Effects of changes at the site of E-cadherin expression as an indicator of colon cancer aggressiveness

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Abstract

The aim of the work was to study the effects of changes in the location of E-cadherin from membrane to cytoplasm and the appearance of metastases and recurrence in patients with colon cancer of pT1 grade. The study group consisted of 34 patients with colon cancer. The material was fixed in 10% buffered, directly following surgery, fixed in formaldehyde and embedded in paraffin blocks by a standard method. Immunohistochemical reactions were performed, using monoclonal E-cadherin antibodies (Novocastra, NCL-E-Cad). Statistical analysis did not show any relation between the change in the location of E-cadherin expression, the patients' sex, and the location of changes. Simultaneously, we observed a strong relationship between the presence of exudate in the vessels from cancer cells, the histological grade and the loss of E-cadherin expression in the main tumour mass ($p<0.01$). We also noted a statistically significant correlation between the presence of lymph node invasion and distant metastases and the E-cadherin cytoplasmic reaction ($p=0.0001$, $p=0.000001$, respectively). A borderline significance of $p=0.06$ was noted in the association between the appearance of recurrence at the postoperative site and the change in location of E-cadherin expression in the main tumour mass from cytoplasm to membrane. On the basis of our results, we can conclude that a change in the location of E-cadherin expression (from membrane cytoplasm) is strongly associated with an increased aggressiveness of CRC, which is related to the appearance of proximal and distant metastases and to recurrence at the postoperative scar.

Key words: colorectal cancer, E-cadherin.

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Introduction

Intercellular associations are frequently analysed in various types of neoplasms. It has been confirmed, that a disturbance of the mechanism of cellular binding may be one of the parameters, indicating the behaviour of neoplastic cells. Adhesion molecules are surface receptors, involved in intercellular interactions and cellular-extracellular interactions [1]. According to their function and shape, they are divided into 4 main groups: cadherins, integrins, selectins, and adhesion molecules of the superfamily of immunoglobulins [2, 3]. E cadherins are membrane proteins. Their cytoplasmic domain interacts with a group of proteins, known as catenins, and join with either ß or γ catenin. The formed complex binds then with α catenin, which has a direct effect on the cytoskeleton [4]. Neoplasms often have a defect in E-cadherin function, due to an absent cytoplasmic domain. More detailed assessment of the role and function of adhesion molecules seems to be a very important area of research. It not only allows a deeper understanding of the biological formation of metastases, but it may also lead to new perspectives with regards to diagnostics and therapy.

Materials and methods

The study was performed retrospectively on postoperative material from 34 patients who underwent surgery at the Department of Surgery, The Śniadecki Hospital, Białystok, Poland, for cancer of the colon with pT1 grade. The observation period lasted 36 months. Postoperatively, the following other examinations were performed: abdominal US, colonoscopy and abdominal CT. On the basis of those examinations, it was decided whether distant metastases were present (all the cases indicated liver metastases). The site of operation was monitored in all the cases. All the cases of recurrence at the post-operative site were confirmed histopathologically. Immunohistochemistry: Slides of 4mm-thick serial sections of the primary tumour were prepared

Table 1. Expression of E-cadherin and chosen parameters.

parameters	n	Expression of E-cadherin		significance
		membranous	cytoplasmic	
Female	9	5(55.6%)	4(44.4%)	p=1
Male	25	12(48.0%)	13(52.0%)	
Colon	7	3(42.9%)	4(57.1%)	p=1
rectum	27	14(51.9%)	13(48.1%)	
G1	20	14(70.0%)	6(30.0%)	p=0.02
G2	12	3(25.0%)	9(75.0%)	
G3	2	0(0%)	2(100%)	
With lymph node metastasis	16	1(6.2%)	15(93.8%)	p=0.0001
Without lym. node metastasis	18	16(88.9%)	2(11.1%)	
With distant metastasis	14	0(0%)	14(100%)	p=0.000001
Without distant metastasis	20	17(85.0%)	3(15.0%)	
With vascular invasion	14	2(14.3%)	12(85.7%)	p=0.0005
Without vascular invasion	20	15(75.0%)	5(25.0%)	
With local recurrence	3	0(0%)	3(100%)	p=0.06
Without local recurrence	31	17(54.8%)	14(45.2%)	

from each patient. In brief, the slides from each patient were dewaxed, using xylene, and transferred to alcohol. They were then placed in citric acid buffer (10 mM) and heated in a microwave oven (700W) for 10 minutes to expose antigens. A Standard avidin-biotin immunoperoxidase (Novostain Super ABC Kit universal) method was used for the detection of E-cadherin protein expression (Novocastra, NCL-E-Cad, Biokom, Poland). Nonspecific mouse IgG was used as negative control. The reaction products were visualized with diaminobenzidine DAB (DAKO S3000, Dako, Poland). Appropriate positive and negative controls were used. The membranous and cytoplasmic immunostaining was observed for E-cadherin. Expression of E-cadherin was semi quantitatively assessed in neoplastic cells of primary tumour and defined as follows: E-cadherin -membranous (> 50% reaction was membranous in the main mass of tumour) and E-cadherin -cytoplasmic (>50% reaction was cytoplasmic in the main mass of tumour). The percentage of E-cadherin positive cells was calculated in, at least, 500 neoplastic cells per sample, using a light microscope (x400). Statistical analysis. The χ^2 and Fisher's exact test were used for statistical analysis. P-values smaller then 0.05 were considered statistically significant.

Results and discussion

Table 1 presents the obtained results. It is apparent that the presence of cancer cells in lymphatic vessels and veins, and also the appearance of lymph node invasion is a known risk factor for malignancy and for the formation of metastases. Moreover, the presence of these changes is strictly associated with the loss of association between cells, for which E-cadherin is responsible. Colon cancer cells, along with cancer cells from other tissues, show decreased adhesive properties. Some authors suggest that the presence of mutations of these molecules in colon cancer [2, 3, 5]. Many authors have analysed the expression of E-cadherin in colon cancer from normal colonic mucous membrane. Some authors have observed either a decrease in the expression or no correlation with the degree of histopathological invasiveness, metastases, or patient's life expectancy [6]. Others, however, have not observed such a correlation [7]. In the presented results, we performed an evaluation of E-cadherin expression in the main tumour mass, analysing the effect of changes in the location of its expression with: vascular invasion, lymph node metastasis, distant metastasis and local recurrence. The results of our study show a changing location of E-cadherin expression. Normal tissue shows a "clean" membrane immunohistochemical reaction, whereas in extra-membrane tumour mass tissue, E-cadherin is similarly observed in the cytoplasm, whilst the cytoplasmic reaction was strongest at the front of invasion. In the present study, statistical analysis did not reveal any association between the changes in E-cadherin expression location and the location of the tumour, or patient's sex. In the present study, we observed a cytoplasmic reaction for E-cadherin in 12/14 cases of vascular invasion. Similarly, out of 17 cases with cytoplasmic reaction, lymph node metastases were found in 15. Moreover, in all our subjects, a cytoplasmic reaction for E-cadherin was associated with the appearance of distant metastases and recurrence at the postoperative scar. Similar results have been observed by El-Bahrawy et al. [7]. When we compare our results to those of other authors, who have noted a decrease in the expression of E-cadherin, we see that the expres-

sion of E-cadherin does not show either any decrease or absence, only a shift from membrane to cytoplasm. This leads to a loss of function. Furthermore, E-cadherin, which is found in recurrent metastases, is found in membrane Mareel et al. [5]. It appears that for cell division ability,it is essential that they bind with one another. Moreover, in our study, the cytoplasmic reaction was observed mainly at the front of invasion, whereas the membrane reaction was found in the main mass of tumour. Similar results have been achieved by El-Bahrawy et al.[7]. The observations and the presented results may indicate a strong effect of a change in the location of E-cadherin expression and the aggressiveness of colon cancer.

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