Clinical Significance of Levels of Molecular Biological Markers in Zones of Invasive Front-Line of Colorectal Cancer

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The role of expression of markers (β-catenin, matrix metalloproteinase 9, collagen IV, and laminin) in primary colorectal adenocarcinomas and their metastases in the liver and lymph nodes of patients with colorectal cancer was studied. High level of matrix metalloproteinase 9 expression in zones of invasive growth of colorectal cancer was associated with high accumulation of β-catenin in cancer cell nuclei in the peripheral zones of 30% studied tumors. The presence of nuclear β-catenin and high content of matrix metalloproteinase 9 in the tumor were associated with abnormal accumulation of laminin in the cytoplasm and with the absence of basal membranes containing collagen IV. These changes were characteristic of colorectal cancer with high invasive metastatic potential. It was found that β-catenin, matrix metalloproteinase 9, laminin, and collagen IV were important markers for prediction of the clinical course of colorectal cancer. The expression of proteins associated with risk of metastases in the liver was coordinated and most pronounced in zone of invasive front-line of tumors.

Key Words: *colorectal cancer; β-catenin; matrix metalloproteinases; laminin; collagen IV; metastases*

Malignant phenotype of human tumors is determined by combination of several disorders, caused by specific functioning of molecular biological markers in the tumor [3,11]. Changes in activities and interactions in the tumor of such factors, as adhesion proteins and cell-cell contact molecules, structural components of basal membranes, and proteolytic enzymes released by tumor and extracellular matrix cells, are clinically significant progress and metastasizing of colorectal cancer (CC). The key molecules involved in these process are β-catenin, matrix metalloproteinase 9 (MMP-9), collagen IV, and laminin.

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Changes in the expression of these factors are detected in tumor cells and in the cells adjacent to the tumor. Analysis of their content in zones of tumor growth and invasion (individually and in various combinations) is important for evaluation of the metastatic potential and clinical prognosis of CC [1,4,9,10,13].

The most significant changes in β-catenin expression in CC are observed in tumor growth zones; these changes are characteristic of tumors with high metastatic potential [1,12]. β-Catenin activates molecules, for example, MMP and laminin, promoting higher invasive activity and dissemination of cancer cells [1].

MMP-9 plays an important role in tumor metastasizing due to its capacity to destroy the basal membranes and stimulate growth factors and angiogenesis [2,14]. It was shown that MMP-positive

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invasive front-line was a marker of poor survival and high probability of early local relapses even in CC patients without metastases in the lymph nodes [9,13].

Recent studies detected a significant relationship between MMP-9 expression and status of collagen IV and laminin (structural components of the basal membrane) [4,5,7]. Some authors noted that the metastatic potential of CC depended on the expression of these proteins in the tumor, particularly in growth and invasion zones [6,8,12].

We analyzed the levels of β-catenin, MMP-9, collagen IV, and laminin in primary and metastatic CC with consideration for the main clinical morphological parameters of the disease and protein expression in the invasive front-line of the tumor.

MATERIALS AND METHODS

Results of clinical observations and analyses of postoperative biopsy specimens from 264 patients with stages I-IV CC are included in the study. A total of 135 patients developed metastases in the liver, which were resected in 110 cases; 129 patients developed no remote metastases during a 3-year follow-up period.

Histological analysis of the material showed adenocarcinomas of various differentiation degrees in all patients. High, moderately, and poorly differentiated tumors were detected in 79 (29.9%), 151 (57.2%), and 34 (12.9%) patients, respectively.

Immunohistochemical staining of paraffin sections of primary tumor tissue and metastases in the lymph nodes and liver was carried out by the biotin-streptavidine immunoperoxidase method using antibodies to β-catenin, MMP-9, collagen IV (Novocastra), and laminin (Dako). The results were evaluated by the semiquantitative method with consideration for staining intensity, number of antigen-positive cells (no specific reaction/weak reaction: -/+; moderate/strong reaction: ++/+++), and immunoreactivity location in cancer cells and tumor stroma.

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

RESULTS

The membrane cytoplasmic and/or nuclear expression of β-catenin was detected in the majority of examined primary tumors of the colon (in 223 of 264; 84.5%). Nuclear localization of β-catenin in the tumors with metastases was more intense at the periphery of large cancer complexes and in small groups and solitary separated cancer cells located in the stroma. In central areas of tumor complexes, β-catenin was often located on membranes or in the cytoplasm of cancer cells and exhibited less intense reaction than in cells at the periphery (Fig. 1). In 84 (31.8%) cases, the content of β-catenin increased in zones of invasive growth of primary colorectal tumor front-line. In addition, the protein was detected in the majority of examined metastases in the regional lymph nodes (in 116 of 127, 91.3%) and in the liver (in 102 of 110, 92.7%). The most intense cytoplasmic expression of β-catenin molecules in the cells of liver metastases was often observed at the interface of the pathological focus in zones of invasive front-line.

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

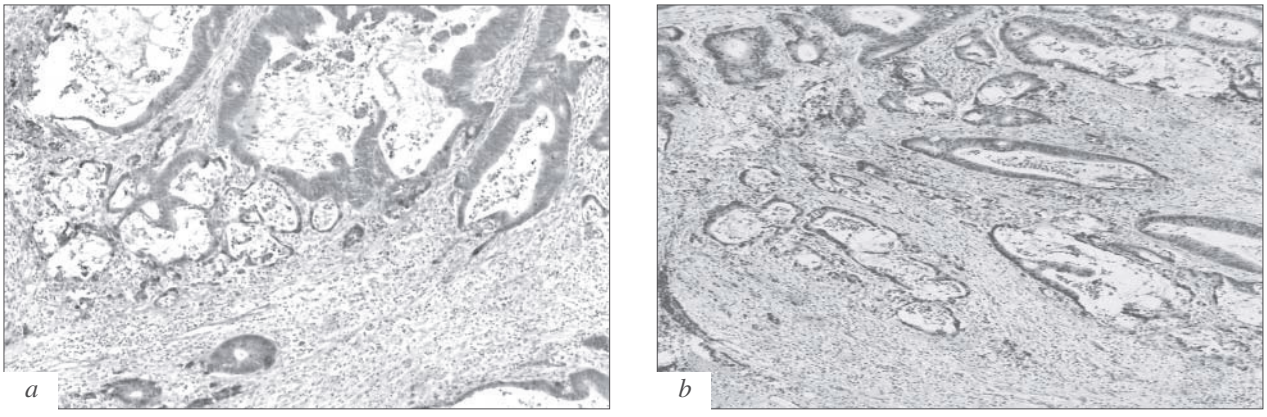

Fig. 1. Expression of protein markers in zones of CC invasive growth front-line. *a*) nuclear cytoplasmic accumulation of β-catenin at the periphery of glandular structures and in small detached cancer complexes (×200); *b*) accumulation of MMP-9 in the cell cytoplasm and extracellular matrix in zones of primary tumor growth (×150).

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

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

Note. *n*: number of cases.

High expression of MMP-9 in the tumors was noted in 133 (50.4%) CC patients in the cytoplasm of cancer cells and stromal components adjacent to tumor complexes (fibroblasts, macrophages, vascular walls, leukocytes). In 77 (29.2%) cases, the accumulation of MMP-9 in cancer cell cytoplasm in glandular structures was most pronounced in zones of invasive growth of metastatic tumors, sometimes in stromal cells adjacent to the invasive edge of the tumor. Cancer complexes at the periphery were more brightly stained with the marker than tumor structures in the central areas. Accumulation of MMP-9 was detected in 57.5% (in 73 of 127) of examined CC metastases in the regional lymph nodes and in 66.4% (in 73 of 110) tumor metastases in the liver. The enzyme accumulated in the cytoplasm of the majority of metastatic cancer cells in the liver irrespective of the type of growth and specific features of the invasive front-line. The protease level in cancer cells was high in the appositional growth of metastases and in infiltrative and mixed types of invasive front-line.

High expression of MMP-9 in the tumor cell cytoplasm was caused by CC progress and was combined with low level of histological differentiation of the tumor (p=0.048), deeper invasion of the tumor (disease stage according to Dukes; p=0.012), and high risk of metastases in the liver (p=0.0001).

Immunohistochemial staining of tumors for collagen IV and laminin showed fine structures of basal membranes around the tumor complexes. The progress of CC was associated with disappearance of positive staining of basal membranes, increased immunoreactivity in the fibrillar components of the stroma, and appearance of laminin in tumor cell cytoplasm.

Abnormal accumulation of laminin in cancer cell cytoplasm correlated with local invasive activity of CC in accordance with the Dukes' stage (A-C; p=0.001), with lower differentiation of the tumor (p=0.002), presence of metastases in the liver (p=0.007), and infiltrative growth (p=0.0001). High level of the marker was detected in separated solitary cancer cells in the invasive front-line of the tumor.

The absence or minimum quantity of collagen IV containing basal membranes was significantly more incident in metastatic CC (p=0.0001) and poorly differentiated tumors (p=0.035).

Comparative study of expression of β-catenin, MMP-9, collagen IV, and laminin in CC showed mutually related changes in the expression of these proteins in the tumors and their levels in zones of invasive front-line.

Cytoplasmic location of laminin in CC was associated with high level of MMP-9 expression in the cells in 67% (63 of 94) tumors. Poor reactivity or absence of basal membranes containing collagen IV was paralleled by high expression of MMP-9 in 42.2% (57 of 135) metastatic CC cases.

High level of MMP-9 expression in zones of CC invasive growth was associated with high accumulation of β-catenin in cancer cell nuclei in the peripheral zones in 30% (52 of 186) tumors (Table 1).

A characteristic feature of the invasive front-line of metastatic CC was accumulation of nuclear β-catenin and high cytoplasmic expression of MMP-9 and laminin in tumor cells, which were often paralleled by the absence of collagen IV-containing basal membranes in these zones.

Specific changes in accumulation of β-catenin molecules in the tumor invasive front-line proved to be the most reliable indicator of a high metastatic potential of CC. Coordinated changes in the levels of proteins (absence of collagen IV-containing basal membranes and cytoplasmic deposition of laminin in parallel with high expression of MMP-9 in the same tumors) characterized CC progress and high risk of remote metastases.

Several phenotypical characteristics related to activity, structure and function of molecular biological factors (β-catenin, MMP-9, collagen IV, and laminin), were intrinsic of zones of CC invasive front-line. These characteristics of protein accumulation in the tumor play an important role in CC progress and demonstrate the important role of markers in tumor cell migration and invasion. The presence of nuclear β-catenin, high content of MMP-9, abnormal accumulation of laminin in cancer cell cytoplasm, and the absence of collagen IV-containing basal membranes are characteristic of CC with a high invasive metastatic potential.

Hence, β-catenin, MMP-9, laminin, and collagen IV are important markers for predicting the clinical course of CC. The expression of proteins associated with the risk of metastases in the liver is coordinated and the most pronounced in zones of invasive front-line of the tumors.

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