found by scanning of the tumor sections at low power (×40), and identifying the areas of invasive carcinoma with the highest number of stained microvessels. Any brown-stained endothelial cell or endothelial cell cluster that was clearly separate from adjacent microvessels, tumor cells, and other connective tissue elements was considered a single, countable microvessel. For each area the number of microvessels identified within high-power fields were counted (×400). Areas of fibrosis, necrosis and inflammation, and vessels with muscle wall were excluded from counting.

Tumors were classified as being positive with regard to the immunoreactivity for tenascin, fibronectin, type IV collagen, and laminin when there was unequivocal immunostaining of the matrix components in at least one representative area of the tumor. The positive tumors were semi-quantitatively scored as 1+, 2+, and 3+ corresponding to weak, moderate and extensive immunoreactivity, respectively.

The immunostaining was assessed from numerically-coded slides without any knowledge of survival or other clinical data. All slides were reviewed and scored in a blind test by two pathologists. Differences in interpretation were reconciled by re-review of slides, separately or jointly at a double-headed microscope. For statistical analysis purposes, the 10% cut-off point for positivity was used for the estimation of Ki-67, 50% for PCNA, and 5% cut-off for p53.

Table III. *Syndecan-1 expression in correlation with clinicopathological data in colorectal cancer.*

	Syndecan-1						*p*-Value
	Epithelial tumor cells			Tumor Stroma			
	+	++	+++	+	++	+++	
Type							
NM*	4	12	60	25	24	29	0.81
Mucinous	1	8	12	3	5	4	0.79
Size							
<5 cm	2	4	39	11	11	11	0.86
>5 cm	5	9	38	17	18	22	0.86
Grade							
1	1	3	13	9	8	10	0.78
2	2	12	46	11	12	11	0.81
3	2	2	16	8	9	12	0.81
Dukes' stage							
B	5	17	35	14	14	15	0.71
C	2	9	29	14	19	18	0.71
Vessel Invasion							
No	4	19	62	11	19	24	0.68
Yes	1	1	8	17	11	9	0.68
Ki67							
<10%	5	13	44	12	15	11	0.32
>10%	2	3	27	16	14	22	0.32
PCNA							
<50%	4	4	10	15	15	10	0.36
>50%	2	15	62	13	18	23	0.36
P53							
<5%	2	7	27	15	14	15	0.34
>5%	3	13	45	13	19	18	0.34

*NM: Non-mucinous.

Table IV. *Syndecan-1 expression in tumor epithelial cells in correlation with angiogenesis-related markers and extracellular matrix components in colorectal cancer.*

	Syndecan-1 expression			*p*-Value
	+	++	+++	
MVD				
Low	1	9	36	0.32
High	3	9	38	0.32
VEGF				
+	1	7	5	0.72
++	1	2	5	0.72
+++	4	8	59	0.001
Tenascin				
+	2	8	20	0.52
++	3	7	23	0.52
+++	1	4	26	0.053
Colagen IV				
+	2	9	13	0.81
++	3	6	22	0.72
+++	1	5	19	0.81
Fibronectin				
–	1	4	4	0.72
+	2	7	22	0.68
++	3	8	46	0.56
Laminin				
+	3	14	16	0.34
++	2	9	18	0.33
+++	1	8	12	0.32

Statistical analysis. The Superior Performance Software System (SPSS) 10.0 for Windows (IL, USA) was used to compare morphological features and protein expression data. Significant differences between expression of target proteins with regard to clinico-pathological parameters were computed by the *t*-test for paired or non-paired values or ANOVA test if the data were normally distributed. Correlation between syndecan-1 and other proteins was computed using the Pearson's correlation coefficient for normally-distributed data or the Kendall's Tau rank correlation coefficient when data did not have a normal distribution. The prognostic significance of syndecan-1 in determining the risk of recurrence was studied with both univariate (log-rank test) and multivariate (Cox proportional hazards) analysis, separately for each group of patients. Overall survival was assessed by the Kaplan Meier method, with the date of primary surgery as the entry data. The end-point was characterized as the length of survival to death from colorectal carcinoma. *p*-Values <0.05 were considered statistically significant.

Results

The expression of syndecan-1 was classified with regard to staining intensity and the percentage of positively-stained tumor cells. In normal colonic mucosa, syndecan-1 was expressed around the basolateral membrane in the cytoplasm of the columnar epithelium and in plasma cells. Syndecan-1 staining was absent from the stroma of normal mucosa. On tumor cells, syndecan-1 was localized around the entire cell membrane and in many cells it appeared to be cytoplasmically-located, with a remarkable reactivity in the lamina propria, in some cases. In the present study, colorectal carcinomas exhibited strong syndecan-1 immunoreactivity in 52/97 (53.6%) cases (Figure 1), moderate in 20/97 (20.6%) (Figure 2), and weak in 5/97 (5.22%), while 20/97 (20.6%) of the cases were totally negative. We observed a progressive decrease in syndecan-1 from normal colonic mucosa to well-, moderately- and poorly-differentiated carcinomas. In contrast to the normal stroma, some tumor specimens, regardless of epithelial tumor expression of syndecan-1, had positive staining for syndecan-1 in the stroma, as well as reactivity of a small number of fibroblasts, neural cells and inflammatory cells mainly macrophages and plasma cells. Stromal cell reactivity, in the vicinity of and within the tumor was also estimated. Seven cases (7.2%) exhibited no staining reaction, 28 (28.9%) exhibited staining of scattered cells, 29 (29.9%) frequent staining, and in 33 (34.02%) cases, most stromal cells exhibited positive staining (Figure 1). In

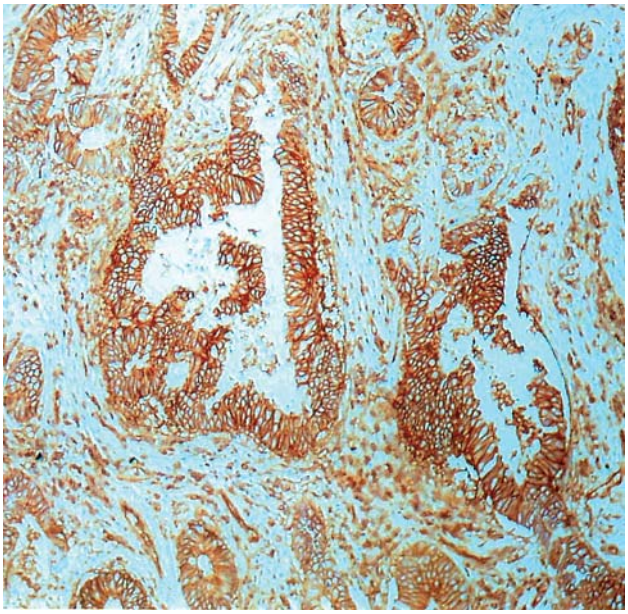

Figure 1. *Strong syndecan-1 immunoexpression in a moderately-differentiated adenocarcinoma of the colon (×100).*

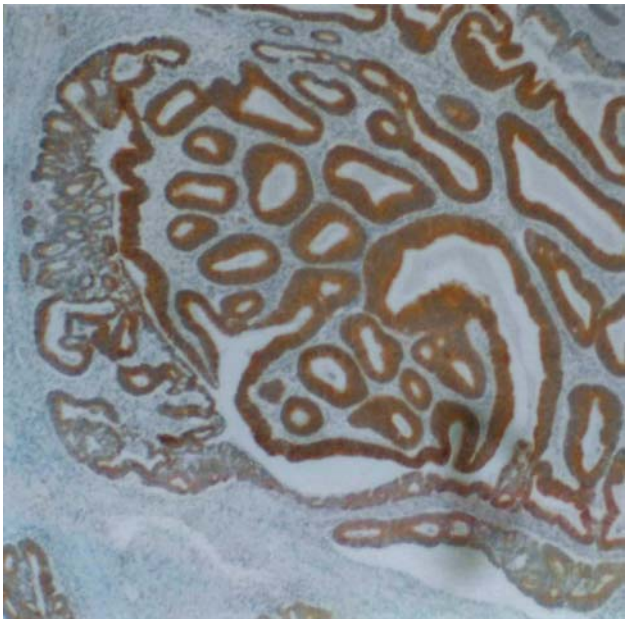

Figure 3. *Strong vascular endothelial growth factor immunoreactivity in moderately-differentiated adenocarcinoma of the colon (×100).*

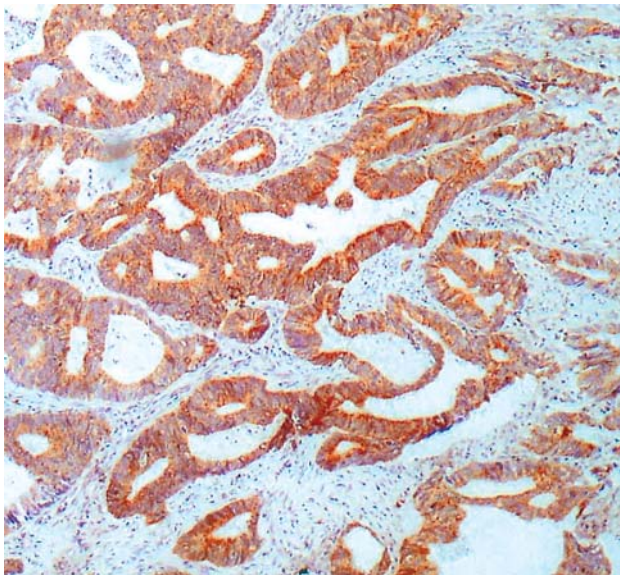

Figure 2. *Moderate syndecan-1 expression in tumor cells of moderate adenocarcinoma of the colon (×100).*

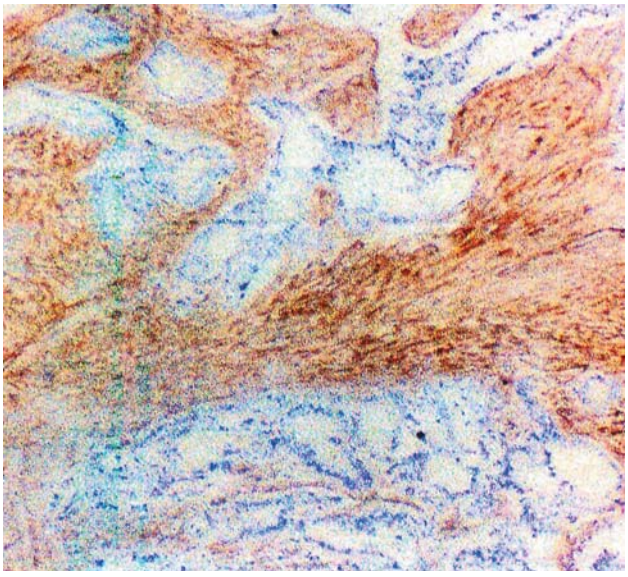

Figure 4. *Strong tenascin staining in the stroma of moderately-differentiated colorectal cancer (×100).*

colorectal cancer, there was no statistically significant association between the level of syndecan-1 expression and age, gender, grade, TNM stage, tumor size or nodal status, in the present study. Epithelial syndecan-1 expression increasing from negative to strong did not correlate to stromal syndecan-1 expression (p=0.41). There was no statistically significant association between the level of stromal syndecan-1 expression and age, gender, TNM stage, tumor size or nodal status (Table III).

VEGF immunohistochemical expression was detected in the cytoplasm and in some cases in the membranes of carcinoma cells, although with different percentages of

stained cells. Specifically, 66 out of the 97 (68.1%) cases were strongly-positive (Figure 3), 17 (17.5%) tumors in patients were moderately-positive, 5 (5.2%) were weakly-positive, and 9 patients (9.3%) were negative. Immunoreactivity in the tumor vessels was observed in 43 (44.3%) cases with strong immunostaining, 17 (17.5%) with moderate, 23 (23.7%) with weak and 14 (14.5%) cases were totally negative. CD34 immunoreactivity was detected in vascular endothelial cells. Microvessels are represented by brown capillaries or small clusters, which stand out sharply from other tissue. At the tumor site the MVD ranged from 15 to 122 (mean SD 56.3±21.3) (Table IV). A positive association was observed between VEGF expression and MVD (p=0.0016).

Strong and well-defined collagen type IV staining was seen in the basement membrane of normal mucosal epithelium and around blood vessel walls. The epithelial cells themselves exhibit negative staining. Small amounts of type IV collagen expression were observed in 24 (24.7%) of the cases, moderate in 31 (31.9%) and strong in 25 (25.8%), while 17 (17.5%) of the cases were negative. In addition, type IV collagen staining was lacking in poorly-differentiated CRCs. No statistically significant association was found between type IV collagen expression and epithelial or stromal syndecan-1 expression.

Normal intestinal mucosa exhibited an organized glandular epithelium overlying a well-defined continuous linear basament membrane that stained for laminin. Small amounts of laminin expression were observed in 33 (34.02%) of the cases, moderate in 29 (29.9%), extensive in 21 (21.6%), while 14 (14.4%) of the carcinomas were completely negative. In some cases, well- and moderately-differentiated tumors exhibited areas of intact basement membrane. An association was found between lack of laminin in CRC and poorly-differentiated carcinomas (p<0.001). There was no statistical significance between syndecan-1 expression and laminin expression (p>0.05).

Expression of tenascin in CRC was studied by immunohistochemistry, and was found to be negative, or rarely weakly-positive in adult colonic mucosa and blood vessels. A small amount of stromal tenascin expression was detected in 30/97 (3.09%) cases, staining was moderate in 33/97 (34.02%), and strong expression was found in 31/97 (31.95%) of the primary tumors (Figure 4). The pattern of expression of tenascin was diffuse and interstitial, forming a fishnet network of fibers around individual tumor cells. A statistically significant difference was found (p<0.001) between staining patterns for poorly differentiated tumors when compared with well- and moderately-differentiated tumors. A borderline statistical relation was found between syndecan-1 tumor epithelial cells and tenascin expression (p=0.053).

Fibronectin expression was negative in 7/97 (7.21%) of the cases and positive in >50% of the areas in 57/97 (58.76%) of the cases. The pattern of fibronectin expression was continuous band-like, surrounding tumor nests in 38.62% of the cases, and showing diffuse distribution in the tumor stroma in 61.04% of the total cases examined.

Syndecan-1 expression levels in tumor cells correlated to VEGF tumor expression (p=0.001), and VEGF vessel expression (p=0.007). No correlation was observed between syndecan-1 expression and MVD. In univariate analysis, syndecan-1 staining intensity correlated well with overall survival (p=0.032), in such a way that patients with tumors of weak staining reaction had a less favorable prognosis. Multivariate analysis of syndecan-1 protein expression did not reveal any prognostic significance.

Discussion

CRC has a high incidence among gastrointestinal tumors. Numerous studies are still discussing and searching for new prognostic markers or marker combinations of tumor development and progression (37). Generally, carcinoma cells are characterized by poor intercellular adhesion, loss of differentiated epithelial morphology and increased cellular motility.

Syndecan-1 is a well-known marker protein that participates in cellular and cell matrix adhesion, cell proliferation, migration and angiogenesis (7, 11). Many studies have already demonstrated that malignant transformation is usually accompanied by altered syndecan-1 expression, indicating that during epithelial tumorigenesis, expression of syndecan-1 is lost (17). In this way, the overall expression of syndecan-1 is markedly reduced in carcinoma cells deriving from carcinoma of the lung (28), uterine cervix (38), larynx (25), liver (24), prostate (27, 40), thyroid (41), and colorectum (26,30), and from squamous cell carcinoma of head and neck (23), and tongue (39), and renal cell carcinoma (42), as compared to their normal counterparts.

In the present study, our findings on surgically resected CRC specimens add to the body of evidence that loss of epithelial syndecan-1 expression is a general feature of carcinoma progression, but in contrast to the majority of studies on CRC (26, 30, 43, 44), our results demonstrated that both CRC epithelial cells and stroma are able to sustain high expression levels of syndecan-1, as 53.6% of the cases exhibited strong positivity in more than 50% of cancer cells, and 34.02% of stromal components (Figure 1). Our findings are in agreement with the study of Lundin *et al*. (31) in which 94% of the carcinomas immunoreacted to syndecan-1, and most recently the report of Pap *et al*., in which positive immunostaining for syndecan-1 occurred in 62.4% of the carcinomas (45). Several mechanisms have been suggested to explain loss of epithelial syndecan-1 expression and tumor progression. Disruption of cell-to-cell and cell-to-matrix adhesion occurs during the development of malignant

epithelial neoplasms (30). Like adhesion molecules, syndecan-1 may have an influence on the tumorigenic activity of cancer cells by altering the adhesion of the ECM and cell morphology. As epithelial syndecan-1 is lost, tumor cells become more migratory by losing their attachment to each other and to the ECM (28). It is unclear whether syndecan-1 expression, seen in the stromal tissue of tumors, is due to the released ectodomain from the cell membrane, or whether it originates from within the stroma itself (31).

The significance of syndecan-1 expression has been studied in many malignancies, and different immunohistochemical expressions have been found in different tumors. For example, there are studies that determined a significant percentage of human breast carcinomas which expressed relatively high levels of several glypicans and syndecan-1, estimated by northern blot analysis (22) and immunohistochemical techniques (46), and reports that showed overexpression of syndecan-1 in the cancerous stroma compared to neoplastic epithelium and the histologically-normal tissue (47). By northern blot analysis, 80% of the samples in pancreatic cancer exhibited moderate- to high-levels of *syndecan-1* mRNA expression (21). By immunohistochemistry, syndecan-1 was abundantly expressed in the cytoplasm and especially on the cell surface of duct-like cancer cells, whereas immunostaining in stromal elements adjacent to cancer cells, was faint or absent (21, 48). Similarly, syndecan-1 *in situ* hibridization signals were evident at moderate to high levels in the majority of cancer cells forming duct-like structures within the pancreatic tumor mass, whereas in the surrounding stroma occasionally very low syndecan-1 signals were observed (21). Ito *et al*. (49), in their study on thyroid cancer, found that epithelial syndecan-1 expression was seen infrequently in well- and poorly-differentiated carcinomas, whereas it was observed in 61.5% of undifferentiated (anaplastic) carcinomas; and they suggested that the role of syndecan-1 in thyroid carcinomas might be unique. Therefore, the biological effects of syndecan-1 might critically depend on the cell type in which syndecan-1 is expressed.

Day and co-workers (30) reported that the expression of syndecan-1 in adenomas and CRCs, is significantly reduced during the late stages of tumor development, especially during transition from moderate to severe dysplasia in adenomas, and of non-invasive to invasive tumors. Consistent with this study is the analysis of Fujiya *et al*. (26) who suggested that loss of syndecan-1 by CRC cells is closely associated with the malignant characteristics of the tumor and with patient survival. Their analysis of syndecan-1 in a panel of patients who underwent curative resection of primary CRC showed that the incidence of lymph node invasion and liver metastasis was higher in patients with syndecan-1-negative tumors; they also noticed a decrease in survival of such patients. These findings were only partially validated in a second study by Lundin *et al*. (31). In this second study, the authors confirmed that the loss of syndecan-1 expression is associated with poor histological differentiation in tumor samples. The data by Lundin *et al*., however, failed to validate the prognostic value of syndecan-1 in CRC. The study of Peretti *et al*. (43) also supports the observations that syndecan-1 is down-regulated in CRC, but again casts doubt on the use of syndecan-1 as a prognostic marker. This is because the authors did not see any correlation between syndecan-1 expression and the histological features or differentiation stage of tumors; the patients in their study are still alive in the 5-year follow-up period, therefore, the investigators did not report on survival outcome. It is also important to note that the studies that have examined the use of syndecan-1 as a prognostic marker (26, 31, 43), were performed in different countries on different continents. In our study, in a univariate analysis, we found that syndecan-1 staining intensity was correlated with overall survival (p=0.032) in a way that patients with reduced staining reaction had a less favorable prognosis. Multivariate analysis did not show any prognostic significance. Hashimoto and co-workers (44) found that syndecan-1 expression was reduced or absent from 87% of adenocarcinomas and only 13% of patients had tumors which stained for syndecan-1 on more than 75% of tumor cells and they noted that syndecan-1 was correlated with a higher TNM stage and lymph node metastasis, and was more common in males; finally, they failed to demonstrate any correlation between the level of either tumor or stromal syndecan-1 and survival. Therefore, other factors such as treatment plans, genetic variations, ethnicity, and immunohistochemical methods (*i.e*. syndecan-1 antibody used), may have influenced the results for the prognostic value of syndecan-1 in CRC progression and metastasis.

Angiogenesis is a multistep process, which involves changes in the ECM, and endothelial cell proliferation, migration and differentiation into capillaries. Studies of tumor biology revealed a complex network of autocrine and paracrine interactions between tumor cells, stromal cells, and endothelial cells, which are in turn influenced by the composition of ECM. Syndecan-1 acts as a co-receptor for several angiogenic and growth factors, including multiple VEGF isoforms and bFGF (6), and has a regulatorial role in angiogenesis (11). Several studies demonstrated VEGF and angiogenesis as being prognostic factors in CRC, VEGF expression being associated with a poor prognosis and therefore with poor overall survival (50). A second finding of our study was the highly significant correlation of strong syndecan-1 expression with VEGF expression of tumor cells (p=0.001) and VEGF expression of vessels (p=0.007). It seems that syndecan-1 acts with VEGF in tumor angiogenesis. In the recent elegant work of Purushothaman *et al*. (51), they found a novel mechanistic pathway of heparanase action. In their study, using multiple myelopma tumor cells, they

demonstrated the mechanism whereby heparanase and syndecan-1 promote angiogenesis. Shed syndecan-1 acts in concert with VEGF, which is also up-regulated by heparanase expression. Together, they drive angiogenesis. These results reveal an important new mechanistic pathway of heparanase action and demonstrated that the heparanase/syndecan-1 axis contributes significantly to tumor angiogenesis in myeloma and perhaps in other types of cancer.

Many properties of the stroma, including ECM components, density of immune and fibroblastic cells, angiogenesis and the production of angiogenic and chemoattractant factors, are known to be regulated as a result of a crosstalk between the tumor and its surrounding host tissue (4). Although the expression of syndecan-1 and other ECM components has been studied in CRC tissues, to our knowledge, there are no reports, to-date, on the relationship between the immunohistochemically determined syndecan-1 and the major basement membrane components. While many immunohistochemical studies have demonstrated quite well that tenascin and fibronectin are up-regulated in cancer tissues, the function of these ECM proteins in cancer invasion and metastasis remains controversial (52). Syndecan-1 can bind several ECM molecules, among which, tenascin (14). In our study we found a borderline-positive correlation between expression of syndecan-1 in the stroma and tenascin expression (p=0.053). We speculate that the maintenance of syndecan-1 expression in CRC leads, in a direct or indirect way, to induction of tenascin production by epithelial cells, which in turn contributes to the formation of tumor stroma necessary for neoplastic growth and infiltration of vascular structures. Hanamura *et al*. (52), in their study of semi-quantitative analysis of mRNA expression in colon cancer revealed significant relationships between the degrees of tenascin and fibronectin mRNA expression, and deep invasion and the presence of lymph node metastasis; they concluded that ECM protein expression may be an indicator of malignant tumor behavior, reflecting remodeling activity of the cancer stroma. In the present study, we failed to demonstrate any correlation between epithelial and/or stromal syndecan-1 expression and collagen type IV, fibronectin and laminin.

In conclusion, the malignant process interferes with normal ECM biosynthesis and can modify the structure and composition of the matrix. To our knowledge, this is the first study of syndecan-1 expression in correlation with ECM components and angiogenesis, and an interesting finding of this study was the relationship of syndecan-1 expression with VEGF and tenascin.

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