Syndecan-1, Epithelial-Mesenchymal Transition Markers (E-cadherin/β-catenin) and Neoangiogenesis-related Proteins (PCAM-1 and Endoglin) in Colorectal Cancer

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Abstract. *The Syndecan-1 protein plays a crucial role in cell proliferation, cell adhesion, cell migration and angiogenesis and, at the same time, its co-expression with E-cadherin is regulated during epithelial-mesenchymal transition (EMT). In colorectal cancer (CRC), the expression of syndecan-1, E-cadherin/β-catenin complex is frequently disturbed. Angiogenesis is critical for the growth and metastatic spread of tumors. In the present study, we focused on the expression of these biological molecules and their prognostic significance in human CRC. Formalin-fixed paraffin-embedded surgical specimens from 69 patients with CRC were immunostained for syndecan-1, E-cadherin, β-catenin, endoglin (CD105) and CD31 (platelet cell adhesion molecule (PCAM-1)). A significant association was found between syndecan-1 with E-cadherin (p<0.0001), as well with β-catenin (p<0.0001). High β-catenin expression appeared to reduce the risk of poor outcome. Endoglin microvascular density (MVD) count was correlated significantly with Dukes' stage (p<0.0001), vessel invasion (p<0.0001), lymph node metastasis (p=0.039), liver metastasis (p<0.0001), recurrence of disease (p=0.010) and poor survival rate (p<0.0001). Endoglin tumor epithelial cell expression was associated with E-cadherin, β-catenin and syndecan-1 (p=0.001, p=0.068 and p=0.005, respectively). In conclusion, changes in the pattern of expression of syndecan-1, EMT markers, E-cadherin/β-catenin, in association with endoglin (CD105), may be involved in tumor progression and prognosis of CRC patients. Further studies are needed to clarify the interaction between these proteins and tumor initiation and progression.*

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Colorectal cancer (CRC) is the most common tumor of the gastrointestinal system, the third most frequent cancer worldwide and the fourth most frequent cause of death (1). In Greece, the last two decades, CRC has a high incidence and mortality rates, in both sexes (2). CRC frequency varies markedly by region and community. This variability is due to differences of dietary and environmental factors. Epidemiological studies showed that CRC is more frequent in communities with a high-fat and low-fiber diet, while the risk is increased in populations migrating from low-risk to high-risk regions (3). The disease, in sporadic colon cancer, reaches the highest incidence at the age of 60-70 years (1, 3). At the time of diagnosis, fifty percent of CRC cases are advanced (1) with metastasis and fatal in most cases.

Except of its high incidence, the molecular pathways involved in colorectal carcinogenesis are not yet well-understood. Basic research has highlighted the understanding of cancer biology and distinguished molecules, such as syndecan-1, an important cell adhesion molecule (4). Syndecans are type I transmembrane proteoglycans with 3 major domains: an extracellular with heparan sulfate chains, a transmembrane and a short cytoplasmic. Syndecans are implicated in the regulation of cell-to-cell and cell-to-extracellular matrix (ECM) adhesion, as well as cell migration. This process is achieved in normal tissues by binding of heparan sulfate chains to ECM molecules and effectors, such as growth factors, cytokines, proteinases and proteinase inhibitors (4, 5). Syndecan-1/CD138, which is mainly expressed by epithelial cells in adult tissues, also

affects tumorigenesis by regulating the molecular mediators of tumor cell survival, proliferation, angiogenesis and metastasis (4). Many studies have shown that the expression of syndecan-1 is deregulated in a number of cancers, while loss of its epithelial expression has been associated with a poor survival in laryngeal, colorectal and gastric cancer (6-9). Syndecan-1 expression is also associated with the maintenance of epithelial morphology and inhibition of invasion (10). Reduced expression of syndecan-1 is related with malignant transformation in hepatocellular carcinoma (11) with bad prognosis in colorectal carcinoma (12) and with poor histological differentiation in prostatic cancer and mesothelioma (13, 14). Furthermore, the expression of syndecan-1 is increased during epithelial regeneration and rearrangement in the stomach (15). Moreover, many studies have demonstrated expression of syndecan-1 in fibroid cells in the stroma of tumors, where its high expression is a poor prognostic marker in human malignancies (16, 17).

The epithelial-mesenchymal transition (EMT) takes place during embryonic development (18) and many studies have focused on its role in tumor progression (19). In EMT, an epithelial cancer cell loses its epithelial characteristics, such as adherens junctions, and gains properties of mesenchymal cells, causing metastasis (20). EMT is classified into three different subtypes (21) according to its function and the pathways involved. Type 1 EMT associates with implantation, embryo formation and organ development. Type 2 EMT associates with inflammation, which, once subsiding, EMT terminates its action. Type 3 or oncogenic EMT occurs in neoplastic cells that have previously undergone genetic and epigenetic changes, promoting invasion and metastasis. EMT is frequently a reversible transition as cells can return to their epithelial phenotype, a process known as mesenchymal-epithelial transition (MET) (22).

An important characteristic of epithelial cells is that they have barrier functions that are facilitated by their tight cell-to-cell interactions (23). Loss of these interactions in epithelial cells causes morphological changes and enhancement of their cellular motility (23). The most significant mediator of cell-to-cell adhesion in epithelial tissues is E-cadherin, also known as uvomorulin, which belongs to a family of cell-surface adherence junctional proteins (23). E-cadherin extends outside the cell membrane and adheres to E-cadherin from neighboring cells through calcium-dependent homophilic interactions. The inner end of the protein chain attaches to actin filaments of the cytoskeleton in cytosol (24). In the middle of this chain, there are catenins (25-27). Alpha-catenin binds to actin by a molecule called Eplin (28), while it is connected to either β-catenin (29) or γ-catenin (25), which in turn is connected to E-cadherin. Moreover, β-catenin (30) and γ-catenin (31) are translocated to the nucleus taking part in the Wingless (Wnt) pathway, where the unbound β-catenin, translocates from the cytosol to the nucleus, in a complex with T cell factor (TCF) and lymphoid enhancer-binding factor (LEF). Beta-catenin regulates the expression of several genes, such as *SNAI1* (32), which enhance the expression of the Forkhead box C2 (*FOXC2*) gene whose overexpression induces EMT (33). In addition, a key change that occurs during EMT is the "cadherin switch" in which the normal expression of E-cadherin is replaced by the abnormal expression of N- or P-cadherin (34). This down-regulation of E-cadherin, as mentioned before, is associated with the release of β-catenin, activating WNT signaling. There is evidence that the malfunction of the E-cadherin/catenin complex induces detachment of cancer cells from their neighboring cells in the primary tumor, thus causing metastasis. Several studies have demonstrated reduced expression of E-cadherin (35, 36) and catenins (37, 38) in a number of carcinomas. Moreover, inactivation of the E-cadherin gene (*CDH1*) and irregular expression of its protein are considered to be connected with the dedifferentiation of gastric cancer cells with diffuse phenotype (39, 40). In conclusion, E-cadherin/catenin-mediated cell adhesion is critical in the development and progression of human carcinomas (41), while, on the contrary, E-cadherin alone acts as a suppressor molecule in cancer invasion and metastasis (27, 42, 43).

To assess the pathological significance and prognostic value of angiogenesis in malignancies it is important to use efficient methods for identifying cancer-related neovascularity in malignancies. In human cancer tissues, the most common method for semi-quantitative evaluation of angiogenesis is to measure microvessel density (MVD) by using endothelial markers. Immunohistochemical expression and antibodies against the endothelial cell markers CD31, CD34 and CD105 has often been used in previous studies on cancer tissues (44-46). MVD assessment using endoglin (CD105) as marker has been suggested as a better predictor of progression and prognosis than respective CD31 or CD34 in a variety of cancers (44-46, 47). Several studies have investigated the clinical significance and pathological role of CD105-MVD in CRC (48-50). CD105 overexpression was always associated with lymphovascular invasion, lymph node metastasis and presence of distant metastases. Its expression in patients with CRC, however, could not be used as a prognostic marker (48-50).

The aim of this study was to evaluate the possible association between the immunohistochemical expression of syndecan-1, EMT markers (E-cadherin/β-catenin), platelet endothelial cell adhesion molecule-1 (PCAM-1/CD31) and endoglin (CD105) in a well-characterized series of a CRC population from a Northwestern region of Greece. To our knowledge, the relationship between the expressions of these molecules has not been considered previously. We also studied the relationship between these antibodies with clinicopathological features (Table I).

Table I. *Clinicopathological features of the cases examined.*

	n	%
Gender		
Male	42	60.87%
Female	27	39.13
Age	40-81	64.58±7.20
Tumor size	2.5-10 cm	5.25±1.58
Histology		
Well-differentiated	3	4.35
Moderate	59	85.51
Poor	7	10.14
Type		
Non-mucinous	61	88.41
Mucinous	8	11.59
Lymphatic invasion		
Yes	64	92.75
No	5	7.25
Venous invasion		
Yes	50	72.46
No	19	27.54
Serosal invasion		
Yes	66	95.65
No	3	4.35
Lymph node metastasis		
Yes	45	65.22
No	24	34.78
Peritoneal invasion		
Yes	9	13.04
No	60	86.96
Liver Metastasis		
Yes	10	14.49
No	59	85.51
Dukes' stage		
B2	24	34.78
C2	33	47.83
D	12	17.39
Recurrence		
Yes	21	33.33
No	42	66.67
Death, colorectal cancer		
Yes	12	19.05
No	51	80.95

Materials and Methods

Patients. Sixty-nine patients with CRC, operated between 2003 and 2005, were retrospectively included in this study. Thus, formalin-fixed, paraffin-embedded tissues from CRCs were selected from the archives of the Pathology Department of General Hospital "Hatzikostas", Ioannina, Greece. The study was approved by the local ethics committee. None of the patients had received neo-adjuvant chemoradiation therapy before surgery. The corresponding hematoxylin and eosin slides were reviewed by two pathologists (A.M. and U.S.). Histological diagnosis of tumors was performed according to World Health Organization (WHO) criteria (51). Pathologic staging was performed according to Dukes' classification (52). Each case was classified according to grade, as well as mucinous differentiation, invasion depth, lymphatic invasion, vessels' invasion, peritoneal dissemination, lymph node involvement and liver metastasis. All CRC patients received standard postoperative 5-fluorouracil (5-FU) adjuvant chemotherapy.

Immunohistochemistry. Immunostaining was performed with a DakoCytomation Autostainer Instrument (DakoCytomation, Glostrup, Denmark). Briefly, 4-μm-thick tissue sections were dewaxed in xylene and rehydrated in decreasing concentrations of ethanol. Endogenous peroxidase activity was blocked by incubation with peroxidase-blocking solution (Dakocytomation) for 5 min. Antigen retrieval consisted of autoclave treatment for sections for 30 min in target retrieval solution (pH 6.0, DakoCytomation). The primary antibodies employed were syndecan-1 (clone DL-101, dilution 1:50; Santa Cruz, Dallas, TX, USA), E-cadherin (clone NCH-38, dilution 1:100, Dako, Glostrup, Denmark); β-catenin (clone 7C2, dilution 1:100; Leica, Nussloch, Germany); CD31 (clone JC70A, dilution 1:40; Dako); and CD105 (clone SN6h, dilution 1:100, Dako). Using an Envision Kit (Dako), the slides were incubated with horseradish peroxidase-labeled polymer conjugated with secondary antibody for 30 min and then with substrate chromogen (diaminobenzidine) solution, followed by light counterstaining with Mayer's hematoxylin. In each case, normal mucosa was used as an internal positive control for E-cadherin and β-catenin. For negative controls, the primary antibodies were omitted.

Immunohistochemical evaluation. The immunohistochemical expression was assessed blindly. The slides were reviewed and scored in a blind test by two pathologists (A.M. and U.S.). Differences in interpretation were reconciled by re-review of slides separately or jointly at a double-headed microscope. Syndecan-1 staining was mainly membranous and often cytoplasmic. The syndecan-1 staining pattern of the tumor stromal component was evaluated using a semi-quantitative score. The membranous or nuclear β-catenin expression was evaluated separately. In the case of E-cadherin only, the membranous staining was considered as positive. The proportion of cells stained, as well as the intensity of syndecan-1, E-cadherin and β-catenin immunoexpression, was assessed. 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 noted. The intensity and percentage was scored and classified into four groups: 0, negative and absence of staining (0%); 1, weak and <10% of staining cells; 2, moderate and 10-50% of staining cells; and 3, strong reactivity and >50% of staining cells. For statistical reasons, the immunoexpression of these molecules was categorized into two groups: negative expression (negative and weak immunoreactivity) and positive expression (moderate and strong immunoreactivity). The syndecan-1 staining pattern of the tumor stromal component was evaluated using a semi-quantitative score as well. For CD31 and CD105, after scanning the immunostained section at low magnification (x40), three areas of tumor with the highest number of distinctly highlighted microvessels ("hot spots") were selected by the two observers at the same time. Then, they independently evaluated the slides by microvessel counting using a 400x magnification. Any single cell or spot that stained by immunohistochemical markers was counted as vessel.

Statistical analysis. Overall survival (OS) was defined as the interval from diagnosis to death or last contact. Clinical, laboratory and histological variables obtained at the time of the study are presented

Table II. *Relationship between the immunohistochemical markers expression used in the present study (Spearman's rho test).*

	CD138 (epithelial)	CD138 (stroma)	E-cadherin	β-catenin	CD31	CD105
CD138 (epithelial)	-	p=0.0025	p<0.0001	p<0.0001	p=0.037	p<0.0001
CD138 (stroma)	p=0.0025	-	NS	p<0.0001	NS	p=0.0028
E-cadherin	p<0.0001	NS	-	p<0.0001	NS	NS
β-catenin	p<0.0001	NS	p<0.0001	-	NS	NS
CD31	p=0.037	NS	NS	NS	-	p=0.0075
CD105	p<0.0001	p<0.0001	p=0.002	p=0.002	p=0.0075	-

as median (range) and proportions. The MVD estimation and the expression level of each protein were separately studied for possible correlation with these variables. Comparisons were performed with the Kruskal–Wallis (one-way ANOVA), Mann-Whitney *U*-test, receiver operating characteristic (ROC) curves, Spearman's correlation coefficient test and Chi-square tests. Survival was calculated by the Kaplan-Meier method, while comparison of survival curves was performed by the log-rank test. The Cox's regression model was used for the multivariate analysis of prognostic factors. The results were considered as statistically significant when $p<0.05$.

The program SPSS (Statistical Package for Social Sciences) version 21.0 (Chicago, IL, USA) was used for statistical analysis.

Results

Clinicopathological features. The study population comprised 69 patients with CRC. The mean age of patients was 64.58±7.20 years (range=40-81). There were 42 male (60.87%) and 27 female (39.13%). Of the tumors, 60 (86.95%) were left-sided, 5 (7.25%) cases were right-sided, 4 (5.80%) tumors were found in the transverse colon. Tumor size varied from 2.5 to 10.0 cm (5.25±1.58). Twenty-four cases (34.78%) were Duke's stage B2, 33 (47.83%) were C2 and 12 cases (17.39%) were Duke's stage D. Three (4.35%) patients had well-differentiated, 59 (85.51%) moderate and 7 (10.14%) poorly differentiated carcinomas; 8 (11.59%) had mucinous-type adenocarcinoma and 61 (88.41%) had non-mucinous adenocarcinoma. Serosal, venous and lymphatic invasion was observed in 66 (95.65%), 50 (72.46%) and 65 (92.75%) patients, respectively. Lymph node involvement was observed in 45 (65.22%) and liver metastasis in 10 (10.49%) cases. Recurrence of the disease was noted in 21 (33.33%) and death from CRC in 12 (19.05%) patients. The clinicopathological features are summarized in Table I.

Immunohistochemical findings

Syndecan-1 expression. In normal colon mucosa, syndecan-1 was expressed strongly around the basolateral membrane of normal columnar epithelium and plasma cells. In contrast, in a large percentage of the adenocarcinomas, syndecan-1 staining was decreased or absent (p=0.001). Loss of syndecan-1 from tumor epithelial cells was most pronounced in poorly differentiated tumors. Out of 69 patients, 43 (62.32%) cases expressed moderate to strong immunoreactions and 26 (37.68%) cases were weak or negative. On tumor cells, syndecan-1 was membranous and in many cells cytoplasmic, while, in normal stromal cells, this marker was typically negative (Figure 1). Specifically, 39 (56.52%) cases had moderate and/or strong expression and 30 (43.48%) cases showed weak or negative expression. A statistically positive correlation was found between syndecan-1 epithelial expression and syndecan-1 stromal expression (Pearson's test, p=0.004). A statistically correlation was observed between syndecan-1 epithelial tumor expression and Dukes' stage (p=0.002), lymphatic invasion (p<0.0001), lymph node metastasis (p=0.002), liver metastasis (p=0.031), E-cadherin expression (p<0.0001 by Spearman's test) and β-catenin expression (p<0.0001 by Spearman's test) (Table II). Syndecan-1 tumor stromal expression was correlated statistically with liver metastasis (p=0.008).

E-cadherin/β-catenin expression. EMT was evaluated by determining E-cadherin and β-catenin expression. Normal expression of these molecules was observed in the intercellular junction areas of normal colonic mucosa. In epithelial neoplastic cells, the intensity of E-cadherin decreased and a change in subcellular distribution (*e.g.* aberrant cytoplasmic expression) was indentified. E-cadherin immunoreactivity was strong in 32/69 (53.6%) cases, moderate in 21/69 (30.45%) and weak in 16/69 (23.19%) cases. Quantitative analysis of staining showed a significant decrease from well-differentiated to poorly differentiated adenocarcinomas (p=0.001). β-catenin also followed the same immunohistochemical pattern as described above but, additionally, cell nucleus expression was found in tumor epithelial cells. Specifically, 24 out the 69 (34.78%) were strongly positive, 16/69 (23.19%) were moderately positive, 27/69 (39.13%) reacted weakly and 2 cases (2.90%) were negative. In 27/69 (39.13%) of tumor cases we observed β-catenin nuclear expression, whereas no nucleus was stained in normal colonic mucosa. Quantitative analysis of staining showed a lower expression of β-catenin in CRC samples compared with normal colonic mucosa (p=0.001).

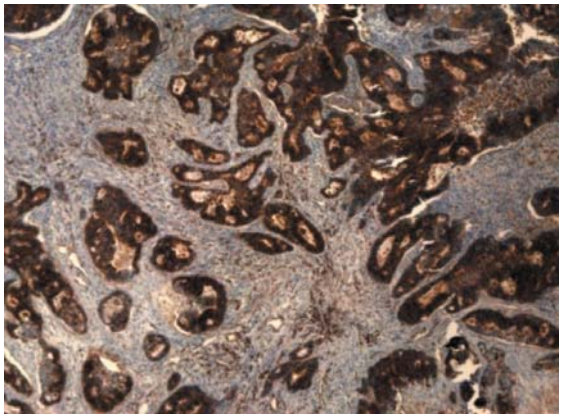

Figure 1. *Immunohistochemical expression in both epithelial and stromal components of colorectal adenocarcinoma (x100).*

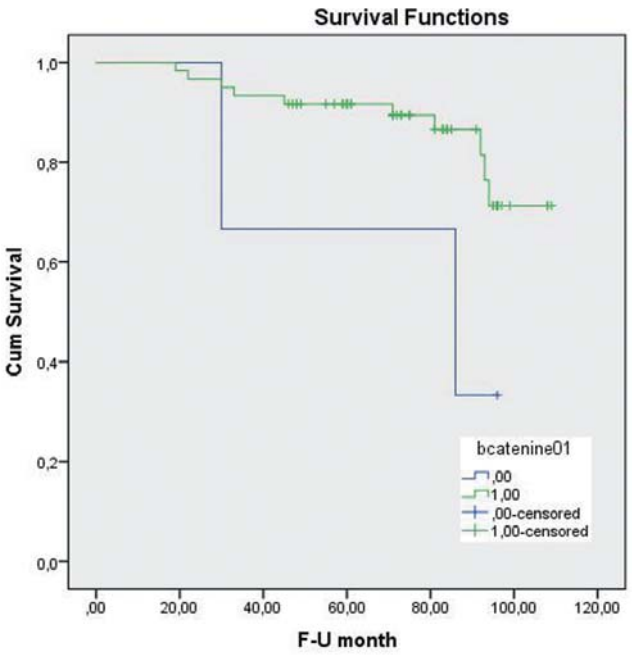

Figure 2. *β-catenin expression (negative/positive) as determinant of overall survival in univariate (Kaplan-Meier's test) analysis in patients with colorectal adenocarcinoma. F-U, Follow-up.*

E-cadherin and β-catenin immunohistochemical scores displayed a positive correlation in CRC (correlation coefficient 0.5, 95% confidence interval (CI)=0.25-0.55, Pearson's test, $p<0.0001$). Moreover, patients with membranous expression of β-catenin had a better OS (Figure 2). Statistically, we found a correlation between β-catenin membranous expression and Duke's stage (Mann Whitney U test, $p=0.019$).

PCAM-1 expression. PCAM-1 (CD31) immunoreactivity was universally detected in small vessels and capillaries. At the tumor site, MVD ranged from 6 to 55 (median=24±8.5). Statistically, an association was observed between CD31 expression and grade of differentiation, peritoneal infiltration and syndecan-1 epithelial tumor expression ($p=0.0016$, $p=0.002$ and $p=0.037$, respectively).

Endoglin (CD105) expression. CD105 was intensely expressed in vascular endothelial cells of tumor ranging from 12 to 79 (median=33±15.7). Microvessel density assessed by endoglin demonstrated significantly more vessels than CD31 ($p=0.0075$ by Spearman's test). CD105 was lower in mucinous adenocarcinomas compared with non-mucinous ($p=0.01$). CD105 immunoreactivity was also expressed in the cytoplasm of neoplastic epithelial cells. Specifically, 11 out the 69 (15.94%) cases were moderately positive, 19 (27.54%) were weakly positive and the rest were negative. A statistical significant relationship, using the Mann Whitney *U*-test, was observed between MVD-CD105 and Dukes' stage ($p<0.0001$), venous invasion ($p<0.0001$), peritoneal infiltration ($p<0.0001$), liver metastasis ($p<0.0001$), lymph node metastasis ($p<0.039$), relapse of the disease after surgical resection and adjuvant chemotherapy ($p<0.010$). Univariate and multivariate analyses showed a positive statistical

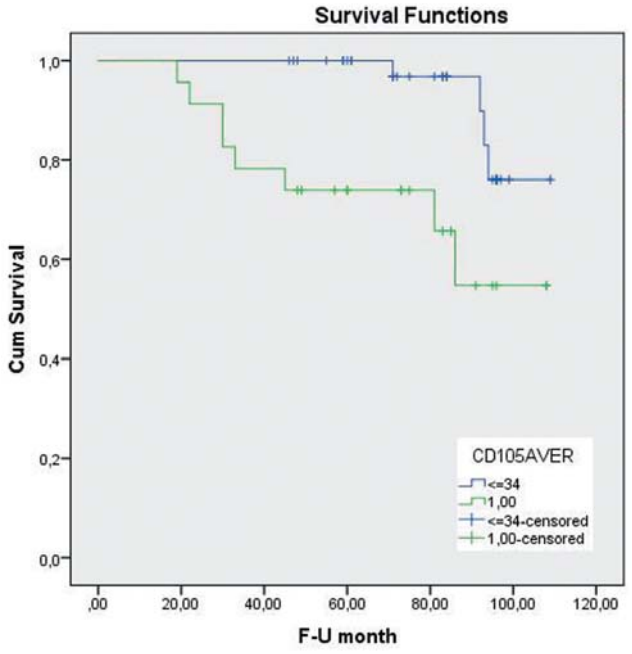

Figure 3. *CD105-MVD-count as determinant of overall survival in univariate (Kaplan-Meier's test) analysis in colorectal adenocarcinoma. F-U, Follow-up.*

relationship between CD105-MVD count and OS ($p<0.0001$) (Figure 3), indicative of CD105 as an independent predictor of survival. A statistical relationship was also noted between syndecan-1 epithelial tumor expression and CD105-MVD count. In addition, a positive correlation was found between CD105 tumor epithelium expression and E-cadherin ($p<0.0001$) and β-catenin ($p=0.068$) expression.

Discussion

This study was undertaken to elucidate the biology of CRC and propose potentially useful prognostic factors than those used by the traditional staging system in therapeutic decision-making (8, 50, 53, 54). Syndecan-1 is a well-known marker-protein involved in cellular and cell-matrix adhesion, cell proliferation, migration and angiogenesis (4). Many studies have previously demonstrated the reduction/loss of syndecan-1 expression during epithelial tumorigenesis (4, 5). The present study demonstrates that loss of syndecan-1 expression from colonic neoplastic epithelial cells associates with lymphatic invasion, lymph nodes' metastasis, liver metastasis and Dukes' stage, but not with patient survival, as already shown in our previous study where patients with weak staining reaction had a more unfavorable prognosis (8). This discrepancy might be explained by differences in methodologies or antibodies used, as well as the number of cases examined. Absence of syndecan-1 epithelial expression is a hallmark of EMT, where neoplastic cells change from an epithelial to a less differentiated mesenchymal phenotype denoting an association with a biologically more aggressive phenotype and a poor clinical outcome (6, 8, 9). In 56.52% (39/69) of cases, stromal immunoreactivity for syndecan-1 was observed. This percentage is in accordance with our previous study and the report of Lundin *et al*. (55). While, in the present study, stromal syndecan-1 immunoreactivity was significantly associated with liver metastasis ($p=0.008$, in our previous study), no statistical significant association was found between stromal syndecan-1 immunoreactivity and various clinicopathological parameters (8). It has recently been shown that the expression of syndecan-1 protein can be increased during epithelial-mesenchymal interaction and is associated with tumor progression and/or metastasis in several malignancies. Furthermore, stromal syndecan-1 can affect the tumor microenvironment by altering extracellular matrix (ECM)-cytoskeleton linkage in the neighborhood of the tumor (4). In addition, we found that stromal syndecan-1 immunoreactivity is remarkably associated with membranous syndecan-1 expression ($p=0.004$), correlated with advanced primary tumors and EMT immunohistochemical positivity. At present, it is difficult to interpret how stromal and epithelial syndecan-1 expressions are related. Further studies are required to validate the statistical and prognostic significance of stromal syndecan-1 immunoreactivity.

Over the last two decades, many studies focused on clarification of the precise role of expression of E-cadherin and β-catenin in colorectal carcinogenesis (3-5). Recent studies have shown that there are three different pathogenetic pathways for the development of CRC: (i) chromosomal instability (CIN) (most cases); (ii) microsatellite instability (MSI); (iii) CpG island methylator phenotype (CIMP) (57, 58). Previous immunohistochemical studies have shown abnormal expression of E-cadherin in the majority of the CRCs (59, 60). Several studies indicate an overall reduction in the expression of E-cadherin compared to adjacent normal mucosa (61), which is in accordance with our results. Therefore, there are no uniform results regarding the predictive value of these proteins. Several studies have indicated that loss of E-cadherin expression is associated with tumor size, histopathology, growth patterns and poor prognosis (59, 60). On the other hand, other authors have not shown any association and/or expression between E-cadherin and conventional staging, tumor differentiation, invasive metastatic potential or prognosis (61, 62), also in agreement with our results. The discrepancies might be explained by differences in methodology or antibodies used, as well as the number of cases investigated.

Beta-catenin is currently believed to be involved in the development of CRC. Abnormal expression of the *APC* gene and mutations at the site of phosphorylation of β-catenin are associated with the overexpression and the subsequent cytoplasmic/nuclear translocation of β-catenin found in CRC (63, 64). In the present study, strong membranous expression of β-catenin indicates improved patients' outcome. The correlation stemming from our study, although at borderline level of statistical significance, clearly indicates a susceptibility of the system investigates. Previous immunohistochemical studies of β-catenin in CRC have shown contradictory results regarding nuclear staining and clinical outcome. In some cases, nuclear staining of β-catenin was predictive of poorer survival (65), whereas in others did not provide any evidence (66, 67), as we also demonstrate in this work.

In this and other studies, there is a positive correlation between syndecan-1 epithelial expression, E-cadherin and β-catenin (68). Our results showed that in advanced Dukes' stages of CRC, the expression level of syndecan-1, E-cadherin and β-catenin significantly decreased. Moreover, β-catenin is translocated to the nucleus suggesting a role in regulation of genes that might be responsible for invasive behavior, metastatic disease and angiogenic potential of the CRC transformed cell (69).

It is commonly accepted that angiogenesis is crucial for tumor growth, invasion and metastasis. The transmembrane glycoprotein CD31 (PCAM-1) is a good marker for endothelial cells and it stains both large and small vessels with equal intensity, as well as blood vessels in normal and

tumor tissue (70, 71). The current study shows that neovascularization is invariably increased in colorectal adenocarcinomas, compared with their corresponding normal mucosa specimens. Abdalla *et al*. have demonstrated that there is a significant association between CD31 and survival; thus, patients with an increased number of microvessels survived longer than those with a low number of microvessels (71). We did not observe any correlation between MVD and tumor stage or patients' survival, which is in agreement with other reports in which MVD was also quantified using the CD31 marker (72, 73). However, we found a significant association between grades of differentiation (p=0.0016), also in accordance with previous findings in breast cancer (51).

Endoglin (CD105), a cell membrane glycoprotein, has been demonstrated to be up-regulated in endothelial cells in *de novo* blood vessels of various tumors compared with those in normal tissues (52). In the present study, CD105 microvessel staining was consistently positive in all cases and its expression was more intense in the tumor microvessels when compared with CD31 immunostaining. CD105 stained small vessels with high sensitivity in or around the tumor, while blood vessels in non-neoplastic tissue did not stain or only weakly stained with CD105. This is in agreement with previous studies where CD105 was expressed mainly in proliferating blood vessels, while CD31 stained all the blood vessels indiscriminately (21, 22). In the present report, using univariate analysis, microvessel count by CD105 showed a statistically significant correlation with the presence of vessel invasion (p<0.0001), peritoneal invasion (p<0.0001), lymph node metastases (p=0.039) and liver metastases (p<0.0001), independently of tumor stage, suggesting that CD105 may be involved in the process of metastasis. CD105 did not associate with patients' age, sex, tumor size, tumor localization and histologic grade. Multivariate analysis confirmed that CD105 was a significant independent prognostic factor for survival (p<0.0001), in agreement with previous reports (71, 72), thus reinforcing the premise that endoglin might be considered for further therapeutic trials as an anti-angiogenic agent. It has been demonstrated that accumulation of mRNA for endoglin (*CD105*) is up-regulated in colon carcinoma tissues compared to normal and dysplastic colon mucosa (53). Similar to our observations, endoglin's overexpression is positively associated with disease progression, confirming that CD105-MVD is a prognostic marker in colon carcinoma (53, 71, 72). We, additionally, noted that CD105-MVD was correlated with relapse of the disease after surgical resection and adjuvant chemotherapy (p=0.010).

Another interesting finding in the present report is the presence of CD105 in the cytoplasm of cancer epithelial cells, which is in accordance with already published data (74), and its association with E-cadherin and β-catenin expression (p<0.0001 and p<0.068, respectively). Recently, CD105 has been reported to play a role in the regulation of adhesion, motility and invasion of normal and transformed cancer cells. It has been suggested that loss of endoglin in cancer cells causes cell detachment and may be associated with cancer progression (75). Finally, CD105 is a specific marker for activated endothelium and mainly reacts with fresh or frozen tissue, while its over-expression in malignant vessels can be used for anticancer therapy in order to improve rectal cancer diagnosis and to monitor the actual therapy (76). The present study demonstrated, for the first time in the literature, the existence of a correlation between the expression of syndecan-1, EMT markers and endoglin MVD-count in human CRC.

Conclusion

The present study showed that: a) expression of syndecan-1 epithelial tumor cells was correlated with the EMT markers E-cadherin and β-catenin; b) syndecan-1 expression was also associated with Dukes' stage, lymphatic invasion, lymph node and liver metastases; c) β-catenin expression appeared to reduce the risk of poor outcome in patients with CRC; d) endoglin (CD105)-MVD count was associated with Dukes' stage, venous invasion, lymph node, peritoneal invasion and liver metastases; e) CD105 epithelial tumor cell expression was associated with syndecan-1, E-cadherin and β-catenin; f) increased CD105-MVD was a strong predictor of disease recurrence and poor patients' survival in multivariate analysis. Further investigations are required to delineate the interactions between these molecular markers in CRC and determine how deregulation of these proteins may alter responses to therapeutic intervention.

Competing Interests

The Authors declare that they have no competing interests.

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