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E-cadherin expression in primary colorectal cancer and metastatic lymph nodes

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Abstract

Colorectal cancer (CRC) is one of the most prevalent malignancies and fourth cause of cancer death worldwide but the current TNM staging system together with clinicopathological characteristics are not sufficient to identify cases that have a poor prognosis. The aim of the current study was to compare E-cadherin expression in primary CRC and lymph node metastases with the one exhibited by normal colic mucosa and normal lymph nodes, and to evaluate its association with disease severity. The authors retrospectively analyzed 65 patients that underwent colectomy for CRC at the First Surgical Clinic, "Sf. Spiridon" Emergency Hospital from Iassy, Romania, over a 10 years period, from January 2004 to December 2013. In all cases, immunohistochemical staining against E-cadherin was performed on primary CRC and associated lymph nodes slices. Primary CRC presented a higher rate (64.62%) of abnormal E-cadherin expression (no staining, cytoplasmic or mixed staining) compared to normal colic mucosa (16.67%) (p<0.001). Both primary CRC and corresponding metastatic lymph nodes displayed a predominantly membranous expression (pure or mixed) of E-cadherin (67.69% and 89.23%, respectively). Well and moderately differentiated tumors displayed an increased E-cadherin expression (44 of 56 cases) compared to poorly differentiated tumors that lacked E-cadherin expression in six out of nine cases. In conclusion, E-cadherin expression abnormalities in CRC are rather qualitative than quantitative and E-cadherin is an important marker of tumor aggressiveness and spreading potential.

Keywords: colorectal cancer, E-cadherin, primary tumor, normal mucosa, metastatic lymph nodes.

Introduction

Colorectal cancer (CRC) is one of the most prevalent malignancies and fourth cause of cancer death worldwide (694 000 deaths in 2012 according to *World Health Organization*) [1]. The current TNM staging system together with clinicopathological characteristics are not sufficient to identify cases of poor prognosis that present a different evolution with lower overall and disease-free survival rates.

The study of genetic and molecular factors in these patients allowed identification of subgroups that express certain markers and need a tailored therapeutic approach including new biological drugs and chemotherapeutics. Studies have shown that deregulation of the E-cadherin-mediated cell adhesion system is involved both in epithelial–mesenchymal transition and tumor progression in many cancers, including CRC, being associated with cellular invasion, angiogenesis and metastatic progression [2].

The aim of this study was to compare E-cadherin expression in primary colorectal cancer and lymph node metastases with normal colic mucosa and normal lymph nodes, and to evaluate their association with disease severity.

Materials and Methods

The authors retrospectively analyzed 65 patients that underwent colectomy for CRC at the First Surgical Clinic, "Sf. Spiridon" Emergency Hospital from Iassy, Romania, over a 10 years period, from January 2004 to December 2013. We compared E-cadherin expression in primary CRC and lymph node metastases with the one exhibited by normal colic mucosa and normal lymph nodes, and to evaluate its association with disease severity. In all cases immunohistochemical staining against E-cadherin was performed on 5 μm sections from paraffin-embedded fragments of the primary tumor and associated lymph nodes removed together with the primary tumor. Sections were deparaffinized using xylene (two baths), and re-hydrated in ethanol baths with decreasing concentrations and finally in distilled water. For antigen retrieval, a buffer solution of citrate 10 mM, pH 6.0 was used. Immunohistochemistry was performed using Novocastra® Liquid Mouse Monoclonal Antibody E-cadherin (Leica Microsystems).

The non-biotinylated polymer system (Novolink®, Leica Microsystems) technique was used for reaction amplification and 3,3'-diaminobenzidine (DAB) solution

as chromogen prior to final Hematoxylin counterstaining. External positive and negative controls were used.

Samples staining were evaluated by two independent observers and graded using two scoring systems proposed by Jawhari *et al.* [3] and Almeida *et al.* [4].

χ^2 test or Fisher's exact test were used for analyzing frequency tables at a 5% significance level (SPSS 21 for Mac).

Results

E-cadherin expression was evaluated in all 65 samples of primary CRC (Figure 1, a–e) and corresponding lymph nodes. The correlation between E-cadherin expression, demographic and clinicopathological features is presented in Table 1.

E-cadherin expression as evaluated using the two scoring systems was compared according to each demographic and clinicopathological variable and proved to associate only with patients' age, negative tumors registering a higher prevalence in younger patients (41.11% in patients <50 years compared to 21.74% in patients ≥50 years). Most tumors were positive for membranous staining for each of the studied variables.

Concerning the metastatic lymph nodes, 37 (56.92%) presented membranous staining, 21 (32.31%) both cytoplasmic and membranous staining, two (3.08%) cytoplasmic staining and five (7.69%) no staining. In the same time, there were also analyzed 13 normal lymph nodes removed together with the metastatic ones and they were all negative for E-cadherin. Normal mucosa that was present in 24 paraffin-embedded fragments registered a membranous staining in 20 (83.33%) cases and no staining in four (16.67%) cases.

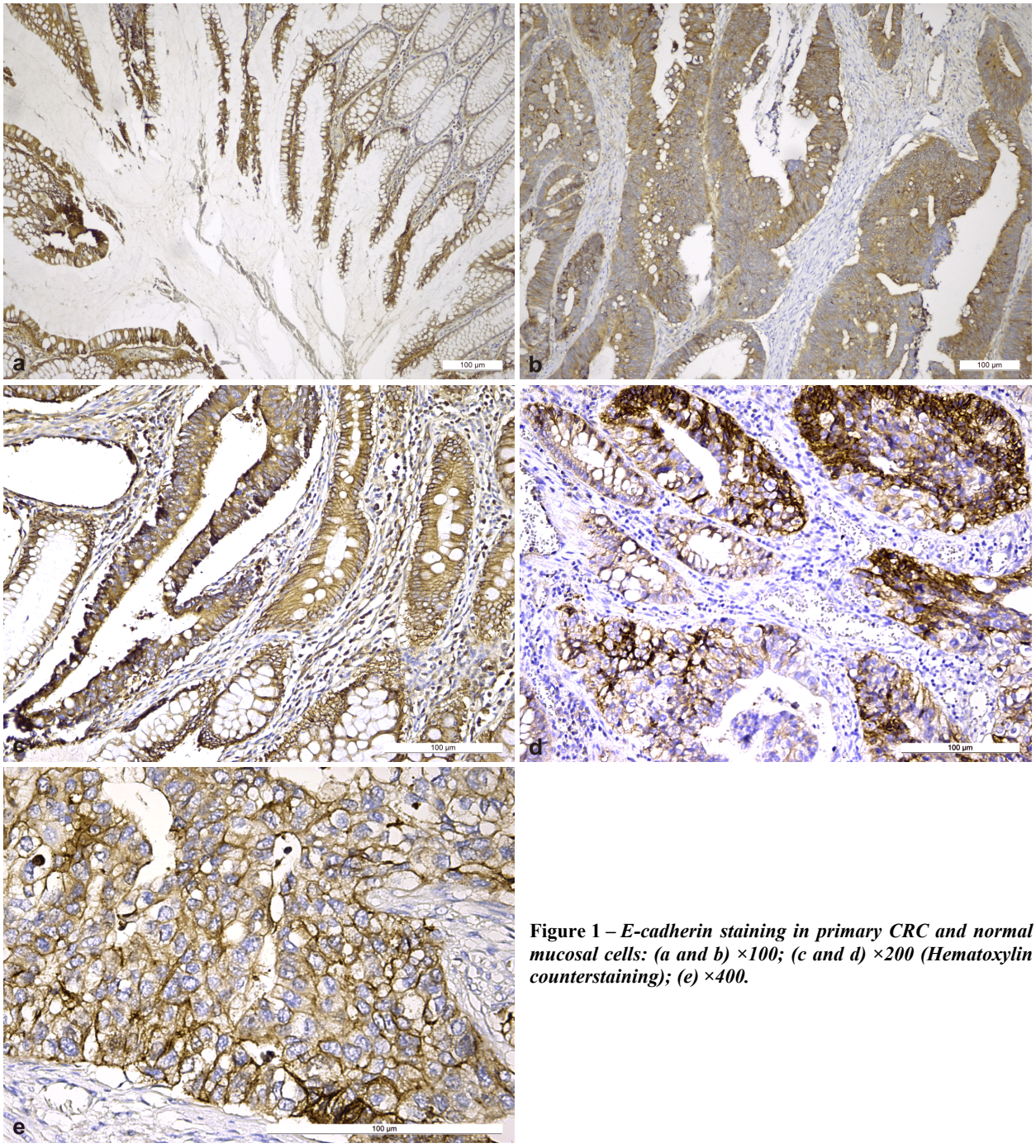

Figure 1 – ***E-cadherin staining in primary CRC and normal mucosal cells: (a and b) ×100; (c and d) ×200 (Hematoxylin counterstaining); (e) ×400.***

Table 1 – ***Correlation of E-cadherin expression in primary tumor with demographic and clinicopathological features***

Variable		No. of cases	E-cadherin score				Jawhari classification	Almeida classification
			0	*1*	*2*	*3*	*p*	*p*
Gender	Males	36	10	1	11	14	0.41	0.56
	Females	29	8	2	10	9		
Age group	<50 years	19	8	0	4	7	<0.001	0.04
	≥50 years	46	10	3	17	16		
CRC location	Right colon	13	4	1	6	2	0.61	0.73
	Transverse colon	3	1	0	0	2		
	Left colon	5	3	1	0	1		
	Sigmoid colon	29	6	1	12	10		
	Not stated	15	4	0	3	8		
Tumor size	<5 cm	19	4	1	7	7	0.48	0.19
	≥5 cm	46	14	2	14	16		

E-cadherin expression comparison between CRC and normal mucosa using Fisher's exact test showed a significantly higher rate on abnormal expression (scores 0, 1 and 2) in CRC (42 cases – 64.62%) compared to normal mucosa (four cases – 16.67%) (p<0.001). When using the other scoring system (Almeida *et al.*) no significant association was noticed between the membranous expression (pure or mixed) and CRC (p=0.20).

E-cadherin abnormal expression registered no significant differences when comparing primary tumor to metastatic lymph nodes (p=0.17) same as membranous expression (p=0.27).

Histological grading showed well and moderately differentiated tumors in 56 cases and poorly differentiated tumors in nine cases (Table 2).

Moderately and well-differentiated tumors mostly displayed membranous (score 3) and mixed (score 2) E-cadherin expression compared to poorly differentiated tumors that were negative in six of nine cases. This association was confirmed by the statistical analysis (p=0.02). Among the poorly differentiated tumors there were three cases with "signet ring" cells, all lacking E-cadherin expression (score 0).

None of the poorly differentiated tumors presented normal E-cadherin expression compared to 23 (41.07%) of the well or moderately differentiated tumors.

Table 2 – ***Correlation of E-cadherin expression with tumor differentiation***

Tumor grade	No. of cases	E-cadherin score				Jawhari classification	Almeida classification
		0	*1*	*2*	*3*	*p*	*p*
Well differentiated	8	2	1	2	3	0.02	0.004
Moderately differentiated	48	10	1	17	20		
Poorly differentiated	9	6	1	2	0		

Discussion

In our study group, 47 (72.3%) of the 65 primary CRC showed E-cadherin expression, in an aberrant pattern in 51% of cases. There was an abnormally increased expression of E-cadherin in CRC compared to normal colic mucosa and this association was statistically significant (p<0.01). Our results confirm those obtained by El-Bahrawy *et al.* (2001 and 2002) and Khoursheed *et al.* [5–7].

On the contrary, when applying Almeida's criteria, no statistically significant difference is noticed between membranous expression and non-membranous expression of E-cadherin (p=0.20) because of grouping a normal (grade 3) and an abnormal (grade 2) pattern into a single entity. Thus, the classification proposed by Almeida for gastric cancer is not applicable in CRC.

The absence of statistically significant difference when using Almeida's criteria portrays an interesting finding of our research, namely that 67.69% (44 cases) of the primary CRC showed membranous expression of E-cadherin, either pure or mixed, compared to 83.33% (20 patients) of the normal mucosa cases, thus suggesting a cytoplasmic redistribution, leading to an abnormal expression pattern in CRC (none of the normal mucosa cases presented cytoplasmic expression). Elzagheid *et al.* showed a predominantly membranous expression of E-cadherin in normal colonic epithelium and mixed expression in primary CRC, findings in accordance with our research. In addition, these authors report a lower disease-free survival rate in case of cytoplasmic expression of E-cadherin in primary CRC [8].

Another study performed by Bezdekova *et al.* showed controversial results, as they found mostly membranous expression of E-cadherin in primary CRC with no cases of cytoplasmic or mixed expression [9].

Other studies with controversial results (Shiono *et al.*, Fang *et al.*) also demonstrated an association between decreased E-cadherin expression and tumor progression in CRC [10, 11].

A very interesting approach was conducted by Masur *et al.* who developed a study of six CRC cell lines and found an association between low E-cadherin expression and increased migration activity of these cells [12]. Our results comparing E-cadherin expression in primary CRC and normal colic mucosa suggest that the change in E-cadherin expression in the primary tumor is qualitative rather than strictly quantitative because the tumors continue to express E-cadherin in an aberrant way. Nevertheless, most primary CRC in our study presented membranous expression of E-cadherin (pure and mixed form) thus suggesting that E-cadherin is an adhesion molecule essential in maintaining cells cohesion also in primary CRC and that loss of E-cadherin membranous expression

is a dynamic event occurring together with epithelial–mesenchymal transition.

There was no statistically significant difference between E-cadherin expression in primary CRC and metastatic lymph nodes in our study (p>0.05). Similar results were obtained by Ikeguchi *et al.* when analyzing E-cadherin expression in primary CRC compared to liver and lymph nodes metastases [13].

Gagliardi *et al.* [14] who compared the expression of E-cadherin in primary CRC and liver metastases found membranous expression in more than 50% of the metastases. The authors suggested that the loss of membranous expression is a transient process by which cells detach from the primary tumor, but that the restatement of membranous expression is essential for the formation of metastasis. Another study carried by Truant *et al.* examined the expression of E-cadherin in liver metastases and showed a vastly increased E-cadherin expression in metastases (85%) compared to the normal liver tissue thus suggesting that neoplastic cells, upon arrival in the metastatic site, regain epithelial phenotype, and membranous E-cadherin expression [15]. Kanazawa *et al.* stated that lymph node metastasis display either a similar or an increased E-cadherin expression compared to primary CRC, and suggested that E-cadherin participates in cell adhesion and consequent formation of neoplastic cells emboli that colonize lymph node tissue [16]. Similar results were found by Batistatou *et al.* who showed that 82% of lymph node metastases from colorectal carcinoma display an E-cadherin expression similar to the primary tumor [17].

E-cadherin expression in the metastatic lymph nodes may be an essential mechanism for cancer cells proliferation in metastatic site and regaining of membranous expression would be an advantageous adaptation for tumor cells in terms of survival.

E-cadherin expression in metastases has also been found in other types of malignancies like gastric carcinoma, lung and esophageal cancer [18, 19]. Bukholm *et al.* found no statistically significant difference between E-cadherin expression in primary infiltrating ductal carcinoma of the breast cancer its metastases [20].

The results of our study are comparable to most of the results found in the literature and suggest that neoplastic cells regain membranous E-cadherin expression upon arrival to lymph nodes (89.23% of metastatic lymph nodes presented pure or mixed membranous expression) in the process known as epithelial–mesenchymal transition.

We also found that poorly differentiated CRC lacked E-cadherin expression in most cases (six of nine cases) unlike well and moderately differentiated CRC, which showed membranous expression of E-cadherin in 78.57% of cases. Pignatelli *et al.* in a study analyzing a cell line of CRC concluded that the E-cadherin expression is required to maintain a well-differentiated glandular pattern and that loss of this expression is associated with undifferentiated tumors and greater spreading capacity [21]. Thus, the loss of E-cadherin expression is associated with less differentiated tumors, the presence in the same case of grouped tumor cells with membranous expression of E-cadherin and isolated cells lacking E-cadherin expression suggests that these isolated cells have excelled the primary tumor after the loss of intercellular adhesion molecules expression, consistent with current evidence on the mechanisms involved in the epithelial–mesenchymal transition.

In another study, Khoursheed *et al.* [7] found that the percentage of E-cadherin expression in well-differentiated CRC was higher than in moderately differentiated tumors. Chen *et al.* [22], working with cell lines of CRC suppressed the expression of E-cadherin in these cells through genetic engineering techniques and found that there was an increase in the replicative potential of these cells and the emergence of resistance to growth inhibitors and increased tissue invasion capacity.

Conclusions

E-cadherin expression in primary CRC is associated with patients' age, negative tumors registering a higher prevalence in younger patients. CRC proved to be associated with an abnormal E-cadherin expression (scores 0–2) compared to normal colic mucosa, indicating that changings in this adhesion molecule in CRC is rather qualitative (cytoplasmic redistribution) than quantitative. Membranous expression of E-cadherin, either pure or associated with cytoplasmic expression, registered no significant differences in primary CRC compared to lymph node metastases and was found to be a peculiar feature of well or moderately differentiated CRC, while the absence of such expression, essential for tumor cells detachment, was associated with poorly differentiated tumors that display a higher spreading potential.

Conflict of interests

The authors declare that they have no conflict of interests.

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