Clinical Significance of the Content of Biomolecular Markers in Invasive Front of Colon Carcinomas

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We studied the expression of β-catenin, matrix metalloproteinase 9, collagen IV, and laminin in invasive front of primary colon adenocarcinomas and their metastases in lymph nodes and liver. Intensive expression of matrix metalloproteinase 9 in zones of invasive growth of the tumor was associated with enhanced accumulation of β-catenin in the nuclei of tumor cells in peripheral zones of 28% studied tumors. The presence of nuclear β-catenin and increased content of metalloproteinase 9 in the tumor were associated with abnormal accumulation of laminin in cell cytoplasm and with the absence of collagen IV-containing basal membranes. These changes were typical of tumors with high invasive and metastatic potential. Our findings suggest that β-catenin, matrix metalloproteinase 9, laminin, and collagen IV are important predictors of clinical course of rectal cancer and that the peculiarities of protein expression related to the risk of liver metastases have concordant patterns and are most pronounced in invasive front of the tumor.

Key Words: *colon cancer; β-catenin; matrix metalloproteinase 9; laminin; type IV collagen*

Malignant phenotype of human neoplasms is determined by complex dysfunction of a number of biomolecular markers [3,11]. Changes in activity and interaction of various factors such as adhesion proteins and cell-cell contact molecules, structural components of basal membranes, and proteolytic enzymes secreted by tumor cells and extracellular matrix are of great clinical significance during colon cancer (CC) progression and metastasizing. β-Catenin, matrix metalloproteinase 9 (MMP-9), collagen IV, and laminin are the key molecules involved in these processes.

Changes in the expression of these factors can be detected in not only tumor cells, but also in cell environment and analysis of their content in zones of tumor growth and invasion (individually in different combinations) is important for evaluation of metastatic potential and clinical prognosis of CC [1,4,9,10,13].

Some studies showed that the most pronounced changes in the expression of β-catenin in CC were observed in the zone of tumor growth and are characteristic for neoplasms with high metastatic potential [1,12]. β-Catenin activates some molecules, including MMP and laminin, increasing invasive potential and dissemination capacity of tumor cells [1].

MMP plays an important role in tumor metastasizing due to destruction of the basal membranes, activation of growth factors, and stimulation of angiogenesis [2,14]. It was demonstrated that MMP-positive invasive front is a marker of low survival rate and high probability of early local relapses in CC patients even in patients without lymph node metastases [9,13].

Recent studies revealed a significant correlation between the expression of MMP-9 and the state of basal membrane structural components collagen IV and laminin [4,5,7]. Some authors noticed that the peculiarities of the expression of these proteins in tumors, especially in growth and invasion zones, affect the metastatic potential of CC [6,8,12].

Here we analyzed the content of β-catenin, MMP-9, collagen IV, and laminin in primary tumors and metastases of CC with consideration for clinical and mor-

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phological parameters of the disease and peculiarities of protein expression in invasive front of the tumor.

MATERIALS AND METHODS

The study included analysis of clinical data and post-operation biopsy material from 264 patients with stage I-IV CC. Liver metastases were detected in 135 cases (in 110 patients they were surgically removed) and in 129 cases no metastases were detected during 3-year follow-up.

Histological examination revealed adenocarcinomas of different differentiation degree in all patients. Highly, medium-, and low-differentiated tumors were detected in 79 (29.9%), 151 (57.2%), and 34 (12.9%) patients, respectively.

Paraffin sections of primary colorectal tumors (n=264) and their metastases in lymph nodes (n=127) and liver (n=110) were immunohistochemically stained by the biotin-streptavidin method using antibodies to β-catenin, MMP-9, collagen IV (Novocastra), and laminin (Dako). The results were evaluated by a semiquantitative method by the intensity of staining, number of antigen-positive cells (specific reaction is absent/weak –/+; moderate/intensive ++/+++), and localization of immunoreactivity in tumor cells and stroma.

The data were processed statistically and significance of differences was evaluated using χ^2 test (the differences were significant at $p<0.05$).

RESULTS

Membrane/cytoplasmic and/or nuclear expression of β-catenin was detected in the majority of the examined primary colorectal tumors (223 cases, 84.5%). In tumors complicated with metastases, nuclear localization of β-catenin was most pronounced along the periphery of large tumor complexes or glandular structures and in small groups and solitary branched out tumor cells located in the stroma. In central areas of tumor complexes, β-catenin was often located on membranes or in the cytoplasm of tumor cells and demonstrated less intensive reaction, than in peripheral cells (Fig. 1, *a*). In 84 (31.8%) cases, the content of nuclear β-catenin was higher in zones of invasive growth of primary colorectal tumors. The protein was also detected in the majority of the studied metastases in regional lymph nodes (in 116 of 127 cases, 91.3%) and liver (in 102 of 110 cases, 92.7%). In cells of liver metastases, the most intensive nuclear and cytoplasmic expression of β-catenin molecules was observed in tumor cells located at the boundaries of the pathological focus in the invasive front.

Intensive expression of β-catenin significantly correlated with the presence of vascular invasion (p= 0.003) and liver metastases (p=0.001) in CC patients.

Enhanced expression of MMP-9 in tumors of was observed in 50.4% CC patients (133 of 264 cases) in the cytoplasm of tumor cells and stroma components around tumor complexes (fibroblasts, macrophages, vascular walls, and leukocytic elements, Fig. 1, *b*). In 77 cases (29.2%), the most pronounced accumulation of MMP-9 in the cytoplasm of tumor cells in glandular structures was observed in the invasive front of metastasizing tumors, sometimes in stromal cells around the invasive edge of the neoplasm. Tumor complexes at the periphery were more intensively stained than central tumor structures. Accumulation of MMP-9 was

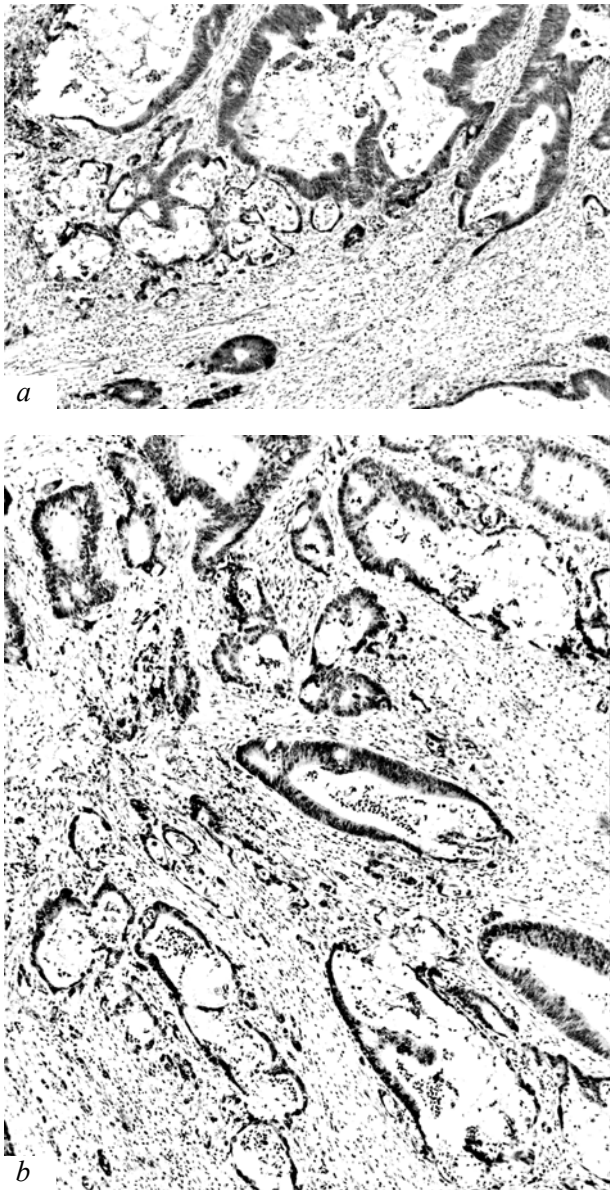

Fig. 1. Expression of protein markers in zones of CC infiltrative front. *a*) nuclear and cytoplasmic accumulation of β-catenin along the periphery of glandular structures and in small branched out tumor complexes, ×200; *b*) accumulation of MMP-9 in cell cytoplasm and extracellular matrix in growth zones of the primary tumor, ×150.

detected in 57.5% (73 of 127 cases) examined CC metastases in regional lymph nodes and in 66.4% (73 of 110 cases) liver metastases. The enzyme was accumulated in the cytoplasm of the majority of metastatic cells in the liver irrespective of the type of growth and peculiarities of the invasive front. Increased content of protease in tumor cells was observed in both apposition growth of metastases and infiltrative and mixed type of the invasive front.

Enhanced expression of MMP-9 in the cytoplasm of tumor cells was associated with CC progression and correlated with low level of histological differentiation of the tumor (p=0.048), deeper tumor invasion (Dukes stage; p=0.012), and high risk of liver metastases (p=0.0001).

Immunohistochemical staining for collagen IV and laminin revealed fine structures of basal membranes around tumor complexes. During CC progression, the positive basal membrane staining disappeared, immunoreactivity of fibrillary components of the stroma increased, and laminin appeared in the cytoplasm of tumor cells.

Abnormal accumulation of laminin in the cytoplasm of tumor cells correlated with local invasion degree of CC according to Dukes staging (A-C; p=0.001), decrease in tumor differentiation degree (p=0.002), the presence of liver metastases (p=0.007) and infiltrative growth (p=0.0001); under these conditions, increased content of the marker was detected in the invasive front of the tumor.

The absence or minimum content of collagen IV-containing basal membranes was more often observed in metastasizing (p=0.0001) and low-differentiated tumors (p=0.035).

Comparison of the expression of β-catenin, MMP-9, collagen IV, and laminin revealed interrelated changes in protein expression in tumors and peculiarities of their content in the invasive front of the tumor.

Cytoplasmic localization of laminin in the tumor was associated with intensive expression of MMP-9 in cells of 67% tumors (63 of 94 cases). Weak reactivity or the absence of collagen IV-positive basal membranes simultaneously with enhanced expression of MMP-9 was observed in 42.2% metastasizing tumors (57 of 135 cases).

Intensive expression of MMP-9 in zones of CC invasive growth was associated with enhanced accumulation of β-catenin in the nuclei of tumor cells in peripheral zones of 28% studied tumors (52 of 186 cases, Table 1).

The invasive front of metastasizing tumors was characterized by accumulation of nuclear β-catenin and enhanced cytoplasmic expression of MMP-9 and laminin in tumor cells, which was often associated with the absence of collagen IV-positive basal membranes in these zones.

TABLE 1. Relationship between Cytoplasmic Expression of MMP-9 and Nuclear Expression of β-Catenin in Invasive Front of CC

Marker	Nuclear β-catenin	
	–/+ (n=102)	++/+++ (n=84)
MMP-9 –/+ (n=109)	77	32
MMP-9 ++/+++ (n=77)	25	52

It should be noted that specific changes in accumulation of β-catenin molecules in the invasive front of the tumor can be a reliable marker of high metastatic potential of CC. The coordinated changes in the content of some proteins, in particular, the absence of collagen IV-positive basal membranes and cytoplasmic deposition of laminin in combination with enhanced expression of MMP-9 in these tumors were typical of the progression of CC and high risk of distant metastases.

Thus, the zone of invasive front of CC is characterized by some phenotypic peculiarities related to activity, structure, and functional state of biomolecular factors, in particular, β-catenin, MMP-9, collagen IV, and laminin. These peculiarities of protein accumulation in the tumor play an important role in progression of CC and accentuate the important role of markers in the processes of migration and invasion of tumor cells. The presence of nuclear β-catenin, increased content of MPP-9, and abnormal accumulation of laminin in the cytoplasm of tumor cells together with the absence of collagen IV-containing basal membranes are typical of CC with high invasive and metastatic potential.

Hence, β-catenin, MMP-9, laminin, and collagen IV are important predictors of clinical course of CC and that the peculiarities of protein expression related to the risk of liver metastases have concordant patterns and are most pronounced in the invasive front of the tumor.

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