ONCOLOGY

Expression of E-Cadherin, β-Catenin, and CD-44v6 Cell Adhesion Molecules in Primary Tumors and Metastases of Colorectal Adenocarcinoma

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Immunohistochemical analysis of the expression of E-cadherin, β-catenin, and CD-44v6 proteins was carried out for evaluating the metastatic potential of colorectal cancer cells. Specific features of expression, distribution, and interactions of adhesive molecules in primary tumors of the large intestine and their metastases in the liver and lymph nodes were studied. Reduction and complete absence of E-cadherin expression were much more often observed in patients with colorectal cancer with metastases in the liver than in patients without metastases. Cytoplasmic immunoreactivity and nuclear translocation of β-catenin were increased in more than 80% cases with colorectal adenocarcinoma with metastases. These changes in the expression of E-cadherin and β-catenin in tumor cells can be regarded as factors of unfavorable prognosis of colorectal cancer. No significant relationship between expression of CD-44v6 protein and metastatic potential of cancer cells was detected.

Key Words: *colorectal adenocarcinoma; adhesive molecules; metastases; immunohistochemistry*

The capacity of tumor cells to invasion and metastasizing is an important mechanism of malignant tumor development in humans [2,4,12]. The study of these processes implies detection of a relationship between clinical manifestations of the disease and activities of some molecules modulating biological behavior of the tumor. Cell adhesion molecules are now most actively studied; disorders in normal expression and functioning of these molecules are often observed in colorectal cancer cells.

E-cadherin (protein belonging to the family of homologous transmembrane Ca^{2+}-dependent glycoproteins) acts as a tumor invasion suppressor; its decreased expression in human malignant tumor cells is associated with high risk of metastases and unfavorable prognosis [4,8-10,13,15].

β-Catenin is an intercellular multifunctional protein, the central regulatory component of the adhesive complex. It activates genes related to tumor development [2-4,6,11,15].

CD44v6 is an adhesive molecule playing an important role in cell interactions between each other and with extracellular matrix; it is expressed in the majority of epithelial tumors and is essential for invasive and metastatic potential of some human tumors [1,5, 7,14].

The aim of this study was to characterize the expression of E-cadherin, β-catenin, and CD44v6 molecules and the degree of impairment of adhesive pro-

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perties in tumor cells during the development of colorectal cancer metastases.

MATERIALS AND METHODS

The study included analysis of clinical observations and biopsy specimens from 129 patients (78 males and 51 females aged 35-78 years) operated for colorectal cancer and in 84 cases subjected to resection of the liver for metastases. The time course of metastases development was followed up for 5 years after radical treatment. All patients were divided into 2 groups: 1) with distant metastases in different periods after the intervention and 2) without distant metastases (according to clinical data). In group 1 a total of 84 primary colorectal tumors, 35 metastases of these tumors in regional lymph nodes, and 92 metastases in the liver (8 of these repeated) were examined. In group 2 a total of 45 colorectal tumors were examined at different stages of the disease.

Immunohistochemical study was carried out using biotin-streptavidin immunoperoxidase method on serial paraffin sections of colorectal primary tumor tissue, adjacent colorectal mucosa, and metastases in the liver and lymph nodes using antibodies to E-cadherin (Clone 36B5), β-catenin (Clone 17C2), and CD 44v6 (Clone VFF-7; all antibodies from Novocastra). First antibodies were detected using biotinylated secondary antibodies and peroxidase-labeled streptavidin (LSAB+Kit, Dako). The reaction was visualized with diaminobenzidine DAB+ (Dako). Cell nuclei were post-stained with Mayer hematoxylin.

Protein expression was evaluated as negative ("—": no reaction; "±": foci, solitary positive cells), weak positive ("+": 25% stained cells), medium and highly positive ("++/+++": 25-50 and >50% cells of medium and high intensity of staining, respectively). The ±/+ reaction was considered as reduced. The type of staining depended on the location of reaction product in cells (cytoplasmic, membrane, mixed, or nuclear).

The morphology of primary tumors and metastases was studied on paraffin sections stained with hematoxylin and eosin. Colorectal adenocarcinoma (CRA) of different degree of differentiation was diagnosed in all cases.

The data were statistically processed using χ^2 test. The differences were considered significant at $p<0.05$.

RESULTS

Expression of E-cadherin was observed in cancer cells of primary CRA (67/129, 52%), some regional (8/35,

TABLE 1. Expression of Adhesive Molecules in Primary CRA

Molecule, group	Number of cases	—/±		+		++/+++	
		abs.	%	abs.	%	abs.	%
E-Cadherin							
1 (metastases)	84	51	61	13	15	20	24
2 (no metastases)	45	11	24	3	7	31	69
β-Catenin							
1 (metastases)	84	7	8	5	6	72	86
2 (no metastases)	45	5	11	9	20	31	69
CD44v6							
1 (metastases)	84	54	64	16	19	14	17
2 (no metastases)	45	21	47	8	18	16	35

TABLE 2. Incidence of Positive Expression of Adhesive Molecules in Primary and Metastatic CRA Cells

Parameter	Number of cases	E-Cadherin		β-Catenin		CD44v6	
		abs.	%	abs.	%	abs.	%
Primary tumors without distant metastases	45	34	76	40	88	24	53
Primary tumors with metastases in the liver	84	33	39	77	92	30	36
Metastases in lymph nodes	35	8	23	32	91	9	26
Metastases in the liver	92	21	26	86	93	21	23

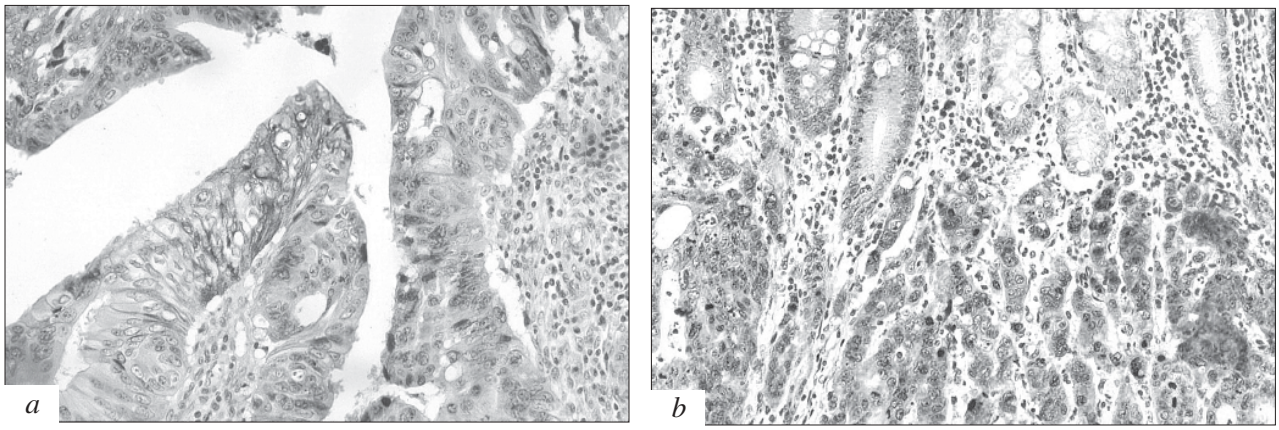

Fig. 1. Expression of E-cadherin and β-catenin molecules in primary colorectal tumors of patients with metastases in the liver. *a*) focus of membrane-cytoplasmic expression of E-cadherin in colorectal adenocarcinoma (CRA) cells, ×120; *b*) nuclear-cytoplasmic expression of β-catenin in CRA cells. Weak staining of epithelium in adjacent colorectal mucosa, ×25.

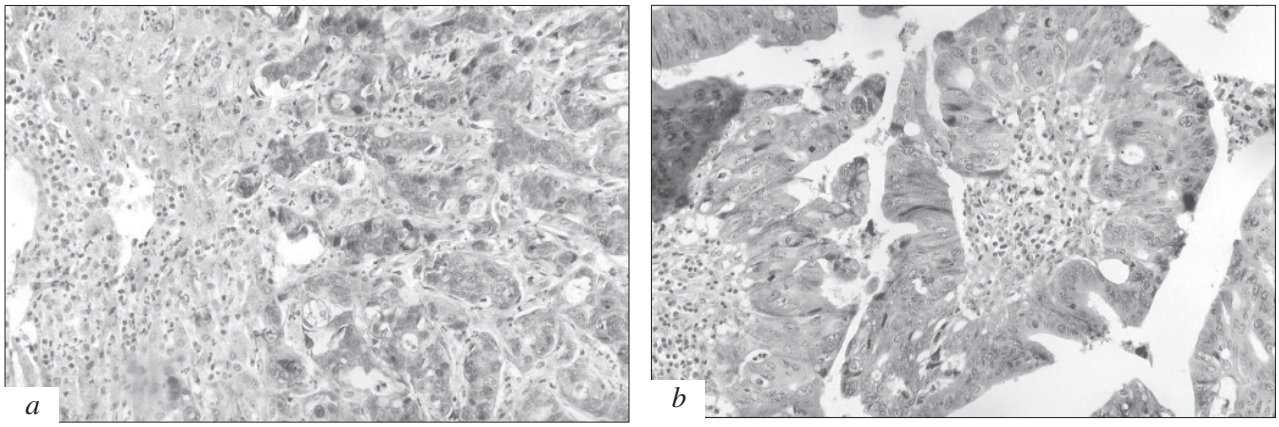

Fig. 2. Expression of β-catenin and CD44v6 in colorectal cancer metastases in the liver. *a*) pronounced nuclear-cytoplasmic expression of β-catenin in cells of CRA metastasis. Weak hepatocyte staining, ×120; *b*) heterogeneous membrane-cytoplasmic expression of CD44v6 in CRA metastasis cells, ×25. Streptavidin biotin immunoperoxidase method. Cell nuclei are post-stained with Mayer hematoxylin.

23%) and distant (18/92, 20%) metastases, and epithelial cells of the colorectal mucosa. Protein expression in primary tumors varied in different groups of patients (Table 1). Immunoreactivity was detected on the surface of cancer cell and/or was detected by diffuse homogeneous or finely granular staining of the cytoplasm. The membrane type of expression, typical of normal colorectal epithelium, was more characteristic of well-differentiated CRA. Accumulation of E-cadherin in cancer cell cytoplasm was observed at different stages of the disease, more often in tumors or at sites of tumors with moderate or poor differentiation. The level of E-cadherin expression in the cytoplasm and/or of cancer cell membranes decreased at higher stages of tumor process and was appreciably lower in tumors with distant metastases (Fig. 1, *a*). In the majority of these cases primary tumor cells and tumor metastases in lymph nodes and liver were characterized by the absence of membrane staining; complete absence of the reaction or foci and low level of cytoplasmic expression of E-cadherin were primarily observed. Expression of E-cadherin in metastases was detected more rarely than in primary foci, but there were no appreciable differences in levels and types of protein expression in primary and metastatic tumor cells.

The expression of E-cadherin in primary tumor cells in patients with and without distant metastases differed significantly. Reduced expression of the protein or its absence were more often detected in patients with colorectal cancer with distant metastases (64/84, 76%) and much more rarely in group 2 patients (14/45, 31%, $p<0.014$). Retained membrane staining of cancer cells was more characteristic of group 2 patients (16/45, 46%) than in group 1 (9/84, 11%; $p=0.012$).

Positive reaction to β-catenin was detected in the majority of primary colorectal tumors (117/129, 91%) and their metastases in the liver (86/92, 93%) and lymph nodes (32/35, 91%). Moderately and strong-positive reaction was often observed in primary tumor cells in both groups of patients (Table 1). The reaction product in cancer cells was located on the membrane,

in the cytoplasm, or both. Nuclear location of β-catenin was detected. Expression of the protein was observed in different variants of adenocarcinoma and did not depend on tumor differentiation. Membrane, cytoplasmic, or mixed staining of different intensity was observed in primary tumors without distant metastases; the nuclei were stained in just solitary cells. Specific reaction on the surface of cancer cell was more intense than in the epithelium of adjacent colorectal mucosa. At later stages of the disease intensive cytoplasmic and nuclear reaction was observed in the majority of primary tumor cells in patients with distant metastases (Fig. 1, *b*). Nuclear β-catenin was usually detected at the periphery of large cancer complexes or glandular structures, or in small groups and solitary cancer cells in the stroma. Cells located in the center of the tumor complexes were less intensely stained with β-catenin than cells at the periphery. Intensive expression of β-catenin molecules was observed in the majority of metastases, the most intense nuclear cytoplasmic reaction in liver metastases was observed in cancer cells located in zones of the invasive front (Fig. 2, *a*). Similar or higher level of nuclear cytoplasmic expression of the protein in comparison with the primary tumor was detected in lymph node metastases.

Nuclear expression of β-catenin in primary tumor cells significantly correlated with the presence of colorectal cancer metastases in the liver and was much more often detected in group 1 patients (70/84, 83%) than in group 2 (15/45, 33%, p=0.01). High and moderate level of cytoplasmic expression of the protein was more characteristic of metastasizing CRA (72/84, 86%) than of tumors without metastases (31/45, 69%), but the differences were statistically insignificant (p=0.528).

A characteristic pattern of expression in patients with distant CRA metastases was a combination of high level of β-catenin (including nuclear expression in invasion zones) and the absence or minimum level of E-cadherin (70% cases for primary cases, 63% for metastases in the lymph nodes, and 73% for metastases in the liver).

Expression of CD44v6 molecules in cancer cells was observed in some CRA (54/129, 42%) and their metastases in the liver (21/92, 23%) and lymph nodes (9/35, 26%). The expression of the protein in primary tumor cells was different in patients of both groups (Table 1). The epithelium of colorectal mucosa was stained mainly in the basal parts of intestinal cryptae. Membrane or cytoplasmic immunoreactivity of cancer cells was noted. Expression of CD44v6 molecules with predominance of cytoplasmic reaction was observed in primary tumors with moderate and poor histological differentiation in patients with and without metastases. Reduction of protein expression was somewhat more frequent in metastasizing tumors. Specific reaction in antigen-positive metastatic cells was heterogeneous for different areas of the cell membrane, sometimes more intense in the basolateral parts of the membrane, while cytoplasmic staining was minor with homogeneous or coarse granular distribution in the cell (Fig. 2, *b*). Expression of CD44v6 molecules in regional and distant metastases did not indicate a constant relationship with the expression in the primary tumor. No significant relationship between protein expression in primary CRA cells in patients with distant metastases (24/45, 53%) and without metastases (30/84, 36%) was detected (p=0.29).

Hence, expression of cell adhesion molecules was observed in primary CRA cells; its incidence differed depending on the presence of distant metastases and was preserved in some metastases of these tumors in the liver and lymph nodes (Table 2).

Immunohistochemical study of E-cadherin and β-catenin in CRA showed that the expression of these molecules was essential for metastatic and invasive potential of the tumor. The detected changes in protein expression can serve as additional criteria of malignancy in evaluating the disease prognosis after surgical treatment and for prescribing adjuvant chemotherapy.

The expression of E-cadherin is reduced in CRA with distant metastases; molecules of β-catenin are accumulated in the cytoplasm and at a certain stage in cancer cell nucleus. These changes in primary tumors during the development of distant metastases sometimes precede clinical manifestations of the disease progress and are factors of unfavorable prognosis in colorectal cancer. Further studies are needed to clear out the role of CD44v6 protein for evaluation of the metastatic potential of cancer cells.

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