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ORIGINAL PAPER

An Immunohistochemical Study of Colon Adenomas and Carcinomas: E-cadherin, Syndecan-1, Ets-1

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Abstract It is thought that dysregulation of E-cadherin, syndecan-1 (CD138) and Ets-1 is involved in carcinoma development. E-cadherin is an important epithelial cell adhesion molecule; syndecan-1 (CD138) is a regulatory proteoglycan in both cell-cell and cell-matrix adhesion and Ets-1 is a proto-oncogene and transcription factor, which takes part in extracellular matrix remodeling. Our goal was to study the changes in the expression of these molecules during colon carcinoma development and progression. We tested 117 colon adenomas and 149 *de novo* and ex adenoma carcinomas of the colon, using the Ultravision Polymer system. The positive reaction rate was 100% for E-cadherin, 98.3% for syndecan-1 and 22.4% for Ets-1 in adenomas, while in carcinomas it was 88.5%, 62.4% and 56.3% respectively. We found decreasing expression of E-cadherin and syndecan-1 throughout colon carcinoma progression and an opposite regulation for the Ets-1 protein. Decrease in expression of syndecan-1 is more pronounced in carcinomas compared to E-cadherin. *De novo* carcinomas have lower E-cadherin and syndecan-1 expression, and higher Ets-1 expression compared to ex adenoma carcinomas. These findings support the hypothesis that there are differences in the carcinogenesis of these tumors.

Keywords Colon adenoma · Colon carcinoma · E-cadherin · Ets-1 · Immunohistochemistry · Syndecan-1

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Introduction

Colorectal cancer (CCR) is the most frequent gastrointestinal tumor in developed countries, while it only ranks fourth (following the cancers of the stomach, liver and esophagus) in developing countries. A constant increase in CCR mortality rates was observed in Romania. Between 1955–59 and 1990–92, CCR mortality rates/100,000 individuals increased by 34% in males and by 38.3% in females. Between 1995 and 2003 a further increase of 22.4% was registered [1].

CCR develops *de novo* from the colon epithelium or arises from different precancerous lesions such as adenomas, aberrant crypt foci (APC), chronic inflammatory bowel disease [2]. Though many attempts have been made at finding surrogate molecular markers for predicting the biological behavior of colorectal cancer, there is still a need to find markers or marker combinations associated with colorectal cancer development and progression.

E-cadherin is a member of the calcium dependent adhesion molecule (CAM) family, mediating homophylic cell-cell adhesion in epithelial tissue and is localized to adjacent cell membranes. Downregulation or complete shutdown of E-cadherin expression are observed in a number of human cancers including breast, esophageal, stomach, endometrium, ovary, thyroid, pulmonary, squamosus head and neck tumors, pancreatic and colorectal

cancers. The decreased expression of E-cadherin has been correlated with a high grade and an advanced stage of these disorders, with poor prognosis [3].

Syndecans are a family of heparan sulphate proteoglycan (HSPG) that are thought to participate in both cell-cell and cell-matrix adhesion. In addition, they may act as receptors for growth factors and thereby may be involved in the control of cell proliferation [4]. Syndecan-1 (CD138) is downregulated in a number of epithelial cancers and pre-malignant lesions, including squamosus carcinoma of the head, neck and lung, laryngeal cancer, hepatocellular and colorectal carcinoma; it is upregulated in multiple myeloma, pancreatic and breast carcinoma. Loss of syndecan-1 expression correlates with a reduced survival in these lesions [5].

The proto-oncogene Ets-1 is a transcriptional factor known to control the expression of a number of genes involved in extracellular matrix remodeling by upregulating matrix metalloproteases (MMPs) and has been postulated to play a role in cell migration and tumor invasion [6–9]. Elevated Ets-1 expression was observed in invasive and metastatic solid tumors including breast, lung, colon, pancreatic, oral and thyroid cancer [7].

According to previous CCR studies, decreased E-cadherin expression [4, 10, 11], decrease or loss of syndecan-1 expression [4, 12, 13], or elevated Ets-1 expression [6, 7, 10] predicts a more invasive behavior and poor prognosis. In this work we studied adenomas of different grade and *de novo* or ex adenoma colon carcinomas. Keeping in mind that such a study has not been performed yet, we set out to perform the comparative immunohistochemical assessment of E-cadherin, syndecan-1 and Ets-1 in colon adenomas and carcinomas, assuming that the combination of these markers will provide new information regarding the multistep carcinogenesis and the biological behavior of these lesions.

Materials and Methods

We included in this retrospective study 117 colon adenomas and 149 colon carcinomas from the Pathology Department of the Clinical County Hospital of Targu-Mures, Romania. The 3 µm thick sections obtained from the formalin fixed and paraffin embedded resection tissue specimens were routinely dewaxed and rehydrated. Antigen retrieval was performed by pressurized steam cooking (citrate solution, pH=6) followed by endogenous peroxidase blocking. We used the following mouse monoclonal antibodies for: CD138 (LabVision, Fremont, CA, U.S.A, clone MI15) in 1:50, E-cadherin (LabVision, Fremont, CA, U.S.A, clone SPM471) in 1:200, Ets-1 (LabVision, Fremont, CA, U.S.A, clone 1G11) in 1:20. Ultravision Labeled Polymer system (LabVision, Fremont, CA, U.S.A) with DAB development was used for detecting primary antibodies.

We determined the frequency (F) of immunopositive tumor cells using the following grading for E-cadherin and syndecan-1: F0: 0–5%, F1: 6–30%, F2: 31–75%, F3: 76–100%, and for Ets-1: F0: 0–9%, F1: 10–29%, F2: 30–50%, F3: >50%; and the signal intensity (I) for E-cadherin and syndecan-1: absent (0), weak (1), moderate (2), strong (3). In case of E-cadherin and syndecan-1 we summed the frequency and intensity values and obtained an immunohistochemical score between 0–6. Based on these results, we determined the following immunohistochemical grading (IG) system: IG0 (absent immunoexpression): F+I= 0–1; IG1 (weak immunoexpression): F+I=2–3; IG2 (moderate immunoexpression): F+I= 4; IG3 (strong immunoexpression): F+I= 5–6. The positive control for E-cadherin and syndecan-1 was normal colon tissue with strong immunoexpression (IG3). In case of Ets-1, we used tonsilla as positive control (according to the manufacturer's recommendations) and the similar method without the primary antibody as negative control. Results were analyzed using the Graph Pad In Stat 3, version 3.06 statistic calculation software (GraphPad Software Inc., San Digeo, U.S.A.) Data were compared using Pearson's chi-square test and Fischer's exact test, and the difference between the means of continuous data was compared using the t-test. We considered the association significant when $p<0.05$, with 95% confidence interval.

Results

Clinicopathological Correlations

The average age of colon carcinoma patients was 64 years (min. value 31, max. value 86), and the population studied consisted of 87 (58%) males and 62 (42%) females. The tumors were located mainly in the left colon (65.7%) and especially in the sigmoid colon (37.5%). Tumor size was 5–135 mm, with an average of 41.4 mm. We studied 103 (69%) non-mucinous adenocarcinomas, 43 (29%) mucinous adenocarcinomas and 3 (2%) undifferentiated carcinomas. Most of the carcinomas were TNM stage II (47.6%) and Dukes stage B (58%) and C (34%). *De novo* developed carcinomas ($n=100$) possessed the same characteristics as ex adenoma carcinomas ($n=49$) regarding the age of the patients, size, localization and stage of the tumors.

The average age of colon adenoma patients was 63 years (min. value 29, max. value 83), and the population studied consisted of 71 (60.6%) males and 46 (39.4%) females. Tumors were located mainly in the left colon (55.5%) and especially in the sigmoid colon (34.1%). We studied 48 (41%) tubular adenomas, 48 (41%) tubulovillous adenomas, 6 (5.1%) serrated adenomas, 6 (5.1%) villous

adenomas, and 3 (2.6%) hyperplastic polyps. Most of the adenomas were non-dysplastic (50%), and the rest had the following distribution: 16% low-grade dysplasia, 23% moderate dysplasia and 11% high-grade dysplasia. There was a strong statistical correlation between the grade of the dysplasia and the histological type of the adenoma ($p<0.0001$) (Fig. 1).

Immunohistochemical Results

Syndecan-1 and E-cadherin were expressed on the basolateral surface of the normal and tumor epithelial cells, and we observed cytoplasmic expression in poorly differentiated cancer cells. Ets-1 exhibited a nuclear immunostaining in tumor cells and was absent in normal tissue.

All adenoma cases showed positive immunostaining for E-cadherin, 98.3% for syndecan-1 and 20.5% for Ets-1. The majority of the adenomas demonstrated IG3 immunoexpression for E-cadherin (89.7%) and syndecan-1 (87.2%) (Fig. 2). Most of the positive Ets-1 immunostaining results were rated IG1 (15.4%) (Table 1).

In comparison to adenomas, carcinomas displayed weaker E-cadherin and syndecan-1 expression and stronger Ets-1 expression (Fig. 2). We obtained positive immunostaining for E-cadherin in 88.6%, for syndecan-1 in 61.1% and for Ets-1 in 56.4% of the carcinomas. Most of the cases showed IG3 immunoexpression for E-cadherin (37.6%) and IG0 for syndecan-1 and Ets-1 (37.6% and 43.6%). However, compared to adenomas carcinomas displayed more Ets-1 positive cases with IG1 (37%) and IG2 (15.4%) immunostaining (Table 1).

Comparing the E-cadherin and syndecan-1 immunohistochemical results of adenomas to those of carcinomas, we noticed that immunohistochemical scores were significantly lower in carcinomas compared to adenomas ($p<0.0001$). Ets-1 presented a reversed correlation, meaning that positive signal was significantly more extended in carcinomas than in adenomas ($p<0.0001$) (Table 2).

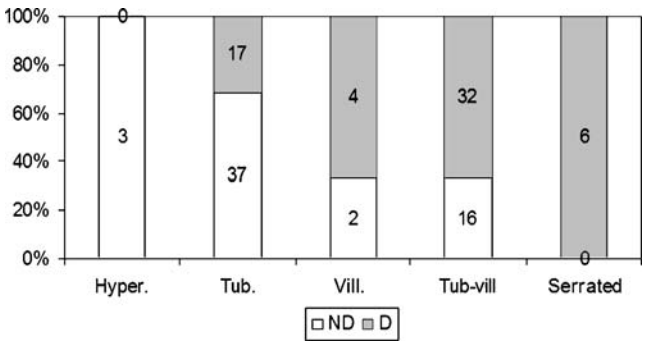

Fig 1 Correlation of grade of dysplasia with histological type of adenomas. Dysplasia was absent in hyperplastic polyps and present in all cases of serrated adenoma. The rate of dysplastic cases (*all grades*) shows a gradually increasing trend from tubular adenomas, through villous adenomas, tubulovillous adenomas to serrated adenomas ($p<0.0001$). ND- non- dysplastic adenomas, D- dysplastic adenomas (low, moderate, high-grade), Hyper- hyperplastic polyps, Tub- tubular adenoma, Vill- villous adenoma, Tub-vill- tubulovillous adenoma, Serrated- serrated adenoma

Comparing E-cadherin and syndecan-1 immunohistochemical results of *de novo* and ex adenoma carcinomas, we observed that immunohistochemical scores were significantly lower in *de novo* carcinomas ($p<0.0001$) (Table 3). In case of Ets-1 we could not demonstrate a significant difference, but there was an increase in its expression in *de novo* carcinomas compared to ex adenoma carcinomas ($p=0.2$) (Table 3). There were no statistically significant differences between the immunostaining of these markers in *de novo* adenomas and those developed synchronously with a colon carcinoma (data not shown).

E-cadherin and syndecan-1 immunohistochemical scores displayed a positive correlation in case of adenomas (correlation coefficient 0.4, 95% CI: 0.27–0.57, $p<0.0001$), as well as carcinomas (correlation coefficient 0.5, 95% CI: 0.43–0.65, $p<0.0001$). This correlation was more pronounced in case of carcinomas. These epithelial markers had an approximately similar expression in adenomas, whereas in carcinomas they demonstrated a decrease in immunoexpression that was more pronounced in case of syndecan-1.

Correlation of Imunohistochemical Data with Classic Prognostic Factors

In case of adenomas, we correlated immunohistochemical scores of the analyzed markers with patient age, gender, location, histological type of the tumor and grade of dysplasia (Table 4). The histological type of adenomas showed a significant correlation with the E-cadherin immunostaining ($p=0.04$). All cases of hyperplastic polyps demonstrated strong E-cadherin expression (IG3), and the rate of cases with moderate immunoexpression (IG2) gradually increased from serrated through tubular, tubulovillous to villous adenomas. We also found a correlation between the grade of dysplasia and E-cadherin expression, moderate (IG2) immunostaining was more frequent in adenomas with moderate and high-grade dysplasia ($p=0.013$). In case of syndecan-1 and Ets-1, we could not demonstrate any correlation with clinicopathological factors.

In case of carcinomas, we correlated immunohistochemical scores of the analyzed markers with patient age, gender and also with location, size, histological type and grade, Duke's and TNM stage of the tumor (Table 5).

In carcinomas, E-cadherin immunoexpression showed a significant correlation with the histological type ($p=0.02$) and grade ($p<0.0001$) of the tumor, pT ($p=0.007$) and pM ($p=0.001$). There was an almost significant correlation with the TNM stage of the tumor ($p=0.06$). Immunostaining was

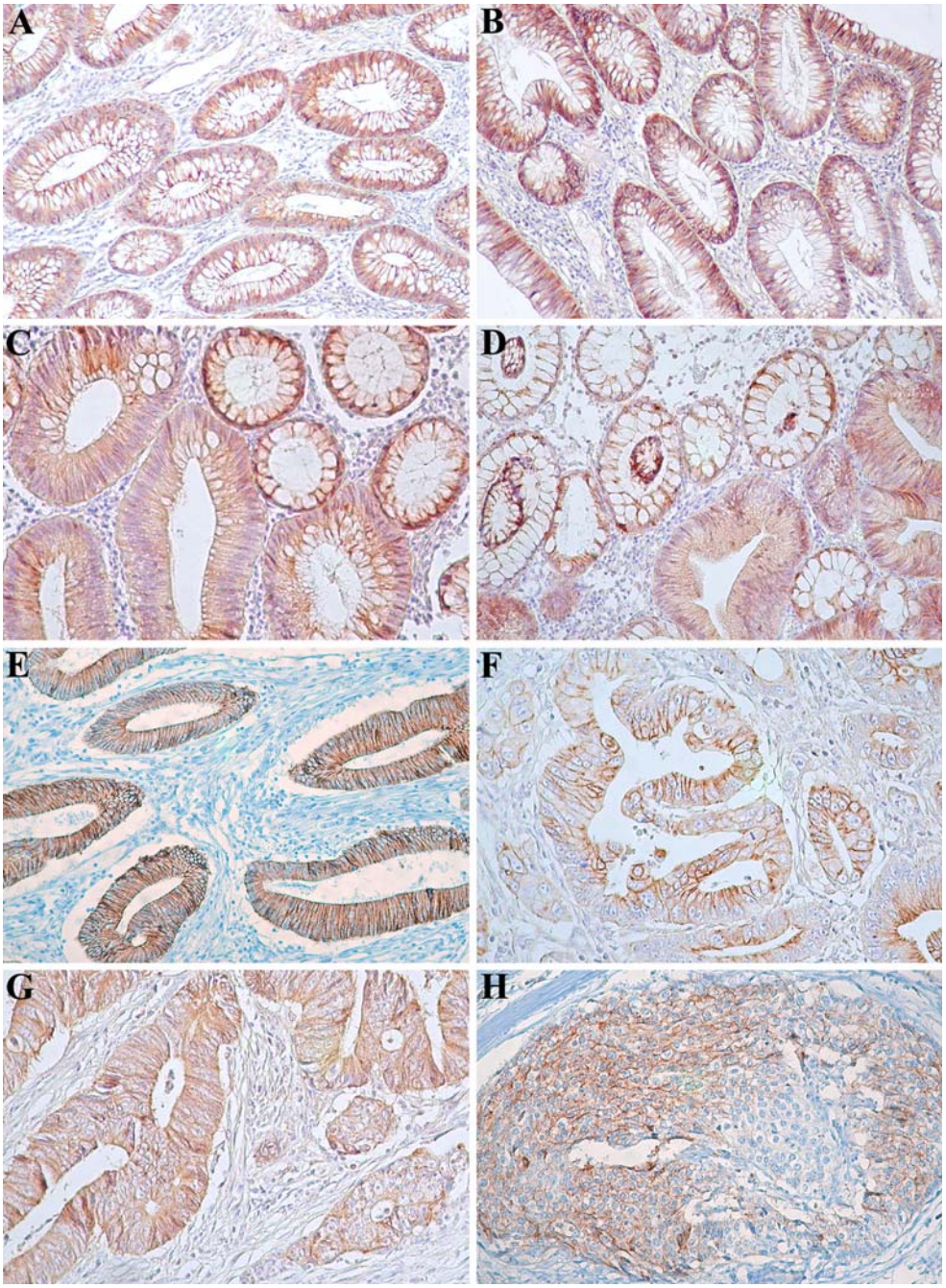

Fig. 2 Strong (IG3) E-cadherin (**a**) and syndecan-1 (**b**) imunoexpression in tubulovillous adenoma on low-power field. IG3 E-cadherin (**c**) and syndecan-1 (**d**) imunoexpression in tubular and tubulovillous adenoma with moderate dysplasia on high-power field. IG3 E-cadherin immunoexpression in a well differentiated adenocarcinoma (**e**) and moderate (IG2) E-cadherin immunoexpression in a moderately differentiated adenocarcinoma (**f**). IG2 syndecan-1 immunoexpression in a moderately differentiated adenocarcinoma (**g**) and weak (IG1) syndecan-1 immunoexpression in a poorly differentiated adenocarcinoma (**h**) on high-power field

weaker or absent in mucinous adenocarcinomas, poorly differentiated adenocarcinomas and undifferentiated carcinomas, and also in advanced TNM stage tumors.

Syndecan-1 presented a significant correlation with pT stage (p=0.02), histological grade (p=0.005), localization (p=0.04) and size of the tumor (p=0.04). We noticed that pT3-4 stage carcinomas, poorly differentiated adenocarcinomas or undifferentiated carcinomas that were located in the right colon and were larger than 40 mm displayed a weaker or absent syndecan-1 expression. Syndecan-1 expression did not correlate with the TNM stage of the tumor (p=0.15).

We pointed out that Ets-1 correlated with patient age (p=0.03), meaning that Ets-1 expression was more frequent in the elderly (>50 years). Imunostaining of Ets-1 also correlated with Duke`s stage of the tumor ($p$=0.02) and distant metastases ($p$=0.005). Ets-1 expression was more frequent in carcinomas with advanced Duke`s stage (C-D) and in tumors with distant metastases.

A comparison has been made between the expression of these markers in 12 lymph node metastases and 2 hepatic metastases and their primary carcinomas. We found an almost significant difference in E-cadherin expression of metastases and corresponding primary tumors (p=0.08), i.e.

Table 1 Immunohistochemical results

	Immuno-histochemical grade	E-cadherin		Syndecan-1		Ets-1	
		n	%	n	%	n	%
Adenomas	IG 0	0		2	1.7%	93	79.5%
	IG 1	0		0		18	15.4%
	IG 2	12	10.3%	13	11.1%	5	4.3%
	IG 3	105	89.7%	102	87.2%	1	0.8%
Carcinomas	IG 0	17	11.4%	58	38.9%	65	43.6%
	IG 1	29	19.5%	43	28.9%	55	37%
	IG 2	47	31.5%	35	23.5%	23	15.4%
	IG 3	56	37.6%	13	8.7%	6	4%

it was weaker in metastases than in primary tumors (data not shown).

We observed progressiveness in E-cadherin and syndecan-1 decrease, and Ets-1 increase starting from adenomas with low-grade, moderate and high-grade dysplasia, through well, moderately and poorly differentiated carcinomas ($p<0.0001$) (Fig. 3).

Discussion

Colon cancer has a high incidence among gastrointestinal tumors. Numerous studies are still discussing and searching for new prognostic markers or marker combinations involved in tumor development and progression [2, 14, 15].

Generally, carcinoma cells are characterized by poor intercellular adhesion, loss of differentiated epithelial morphology and increased cellular motility [16–18]. They also feature downregulation or complete shutdown of E-cadherin expression caused by mutation of the E-cadherin gene (CDH1) or other mechanisms that interfere with the integrity of the adherens junctions [3]. Numerous studies demonstrate E-cadherin implication in tumor progression and invasion by participating in multiple signaling pathways. Activation of the Wnt signaling pathway (β-catenin/TCF dependent signaling mechanism) in SW480 colorectal tumor cell line depends on the presence of the cadherin cytoplasmatic domain, regardless of the presence or absence of its extracellular domain [19].

Another well known marker that participates in cellular and cell-matrix adhesion is syndecan-1 [5]. The activation of multiple intracellular signaling pathways can cause shedding of the syndecan-1 ectodomains from the cell surface, facilitating cell motility [20, 21]. As our antibody detects the extracelullar domain of syndecan-1, the absence of the protein from the cell surface can be caused by increased shedding.

In this immunohistochemical study we compared the changes of E-cadherin, syndecan-1 and Ets-1 expression in 117 colon adenomas and 149 colon carcinomas. As previous studies also demonstrated [4, 22], in adenomas E-cadherin and syndecan-1 expression was weaker or similar compared to normal tissue, but stronger compared to cancer tissue. Evaluating the differences between these two epithelial markers, we concluded that they had an approximately similar expression in adenomas. We observed that the number of cases showing moderate immunoexpression gradually increased from serrated through tubular and tubulovillous adenomas and reached its maximum in villous adenomas. Hyperplastic polyps very rarely become malignant, while in 20% to 70% of the cases villous adenomas may undergo a malignant transformation [14]. In accordance with the results of a previous study [4], E-cadherin expression showed a similar trend starting from adenomas without and low-grade dysplasia to adenomas with moderate and high-grade dysplasia.

In case of carcinomas, syndecan-1 and E-cadherin expression was weaker or absent in poorly differentiated and undifferentiated carcinomas and carcinomas with deep

Table 2 Comparison of colon adenoma and carcinoma immunohistochemical results

		Mean IG	95% CI	SD	t-test
E-cadherin	adenoma	5.36	5.36±0.12	0.66	$p<0.0001$
	carcinoma	3.69	3.69±0.25	1.58	
Syndecan-1	adenoma	5.26	5.26±0.16	0.88	$p<0.0001$
	carcinoma	2.18	2.18±0.3	1.9	
Ets-1	adenoma	0.26	0.26±0.1	0.57	$p<0.0001$
	carcinoma	0.79	0.79±0.13	0.84	

SD standard deviation, *IG* immunohistochemical grade

Table 3 Comparison of ex adenoma and de novo carcinoma immunohistochemical results

	Colon carcinoma	Mean IG	95% CI	SD	t-test
E-cadherin	de novo	3.3	3.3±0.33	1.69	$p<0.0001$
	ex adenoma	4.4	4.4±0.32	1.13	
Syndecan-1	de novo	1.73	1.73±0.34	1.74	$p<0.0001$
	ex adenoma	3.02	3.02±0.55	1.92	
Ets-1	de novo	0.86	0.86±0.17	0.86	$p=0.2$
	ex adenoma	0.67	0.67±0.22	0.8	

SD standard deviation, *IG* immunohistochemical grade

invasion. In addition, E-cadherin decrease was associated with the presence of the mucinous component and distant metastases, while syndecan-1 decrease correlated with large size and localization on the right colon. There are several studies supporting the correlation between decreased expression of these two markers and the following clinicopathological characteristics: histological grade and type [4, 10–13, 23], lymph node and distant metastases [12, 22, 23], TNM stage [22, 23], Duke's stage [13] of colon cancer and age [11], gender of the patient [22]. These didn't report connections between syndecan-1 and size or localization of the tumor. Although, the prognostic value of tumor location and size is still highly controversial, there are studies suggesting a worse outcome of large size tumors of the right colon [14]. Our results complement this opinion, showing decreased E-cadherin expression and decreased or absent syndecan-1 expression in large size tumors of the right colon. In a few CCR studies E-cadherin and syndecan-1 expression was not associated with clinicopathological factors [18, 24].

A study performed in 1999 discusses the comparative immunohistochemistry of syndecan-1 and E-cadherin in 59 colorectal adenomas and 20 colorectal carcinomas arising from adenomas. Their results were similar to ours: in carcinomas there is a decrease of these two markers compared to adenomas, this decrease being more pronounced for syndecan-1. Based on these results, the authors presumed that alteration of syndecan-1 expression appears before the alteration of E-cadherin mediated adhesions [4]. In addition, both in adenomas and carcinomas we observed a positive correlation between the expression of these two markers.

De novo carcinomas appeared to be more aggressive than ex adenoma carcinomas, since they had a tendency to more deeply invade the colonic wall at a smaller size [16]. Comparing clinicopathologic characteristics of *de novo* carcinomas to ex adenoma carcinomas we did not notice

Table 4 Correlation of immunohistochemical results with clinicopathological factors of colon adenomas

Colon adenomas	E-cadherin			Syndecan-1			Ets-1		
	IG 0	IG 1-3	p value	IG 0	IG 1-3	p value	IG 0	IG 1-3	p value
Grade of dysplasia									
Non dysplastic	0	58	$p=0.01$	1	57	$p=0.41$	45	13	$p=0.54$
Low	0	19		0	19		17	2	
Moderate	0	27		1	26		22	5	
High	0	13		0	13		9	4	
Histological type									
Villous	0	6	$p=0.04$	0	6	$p=0.39$	6	0	$p=0.4$
Tubulovillous	0	48		1	47		33	15	
Tubular	0	48		1	47		40	8	
Serrated	0	6		0	6		5	1	
Hyperplastic	0	3		0	3		3	0	
Localization									
Right colon	0	18	$p=0.33$	1	17	$p=0.5$	13	5	$p=0.38$
Left colon	0	64		1	63		51	13	
Gender									
Male	0	71	$p=0.89$	0	71	$p=0.19$	56	15	$p=0.54$
Female	0	46		2	44		37	9	
Age (years)									
31–59	0	48	$p=0.5$	0	48	$p=0.61$	38	10	$p=0.85$
60–86	0	69		2	67		55	14	

IG immunohistochemical grade

Table 5 Correlation of immunohistochemical results with classic prognostic factors of colon carcinomas

Colon Carcinomas	E-cadherin			Syndecan-1			Ets-1		
	IG 0	IG 1-3	p value	IG 0	IG 1-3	p value	IG 0	IG 1-3	p value
TNM stage									
0–2	7	68	$p=0.06$	31	44	$p=0.15$	33	42	$p=0.08$
3–4	9	46		27	28		24	31	
Degree of invasion									
pT1-2	2	9	$p=0.007$	6	5	$p=0.02$	2	9	$p=0.42$
pT3-4	13	114		51	76		56	71	
Lymph node metastases									
pN0	8	65	$p=0.14$	32	41	$p=0.59$	31	42	$p=0.9$
pN1-2	7	46		25	28		23	30	
Distant metastases									
pM0	11	116	$p=0.001$	50	77	$p=0.33$	53	74	$p=0.005$
pM1	4	7		7	4		5	6	
Duke's stage									
A-B	7	73	$p=0.12$	29	51	$p=0.38$	35	45	$p=0.02$
C-D	8	50		28	30		23	35	
Histological grade									
Well diff.	0	29	$p<0.0001$	10	19	$p=0.005$	11	17	$p=0.2$
Moderate diff.	7	94		33	68		49	53	
Poorly diff.	8	6		11	3		4	10	
Undiff.	2	1		2	1		1	2	
Histological type									
NMA	15	115	$p=0.02$	50	80	$p=0.9$	57	73	$p=0.78$
MA	0	16		6	10		8	8	
Tumor size (mm)									
5–40	10	76	$p=0.56$	31	55	$p=0.04$	41	45	$p=0.67$
41–160	7	55		27	35		25	37	
Localization									
Right colon	8	42	$p=0.53$	25	25	$p=0.04$	18	33	$p=0.5$
Left colon	9	90		32	67		47	51	
Gender									
Male	11	76	$p=0.29$	36	51	$p=0.86$	32	54	$p=0.26$
Female	6	56		22	40		32	30	
Age (years)									
31–59	6	37	$p=0.94$	17	26	$p=0.85$	26	24	$p=0.03$
60–86	11	95		41	65		39	60	

Diff. differentiated, *NMA* non-mucinous adenocarcinoma, *MA* mucinous adenocarcinoma, *IG* immunohistochemical grade

any differences. Opposed to our results, a study performed on 106 pT1 CCRs, had shown that *de novo* CCRs were smaller in size and displayed poor differentiation more frequently compared to ex adenoma CCRs. Their results, obtained by analysis of E-cadherin expression in these tumor groups, were similar to ours: E-cadherin expression was lower in *de novo* carcinomas [16]. Syndecan-1 immunoexpression in these tumor groups, a subject not yet approached in previous studies, is also lower in *de novo* carcinomas. In addition, our results support the possibility of a difference between the carcinogenetic process of *de novo* and ex adenoma carcinomas.

Numerous studies discuss colorectal carcinogenesis, describing two major groups: the first group follows the adenoma-carcinoma sequence, initially described by Vogelstein, and is based on disorders resulting from chromosomal instability; the second group is characterized mostly by epigenetic transformations with microsatellite instability [25]. Some studies claim that in CCRs classified as MSS/MSI-L E-cadherin expression is lower than in those classified as MSI-H tumors [26].

The reexpression of E-cadherin in lymph nodes and distant metastases may play an important role in the growth of the cancer cells in the metastatic foci, offering them a

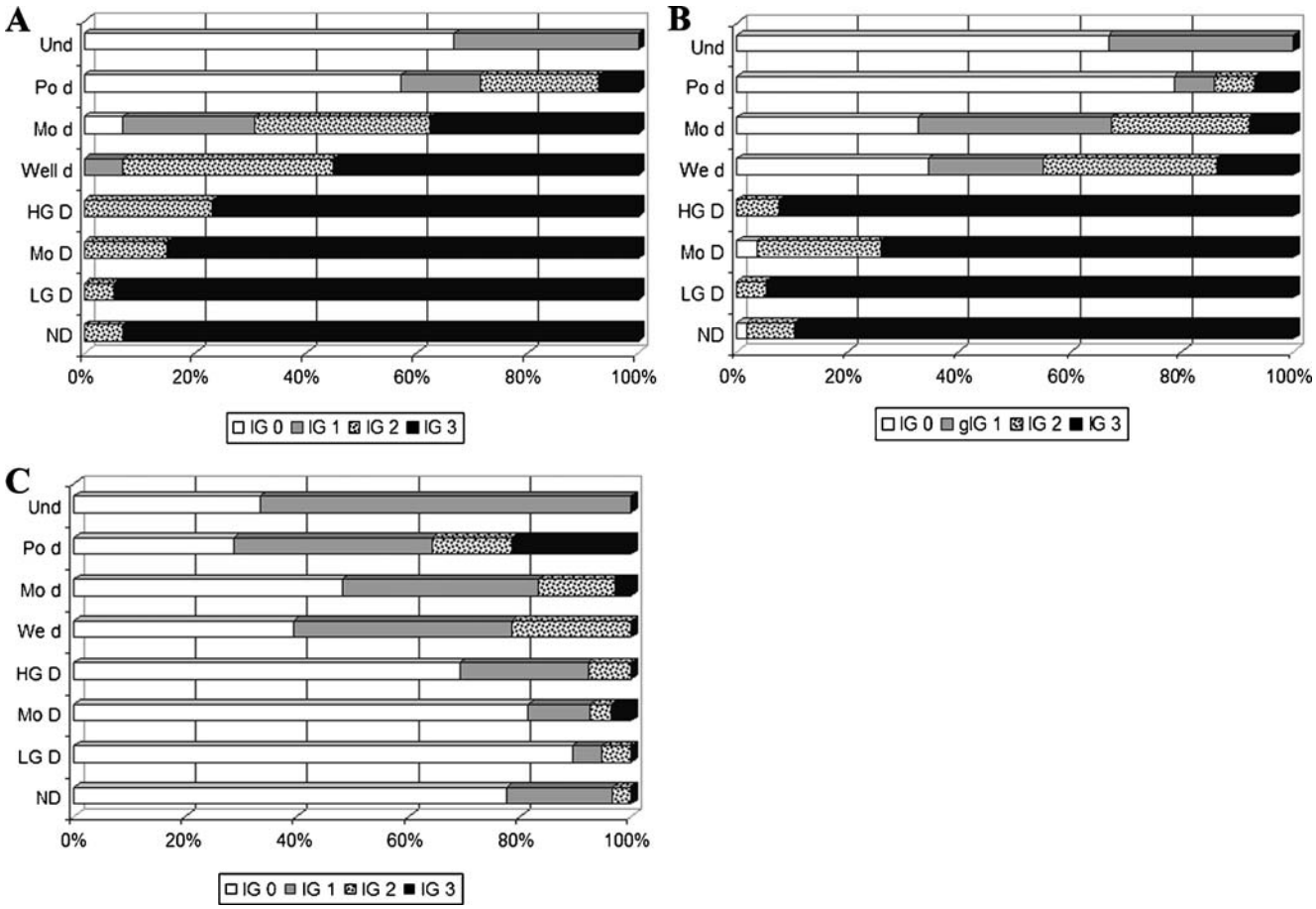

Fig. 3 Immunohistochemistry results plotted against the grade of dysplasia of adenomas and histological grade of carcinomas of the colon. Weak (IG1) or absent (IG0) E-cadherin (**a**) and syndecan-1 (**b**) immunoexpression is more frequent in poorly differentiated or undifferentiated adenocarcinomas, while strong (IG3) or moderate (IG2) immunoexpression is more common in adenomas with or without dysplasia. Positive Ets-1 immunoexpression (IG1, IG2, IG3) (**c**) is detectable in adenomas with or without dysplasia and in carcinomas, displaying higher frequency of occurrence in poorly differentiated and undifferentiated adenocarcinomas. Along the adenoma-carcinoma sequence the number of E-cadherin (**a**) and syndecan-1 (**b**) negative cases increases and that of the Ets-1 (**c**) cases decreases. ND- non-dysplastic adenomas, LG D- adenomas with low-grade dysplasia, Mo D- adenomas with moderate dysplasia, HG D- adenomas with high- grade dysplasia, We d- well differentiated adenocarcinoma, Mo d- moderately differentiated adenocarcinoma, Po d- poorly differentiated adenocarcinoma, Und- undifferentiated carcinoma

survival advantage [11, 17]. In agreement with a previous study we observed that E-cadherin expression was more pronounced in primary tumors than in their metastases [11]. Another study reports enhanced expression of E-cadherin in lymph node metastases of Duke's C stage CCRs compared to primary tumors [17].

Ets-1 was originally characterized as a v-ets retroviral gene of the avian leukemia retrovirus, E26 [6, 7]. In a variety of cell types, including endothelial cells, vascular smooth muscle cells and epithelial cancer cells, Ets-1 promotes invasive behavior [27]. Several studies have demonstrated co-expression of Ets factors and presumptive Ets target genes, including MMPs (MMP-1, MMP-2, MMP-3, MMP-7, and MMP-9) [6–9], probably involved in the shedding of E-cadherin and syndecan-1 [20, 28].

In our study Ets-1 immunostaning did not correlate with any clinicopathological factor of adenomas, but it was present in adenomas with and without dysplasia, so in our conclusion it may have an early involvement in carcinogenesis. In colon carcinomas we found a higher incidence of Ets-1 expression in older patients, advanced Duke`s stage and in the presence of distant metastases. It has been reported that Ets-1 expression was absent in normal colon and hyperplastic polyps, in adenomas with low-grade to moderate dysplasia; it was present in adenomas with high-grade dysplasia and in carcinomas, wherein its expression was associated with advancing tumor grade, lymph node metastasis, lymphatic and venous invasion and poor prognosis [6, 7]. In colorectal adenomas and carcinomas Ets-1 mRNA was observed in 38.9% of the cases, and the expression was not correlated significantly with any of the clinicopathological characteristics [8].

We found that Ets-1 expression was increased in carcinomas compared to adenomas, and was more pronounced in

de novo carcinomas compared to ex adenoma carcinomas. Its expression displayed an inverse correlation with that of E-cadherin and syndecan-1. This indicates a much more invasive nature of *de novo* carcinomas and suggests that these tumors follow a different carcinogenetic process.

We observed a gradual increase in the number of low expression E-cadherin and syndecan-1 cases and high expression Ets-1 cases starting from adenomas with low-grade, moderate and high-grade dysplasia through well and moderately differentiated adenocarcinomas, peaking in poorly differentiated adenocarcinomas. Based on these data, we concluded that these markers might be involved in colon carcinogenesis.

In **conclusion**, we found decreasing expression of E-cadherin and syndecan-1 during colon carcinoma progression and an opposite regulation for Ets-1 protein. *De novo* carcinomas have lower E-cadherin and syndecan-1 expression and higher Ets-1 expression compared to ex adenoma carcinomas. These findings support the hypothesis that there are differences in the carcinogenesis of these tumors.

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