Prognostic Significance of Expression of Matrix Metalloproteinase in Colorectal Adenocarcinomas and Their Metastases

V. V. Delektorskaya, A. G. Perevoshchikov, D. A. Golovkov, and N. E. Kushlinskii

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Expression of matrix metalloproteinases 2 and 9 was studied in primary tumors and their metastases in patients with colorectal cancer. The correlation of immunoreactivity with clinical morphological signs, prognosis of the disease, and development of metastases in the liver was analyzed. The level of expression and distribution of markers in patients with colorectal cancer with metastases in the liver differed from that in control patients without metastases. Enhanced expression of matrix metalloproteinases 2 and 9 was detected in colorectal cancer patients with distant metastases. Increased expression of matrix metalloproteinase 9 was associated significantly with low histological differentiation of the tumor, deeper tumor invasion, and was more often observed in tumors of colorectal cancer patients with unfavorable prognosis. Thus, matrix metalloproteinase 9 is a valuable marker for clinical observation and prognosis in patients with this location of the tumor process.

Key Words: *matrix metalloproteinases; colorectal cancer; metastasis; immunohistochemistry*

Colorectal cancer (CC) is one of the most incident malignant diseases of humans. The percent of advanced forms complicated by the development of distant metastases remains high. The study of the factors determining the invasive metastatic potential of the tumor is important for individual prediction of the disease course and treatment of CC metastases. Proteolytic destruction of the main extracellular matrix proteins is the central mechanism of tumor invasion and metastasizing [1,2,11]. Matrix metalloproteinases (MMP), specifically MMP-2 and MMP-9, essential for tumor process dissemination and belonging to the most perspective prognostic factors [4,9,10], are involved in this process. MMP-2 and MMP-9 play an important role in the development of invasive processes; they hydrolyze the main component of the basal membranes, type IV collagen [6,11]. Hyperexpression of MMP-2 and MMP-9 is often detected in human tumors and, as was found, correlates with poor prognosis [3,5, 7,8]. The effect of MMP-2 and MMP-9 on the clinical course of colorectal tumors and disease prognosis is little studied.

We analyzed the expression of MMP-2 and MMP-9 in the tissue of colorectal adenocarcinoma and its metastases in patients with different clinical and morphological parameters and prognosis.

MATERIALS AND METHODS

The study was carried out on postoperative material from 165 CC patients with different stages of tumor process. In 92 patients, synchronous or metachronous metastases in the liver (54 and 38 cases, respectively) were detected and removed (meta-

N. N. Blokhin National Cancer Research Center, Russian Academy of Medical Sciences, Moscow

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static group) and 73 patients (control group) had no distant metastases. Repeated metastases in the liver were studied in 6 cases and relapses of colorectal tumors in 12 cases. All tumors had histological structure of adenocarcinomas of various differentiation degrees, including well, moderately, and poorly differentiated variants. Immunohistochemical staining was carried out by the biotin-streptavidin immunoperoxidase method on paraffin sections of primary tumor tissue (n=165), metastases in the lymph nodes (n=45) and liver (n=98) using antibodies to MMP-2 (1:40 working dilution) and MMP-9 (1:80 working dilution) (Novocastra).

The results were evaluated by the semiquantitative method with consideration for staining intensity ("—" no reaction; "+" weak reaction; "++" moderate reaction; and "+++" intensive reaction), number of antigen-positive cells, and location of immunoreactivity in cancer cells and tumor stroma. The data were processed statistically using χ^2 test (the differences were considered significant at $p<0.05$).

RESULTS

The expression of MMP-2 and MMP-9 in normal colorectal cells was weakly positive. High expression of MMP-2 and MMP-9 was detected in colorectal adenocarcinomas at different stages of tumor process (in cancer cell cytoplasm and in the adjacent extracellular stroma). Accumulation of MMP-9 was detected in 50.3% colorectal adenocarcinomas and MMP-2 in 38.8% cases. Expression of MMP-9 in the tumor glandular structures (Fig. 1, *a*) was often more pronounced than expression of MMP-2, being particularly manifest in the cytoplasm of tumor cell in the invasive front-line, sometimes in the stromal cells around the invasive edge of the tumor. Cancer complexes at the periphery and individual tumor cells were more intensely stained with the marker than cells located in central areas. In the extracellular matrix the proteases were located in fibroblasts, macrophages, vascular walls, leukocytic elements; MMP-2 was detected in the tumor stroma in 52.1% cases and MMP-9 in 39.4% cases. Intensive immunoreactivity of the cytoplasm (++/+++) was more typical of poorly differentiated tumor cells, while reduced (-/+) or focal expression was more often observed in structures of moderately and well-differentiated adenocarcinomas. The differences in the levels of enzyme expression in tumors of different differentiation degree were significant only for MMP-9 (p=0.011; Table 1). Focal expression of proteases, as a rule in sites of low-differentiated cells, was observed in colorectal ade-

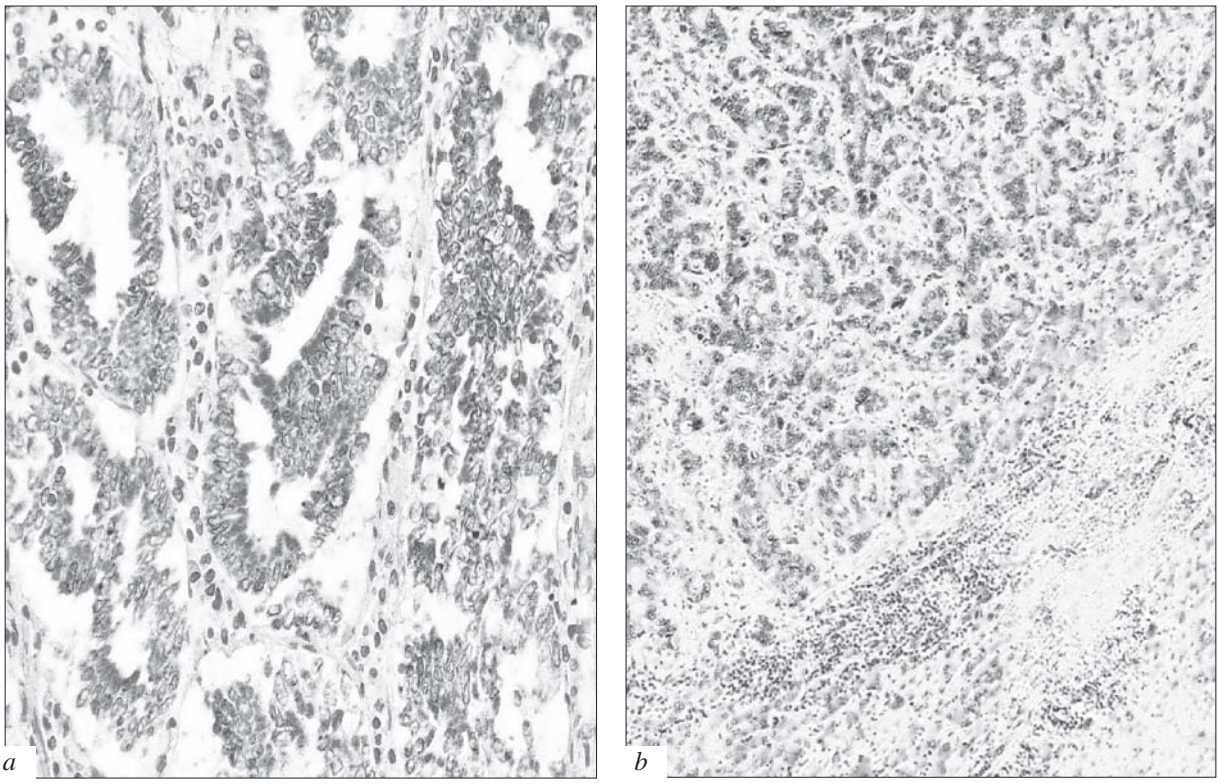

Fig. 1. Immunohistochemical study of primary tumors and metastases of colorectal adenocarcinomas. *a*) expression of MMP-9 in the cytoplasm of primary tumor cancer cells (×400); *b*) expression of MMP-9 in the cytoplasm of CC metastasis cells in the liver (×225).

TABLE 1. Analysis of MMP-2 and MMP-9 Expression in Colorectal Adenocarcinomas of Patients with Different Clinical and Morphological Parameters of the Disease

Sign		Expression in cancer cells					
		MMP-2			MMP-9		
		high (n=64)	low (n=101)	p	high (n=83)	low (n=82)	p
T stage							
	T1, T2	18	38		23	33	
	T3,T4	46	63	0.277	60	49	0.125
Differentiation degree							
	high/moderate	40	72		50	62	
	low	24	29	0.314	33	20	0.011*
Duke's stage	A/B	19	42		24	37	
	C/D	45	59	0.168	59	45	0.046*
Metastases in lymph nodes							
	+	37	47		46	38	
	—	27	54	0.210	37	44	0.312
Metastases in liver							
	+	43	49		57	35	
	—	21	52	0.028*	26	47	0.001*
Lymphoid infiltration							
	+	29	41		38	32	
	—	35	60	0.663	45	50	0.471
Invasion of vessels							
	+	35	46		45	36	
	—	29	55	0.325	38	46	0.242

Note. "+": detected; "—": not detected. *p<0.05.

nocarcinomas with pronounced mucus production. The intensity of staining of extracellular matrix components did not depend on tumor differentiation, but depended on the type of stroma and degree of desmoplastic reaction. In the group of patients with metastatic CC, the content of MMP in tumors significantly increased. High expression of MMP-2 and MMP-9 was detected in 46.7 and 61.9% tumors in CC patients with distant metastases *vs.* 28.8 and 35.6% in patients without metastases in the liver (p=0.028 and p=0.001, respectively). Positive expression of proteases in the tumors was more incident in CC patients with synchronous metastases in the liver (MMP-2: 27/54, 50%; MMP-9: 34/54, 63%) compared to the group with metachronous metastases (MMP-2: 16/38, 42.1%; MMP-9: 23/38, 60.5%).

The expression of proteases increased with the depth of invasion (disease stage according to Duke), the differences detected for MMP-9 being significant (p=0.046). The level of enzyme expression increased at higher stages of the disease (T3, T4 *vs.* T1, T2) and in patients with invasion into blood vessels and metastases in lymph nodes, but the differences did not reach the level of significance.

Analysis of enzymes accumulation in metastatic tumors showed high level of immunoreactivity in some CC metastases in the lymph nodes (MMP-2: 21/45, 46.7%; MMP-9: 26/45, 57.8%) and in the liver (MMP-2: 45/98, 45.9%; MMP-9: 62/98, 63.3%). The enzymes accumulated mainly in the cytoplasm of metastatic tumor cells (Fig. 1, *b*). Immunoreactivity in synchronous metastases in the liver was somewhat higher than in metachronous ones, for both MMP-2 (27/54, 50% and 18/38, 47.4%) and MMP-9 (25/38, 65.8% and 37/54, 68.5%, respectively). High level of immunoreactivity was detected in repeated metastases in the liver (MMP-9: 6/6, 100%; MMP-2: 3/6, 50%) and in relapsing colorectal tumors (MMP-9: 9/12, 75.0%; MMP-2: 5/12, 41.7%).

Changes in enzymes expression in metastases were similar to changes in the primary tumor, but accumulation of proteases in the extracellular matrix components was less pronounced.

TABLE 2. Survival of CC Patients and Expression of MMP-2 and MMP-9 in Colorectal Adenocarcinoma

Expression			CC patients (n=124)		p
			favorable prognosis (survival >5 years, n=54)	unfavorable prognosis (survival <5 years, n=70)	
MMP-2	in tumor cells	high	26	38	0.619
		reduced	28	32	
	in tumor stroma	positive	32	49	0.291
		negative	22	21	
MMP-9	in tumor cells	high	25	47	0.032*
		reduced	29	23	
	in tumor stroma	positive	22	33	0.597
		negative	32	37	

Note. *p<0.05.

Analysis of relationship between changes in the expression of MMP-2 and MMP-9 in the tumor and postoperative prognosis (5-year survival) in CC patients was based on the results of observation of 124 of the 165 patients included in the study (Table 2).

High enzyme expression in tumor cell cytoplasm and in tumor stroma was more often observed in CC patients with unfavorable prognosis (survival <5 years). However, only expression of MMP-9 in cancer cells significantly correlated with postoperative prognosis (p=0.032). High expression of this protease in tumor cells of CC patients with good prognosis (survival >5 years) was significantly more rare than in patients with unfavorable prognosis (25/54, 46.3%, and 47/70, 67.1%, respectively). The correlation between MMP-2 expression and disease prognosis was weaker.

MMP-2 and MMP-9 play an important role in the development and metastasizing of CC and are essential for invasive potential of cancer cells in primary tumors and metastases. The results indicate that high expression of MMP-9 is associated with possible progress of the tumor process and with low histological differentiation of the tumors, deeper invasion, unfavorable prognosis, and high risk of metastases in the liver. Hence, MMP-9 is a valuable marker for clinical observation and evaluation of the prognosis in patients with this location of tumor process.

REFERENCES

1. S. Curran and G. I. Murray, *Eur. J. Cancer*, **36**, No. 13 (Spec.), 1621-1630 (2000).
2. M. Egeblad and Z. Werb, *Nat. Rev. Cancer*, **2**, No. 3, 161-174 (2002).
3. M. Illemann, N. Bird, A. Majeed, *et al.*, *Mol. Cancer Res.*, **4**, No. 5, 293-302 (2006).
4. N. Johansson, M. Ahonen, and V. M. Kahari, *Cell. Mol. Life Sci.*, **57**, 5-15 (2000).
5. Y. Matsuyama, S. Takao, and T. Aikou, *J. Surg. Oncol.*, **80**, No. 2, 105-110 (2002).
6. O. R. Mook, W. M. Frederiks, and C. J. Van Noorden, *Biochim. Biophys. Acta*, **1705**, No. 2, 69-89 (2004).
7. Y. Nagakawa, T. Aoki, K. Kasuya, *et al.*, *Pancreas*, **24**, No. 2, 169-178 (2002).
8. S. Papandopoulou, A. Scorilas, N. Arnogianaki, *et al.*, *Tumour Biol.*, **22**, No. 6, 383-389 (2001).
9. T. Turpeenniemi-Hujanen, *Biochimie*, **87**, Nos. 3-4, 287-297 (2005).
10. S. O. Yoon, S. J. Park, C. H. Yun, and A. S. Chung, *J. Biochim. Mol. Biol.*, **36**, No. 1, 128-137 (2003).
11. S. Zucker and J. Vacirca, *Cancer Metastasis Rev.* **23**, No. 1-2, 101-117 (2004).