TÜBİTAK

Turkish Journal of Medical Sciences

http://journals.tubitak.gov.tr/medical/

Research Article

Turk J Med Sci
(2016) 46: 1168-1176

doi:10.3906/sag-1412-85

Proliferation and differentiation markers of colorectal adenocarcinoma and their correlation with clinicopathological factors

Olivera MITROVIĆ AJTIĆ[1,*], Slobodan TODOROVIĆ[2], Miloš DIKLIĆ[1], Tijana SUBOTIČKI[1], Bojana BELESLIN-ČOKIĆ[3], Gordana JOVČIĆ[1], Vladan ČOKIĆ[1]

[1]Institute for Medical Research, University of Belgrade, Belgrade, Serbia
[2]Department of Surgery, Medical Center Bezanijska kosa, Belgrade, Serbia
[3]Clinic of Endocrinology, Diabetes and Diseases of Metabolism, Clinical Center of Serbia, Belgrade, Serbia

Received: 17.12.2014 • **Accepted/Published Online:** 07.11.2015 • **Final Version:** 23.06.2016

Background/aim: The purpose of this study was to investigate proliferation and differentiation markers in colorectal adenocarcinoma and their correlation with clinicopathological factors.

Materials and methods: Samples were collected from 38 patients with colorectal adenocarcinoma and 10 healthy controls. E-cadherin, carcinoembryonic antigen (mCEA), cyclin B1, vascular endothelial growth factor (VEGF), and erythropoietin (EPO) receptor (EPOR) were examined by immunohistochemistry; VEGF and EPO were examined by real-time PCR.

Results: The tumor samples were mostly characterized by large dimension (pT3), moderate level of differentiation (G2), negative lymph node status (N0), and no metastasis. Cyclin B1 and VEGF gene and protein expressions were significantly higher in tumor tissues than in control tissues; E-cadherin expression was significantly decreased in tumor samples and in positive correlation with mCEA. EPO was almost undetectable in tumor tissues of colorectal adenocarcinoma. Significant positive correlation was detected between tumor size and cyclin B1, tumor grade, and lymph node status.

Conclusion: Decreased expression of EPO, high levels of VEGF and cyclin B1 expression, predominant moderate tumor differentiation, absence of metastasis, and negative lymph node status may suggest low level of aggressiveness, better prognosis, and longer patient survival.

Key words: Adenocarcinoma of colon, E-cadherin, mCEA, cyclin B1, erythropoietin receptor, vascular endothelial growth factor, erythropoietin

1. Introduction

Colorectal cancer is the second most prevalent malignancy in Europe (1). In 2013 in Serbia the estimated new cases of colorectal cancer were 3900, making it the second most frequent malignant tumor in the country (2). The expression of E-cadherin, carcinoembryonic antigen (mCEA), cyclin B1, erythropoietin (EPO), EPO receptor (EPOR), and vascular endothelial growth factor (VEGF) indicate the presence, extent, and response to therapy of this neoplasm and define the survival period (3,4). mCEA is one of the most extensively studied tumor markers in colon cancers. During the early stage of colorectal cancer mCEA has a low sensitivity and expression; it becomes more prominent during the process of tumor development (4). E-cadherin belongs to a family of transmembrane glycoproteins involved in the mediation of calcium-dependent cell-cell adhesion in epithelial tissues (5). Previous studies have shown a fundamental association between downregulation of E-cadherin expression in tumor cells and the progression of an invasive tumor (6). According to previous results, there is a negative correlation between the level of E-cadherin expression and tumor grade (7–9).

EPOR is highly expressed in endothelial cells, neurons, the uterus, and various solid tumors (10). EPO and EPOR have a significant role in the process of neoangiogenesis, stimulating survival of hypoxic cancer cells and tumor growth (11). It has been shown that EPO and EPOR expressions are more prominent in adenocarcinoma of the colon than in mucinous carcinomas; they are also more prominent in moderately differentiated carcinomas than in poorly differentiated ones (12,13). Besides EPO and EPOR, VEGF is considered one of the most important angiogenic agents overexpressed in colon adenocarcinoma

* Correspondence: oliveram@imi.bg.ac.rs

(14). In addition to tumor tissues, VEGF is expressed in noncancerous colonic tissue (14,15).

Cyclin B1 belongs to the cyclin family of proteins involved in the control of normal cell proliferation (16). Cyclin B1 is the component of the maturation/mitosis-promoting factor with an important role in the G_2–M transition (16,17). Cyclin B1 expression is irregular with minimal expression in the G_1 phase, and maximal at the G_2–M transition, which results in early entry into mitosis, uncontrolled cell proliferation, and tumor growth (16,17). Previous studies reported that cyclin B1 was detected in breast, prostate, colon, and oral cancers; high levels of its expression are associated with poor survival of patients (18,19).

The aim of our study was to investigate the immunohistochemical expression of E-cadherin, mCEA, cyclin B1, VEGF, EPO, and EPOR in adenocarcinoma of the colon and their correlation with basic clinicopathological characteristics. In addition to protein studies of angiogenic factors EPOR and VEGF, we also performed their gene expression studies concomitantly in cancer and neighboring healthy tissues. Using this approach, we wanted to shed light on the proliferation, differentiation, and angiogenesis markers in adenocarcinoma of the colon as potential diagnostic and prognostic factors.

2. Materials and methods

2.1. Subjects

The present study was performed in accordance with the regulations of the local ethics committee. We included 38 patients with adenocarcinoma of the colon who underwent surgery. We also included 10 controls for immunohistochemistry and 38 control samples for RT-PCR.

2.2. Immunohistochemical procedure

The examined tumor tissue specimens were fixed in 10% buffered (PBS) formalin and embedded in paraffin. Tissue blocks of the tumor (five tissue sections of each paraffin block) were transversely cut in 5-μm-thick serial sections. Five consecutive sections from each level, taken at intervals of 20–50 μm, were stained with different antibodies. For immunohistochemical analysis, polyclonal and monoclonal antibodies against mCEA (monoclonal mouse anti-human antibody, Novocastra, Leica Biosystems Ltd, Clone: 12–140-10), E-cadherin (monoclonal mouse anti-human antibody, Dako, LOT NO: 00023), cyclin B1 (monoclonal mouse anti-human antibody, Dako, LOT NO: 098), EPOR (rabbit polyclonal anti-mouse antibody, Santa Cruz Biotechnology, LOT NO: D1210), VEGF (BD Pharmingen, cat. no. 555036, dilution 1:1000) were used. The tissue samples were fixed in 10% buffered formalin solution and embedded in paraffin. The tissue sections were cut at 5 μm, heated at 56 °C for 60 min, then deparaffinized and rehydrated through a series of xylenes and alcohols, followed by an epitope retrieval step. Tissue sections were treated with 3% H_2O_2 solution in PBS to block endogenous peroxidase activity. Then tissue sections of colon cancer and control tissues were heated in a microwave oven (at 680 W, in 10 mmol/L citrate buffer with a pH of 6.0 for 21 min) for epitope retrieval. The next step was incubation of tissue sections with appropriate antibodies (against mCEA, E-cadherin, cyclin B1, VEGF, and EPOR) in a humid chamber for 60 min at room temperature. Immunostaining was performed using the streptavidin–biotin technique (LSAB+/HRP Kit, Peroxidase Labeling, K0690, DakoCytomation). The immunoreactivity complex was visualized with Dako Liquid DAB $^+$ Substrate/Chromogen System (Code No. K 3468) and counterstained with Mayer's hematoxylin (Merck). Tumor tissue sections with omitted primary antibody were used as negative controls. Tumor tissue sections known to express mCEA, E-cadherin, cyclin B1, and EPOR were used as positive controls for immunohistochemical staining.

2.3. Scoring system

EPOR and mCEA expressions of 0 and 2 grades and E-cadherin expression of 0 and 1 grades were considered negative expression, while in other cases the expression of those three markers was considered positive. Tissue samples for EPOR, mCEA, and E-cadherin were scored on a scale representing the estimated proportion and intensity of positive-staining tumor cells (with a range of 0–6). The scores for EPOR, mCEA, and E-cadherin were determined by adding intensity for the immunoreactivity staining of tumor cells (0, none; 1, weak; 2, intermediate; and 3, strong) and the average number of staining tumor cells. The number of cyclin B1 immunoreactive cells was determined in the surface and glandular epithelium using a computer-supported imaging system connected to a light microscope (Olympus AX70) with objective magnification of 40×. The intensity of the cyclin B1 immunostaining was evaluated by dividing staining reactions in three score groups: 0: no immunostaining cells; 1: weak to moderate staining intensity in 1% to 50% of tumor cells; 2: strong staining intensity in >50% of tumor cells. Immunoreactivity for VEGF expression was evaluated according to the degree of staining, with staining intensities scored as: 0, no positive staining cells; 1, less than 10% of cells stained; 2, 11% to 50% of cells stained; 3, more than 50% of cells stained. The group of patients with positive VEGF expression was defined by observation of staining of more than 10% of the tumor area (scores 2 and 3).

2.4. Real-time PCR

We used the RNeasy protocol for the isolation of total RNA from 38 tumors and 38 healthy tissues according to the manufacturer's instructions (Qiagen GmbH).

Concentration and integrity of total RNA was assessed using Ultrospec 3300 spectrophotometer (Amersham Pharmacia). Equal amounts of RNA from different samples were transcribed into cDNA using the Maxima First Strand cDNA Synthesis kit (Thermo Fisher Scientific) according to the manufacturer's instructions. Quantitative real-time PCR analyses were performed using a 4800 Light Cycler (Roche) and Taqman oligonucleotides probes for VEGF. The oligonucleotides probe 5' CCA AGT GGT CCC AGG CTG CAC C 3' was fluorescently labeled on the 5' end with 6-carboxy-fluorescein and on the 3` end with 6-carboxy-tetramethyl-rhodamine. VEGF primers were: forward 5' TTG CTG CTC TAC CTC CAC CAT 3' and reverse 5' CAC TTC GTG ATG ATT CTG CCC 3'. PCR reaction conditions consisted of 50 °C for 2 min and 95 °C for 4 min, followed by cycling between a melting temperature of 95 °C for 15 s and an anneal-extension temperature of 60 °C for 1 min, repeated for 40 cycles. Quantification of the corresponding mRNA transcript was determined by comparison with VEGF specific cDNA standards. β-Actin was used as an internal control for the total amount of RNA analyzed.

2.5. Statistical analysis

The results are expressed as means ± standard deviation (SD). Values were compared using independent samples t-tests with SPSS 19 for Windows. $P < 0.05$ was considered significant.

3. Results

3.1. Clinicopathological parameters and correlation with biological markers

The ages of patients ranged from 54 to 86 years (mean ± SD, 72.8 ± 8 years). The tumor size ranged between 1.5 and 10 cm, and the histological grades were G1, G2, and G3 in 18.4% (7/38), 76.3% (29/38), and 5.3% (2/38) of patients, respectively. In 23 patients (60.6%) lymph node status was negative, while positive lymph node status was detected in 15 patients (39.4%). Distant metastasis was found in only 1 (2.6%) patient (Table 1). Statistical analyses showed a significant positive correlation between tumor size and positive lymph node status ($r = 0.483$, $P < 0.01$), and between tumor size and histological tumor grade ($r = 0.342$, $P < 0.05$, Table 2). Significant positive correlation was also found between tumor size and cyclin B1 tumor expression ($r = 0.414$, $P < 0.01$, Table 2) and between E-cadherin and mCEA expression ($r = 0.409$, $P < 0.05$, Table 2). Significant negative correlation was detected between VEGF protein expression and lymph node status ($r = -0.329$, $P < 0.05$, Table 2).

3.2. E-cadherin, mCEA, and cyclin B1 immunoexpression

Positive E-cadherin protein expression in tumors was detected in 30 patients (78.9%) and in all 10 controls (10/10, 100%, Table 3). In our study group the highest level of E-cadherin expression, with scores of 5 and 6, was detected in 17 patients (17/38, 44.7%, Table 3); moderate level of expression (4) was found in 9 (9/38, 23.7%) patients; and low level of expression (3) was detected in 4 patients (4/38, 10.5%, Table 3). Statistical analysis showed significant differences in E-cadherin expression between tumor and control samples ($F = 4.257$, $P < 0.05$, Figure 1). High level of positive expression (5 and 6) for mCEA was detected in 29 patients (29/38, 76.3%), while minimum level of positive expression was detected in 3 patients

Table 1. Clinicopathological characteristic of adenocarcinoma of the colon.

Clinicopathological parameters	Adenocarcinoma of the colon
Tumor size	
pTx	0
pT0	0
pT1	4 (10.5%)
pT2	6 (15.8%)
pT3	26 (68.4%)
pT4	2 (5.3%)
Unknown	0
Histological grade	
G1	7 (18.4%)
G2	29 (76.3%)
G3	2 (5.3%)
Unknown	0
Lymph node status	
Nx	0
N0	23 (60.6%)
N1	11 (28.9%)
N2	4 (10.5%)
N3	0
Unknown	0
Metastasis	
Mx	0
M0	37 (97.4%)
M1	1 (2.6%)
Unknown	0

Table 2. Correlation between E-cadherin, mCEA, EPOR, cyclin B1, VEGF gene and VEGF protein expressions, and clinicopathological characteristics.

	E-cadherin	mCEA	Cyclin B1	EPOR	VEGF gene	VEGF IHC	Tumor size	Lymph node status	Grade	Metastasis	Age
E-cadherin	1	**0.409***	–0.195	–0.062	–0.112	–0.118	–0.078	–0.203	0.164	–0.038	–0.184
mCEA	**0.409***	1	–0.115	0.041	–0.071	–0.231	0.000	0.000	0.111	–0.211	0.049
Cyclin B1	–0.195	–0.115	1	–0.034	0.054	–0.071	**0.414****	0.191	0.275	0.247	–0.048
EPOR	–0.062	0.041	–0.034	1	–0.164	–0.195	–0.107	–0.056	–0.291	0.077	0.201
VEGF gene	–0.112	–0.071	0.054	–0.164	1	–0.245	–0.245	–0.079	0.019	0.142	0.003
VEGF IHC	–0.118	–0.231	–0.071	–0.195	–0.245	1	–0.180	**–0.33***	–0.085	0.108	–0.081
Tumor size	–0.078	0.000	**0.414****	–0.107	–0.245	–0.180	1	**0.483****	**0.342***	0.098	–0.053
Lymph node status	–0.203	0.000	0.191	–0.056	–0.079	**–0.33***	**0.483****	1	0.112	0.204	0.015
Grade	0.164	0.111	0.275	–0.291	0.019	–0.085	**0.342***	0.112	1	0.046	0.034
Metastases	–0.038	–0.211	0.247	0.077	0.142	0.108	0.098	0.204	0.046	1	0.119
Age	–0.184	0.049	–0.048	0.201	0.003	–0.081	–0.053	0.015	0.034	0.119	1

Values in bold are significant: * $P \leq 0.05$; ** $P \leq 0.01$.

(3/38, 7.9%, Table 3, Figure 2). Moderate level (4) of mCEA expression was detected in 6 patients (6/38, 15.8%, Table 3). Statistical analysis did not show that mCEA expression was significantly higher in tumor samples compared with control patients (Figure 1). Positive expression of cyclin B1 in more than 50% of tumor cells was detected only in 6 tumor samples (6/38, 15.8%), while in 32 patients (32/38, 84.2%) the number of cyclin B1 positive cells was less than 50% (Table 3). In all 10 control cases the average number of cyclin B1 positive cells was less than 30% (Table 3). Statistical analysis showed significant differences in cyclin B1 nucleus accumulation between tumor and control tissues ($F = 7.716$, $P < 0.05$, Figure 1). Namely, cyclin B1 expression was significantly higher in tumor than in control samples (Figures 1 and 2).

3.3. VEGF and EPOR gene and protein expressions in adenocarcinoma of the colon

Negative expression of EPOR was detected in 26 tumor samples (26/38, 68.4%), while maximal positive expression, scored as 6, was detected in 4 patients (4/38, 10.5%, Table 3). In control samples positive EPOR expression was detected in 7 samples (7/10, 70%, Table 3). Similar to the results for cyclin B1, statistical analysis showed significant differences in EPOR expression between tumor and control patients ($P < 0.05$, Figures 1 and 2). VEGF positive protein expression was detected in 35 tumor tissue samples (35/38, 92.1%, Table 3). Eight control samples were negative for VEGF protein expression and had a score of 1 (8/10, 80%, Table 3). The VEGF protein levels were significantly higher in tumor samples than in control samples (Figure 2). Statistical analysis showed that VEGF gene expression was significantly higher in tumor than in control samples (Figure 3). In contrast, EPO and EPOR genes were expressed only in traces in tumor and control samples (not shown).

4. Discussion

The presented findings showed that cyclin B1, EPOR, and VEGF gene and protein expressions were significantly higher in tumor than in control samples, while E-cadherin expression was significantly decreased in tumor samples, accompanied by positive correlation with mCEA. Significant positive correlation characterized the relationship between tumor size and cyclin B1 and that between tumor grade and lymph node status. Positive correlation was also observed between E-cadherin and mCEA expression. Cyclin B1 and VEGF gene and protein expressions were significantly increased in tumor samples in comparison with their expressions in control patients.

The main role of E-cadherin in the process of normal tissue development was verified with the appearance of lethality in E-cadherin gene knockout mice at an early stage during embryogenesis (20). Dysregulation of E-cadherin expression was essential in the development of neoplastic tissue, normal growth, loss of differentiation, and increased cell proliferation related to invasive behavior (6). It was shown that invasive behavior and the appearance of metastasis in breast and bladder cancer

Table 3. E-cadherin, mCEA, cyclin B1, VEGF, and EPOR expression in tumors and controls.

	E-cadherin expression in tumors		E-cadherin expression in controls	
0	5 (13.2%)		0	
2	3 (7.9%)		0	
3	4 (10.5%)		1 (10%)	
4	9 (23.7%)		0	
5	6 (15.8%)		3 (30%)	
6	11 (28.9%)		6 (60%)	
E-cadherin (tumors)		–	8/38 (21.1%)	
		+	30/38 (78.9%)	
E-cadherin (controls)		–	0/5	
		+	10/10 (100%)	
	mCEA expression in tumors		mCEA expression in controls	
0	0		0	
2	0		0	
3	3 (7.9%)		0	
4	6 (15.8%)		4 (40%)	
5	10 (26.3%)		4 (40%)	
6	19 (50%)		2 (20%)	
mCEA (tumors)		–	0	
		+	38/38 (100%)	
mCEA (controls)		–	0/10	
		+	10/10 (100%)	
	EPOR expression in tumors		EPOR expression in controls	
0	16 (42.2%)		0	
2	10 (26.3%)		3 (30%)	
3	4 (10.5%)		2 (20%)	
4	3 (7.9%)		0	
5	1 (2.6%)		2 (20%)	
6	4 (10.5%)		3 (30%)	
EPOR (tumors)		–	26/38 (68.4%)	
		+	12/38 (31.6%)	
EPOR (controls)		–	3/10 (30%)	
		+	7/10 (70%)	
	Cyclin B1 expression in tumors		Cyclin B1 expression in controls	
≤50%	32 (84.2%)		10 (100%)	
>50%	6 (15.8%)		0	
	VEGF expression in tumors		VEGF expression in controls	
0	0		0	
1	3 (7.9%)		8/10 (80%)	
2	20 (52.6%)		2/10 (80%)	
3	15 (39.5%)		0	

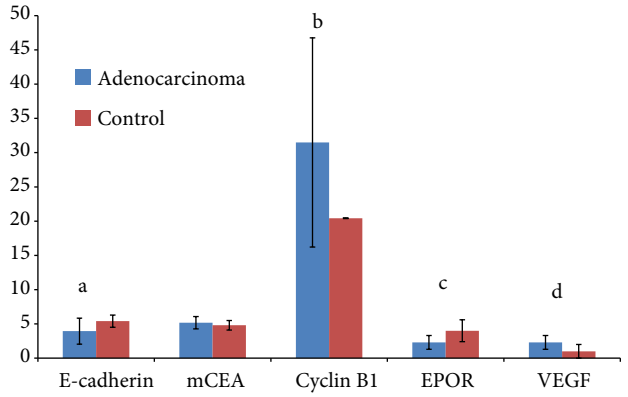

Figure 1. Immunohistochemical expression of E-cadherin, mCEA, cyclin B1, VEGF, and EPOR in colorectal adenocarcinoma and controls. Control vs. adenocarcinoma of the colon: a) E-cadherin ($P < 0.05$); b) Cyclin B1 ($P < 0.05$); c) EPOR ($P < 0.05$); d) VEGF ($P < 0.01$).

were associated with decreased expression of E-cadherin (21,22). Our results are in agreement with these studies because E-cadherin levels were significantly higher in healthy controls than in tumor samples.

The mCEA levels in the serum of patients with colorectal, gastric, pancreatic, lung, and breast carcinoma were increased compared with those in the serum of healthy controls (23). In the same study it was confirmed that increased levels of serum mCEA showed 100% sensitivity in differentiating metastatic liver disease from colorectal cancer. Our results also demonstrated that mCEA expression was higher in tumor samples than in control ones. Moreover, serum concentrations of mCEA were higher in patients with well-differentiated tumors than in patients with poorly differentiated colon cancers (24). Our results showed a positive, but not significant, correlation between mCEA and tumor grade.

During the process of tumor growth, the lack of blood supply created a hypoxic environment that induced the release of hypoxia inducible factor-1 (HIF-1) to stimulate angiogenesis by activating VEGF expression (25,26). Two studies confirmed that high levels of VEGF expression indicated poor prognosis in patients with colorectal cancer, invasive tumor behavior with increased rates of vascular-lymphatic and lymph node metastasis, and increased incidence of distant metastasis (27,28). The results of the present study also confirmed significantly higher levels of VEGF gene and protein expressions in tumor samples of colon adenocarcinoma than in control samples.

EPO and EPOR are expressed in numerous normal nonhematopoietic cells as well as in cancer tissues, especially at the surface of endothelial cells and various solid tumors (10,12). EPO, EPOR, and VEGF play a key role in the process of neoangiogenesis, supporting survival of hypoxic cancer cells and tumor growth. Lee et al. (29) showed that higher expression of VEGF and EPO were associated with resistance to treatment, increased tumor aggressiveness, and poor prognosis in patients with cancer. In our study EPOR protein expression was significantly higher in tumor samples than in control ones, but EPO gene expression was found only in traces. This could be related to the small invasiveness of the tumor and the absence of a hypoxic state.

Despite significant progress, the role of E-cadherin, mCEA, cyclin B1, and VEGF in colorectal cancer cells remains unclear, especially in association with EPO and EPOR expression. This is the first immunohistochemical study to report E-cadherin, mCEA, cyclin B1, VEGF, and EPOR expressions and their correlations with clinicopathological factors in colorectal adenocarcinoma. The present findings showed that cyclin B1 and VEGF expressions were significantly elevated in tumor samples, and E-cadherin expression was significantly decreased in tumor samples and positively correlated with mCEA. A significant positive correlation was found between cyclin B1 and tumor size, tumor size and grade, and tumor size and lymph node status. Since our study was carried out for 1 year, we could not reach a conclusion about the prognostic significance of the studied markers. However, decreased expression of EPO and EPOR, increased VEGF and cyclin B1 expression, and predominant moderate differentiation of the tumor could suggest a low level of aggressiveness, good prognosis, and long period of patient survival. Observation of the same patient population for an extended period could give valuable information about the relationship between these factors and survival.

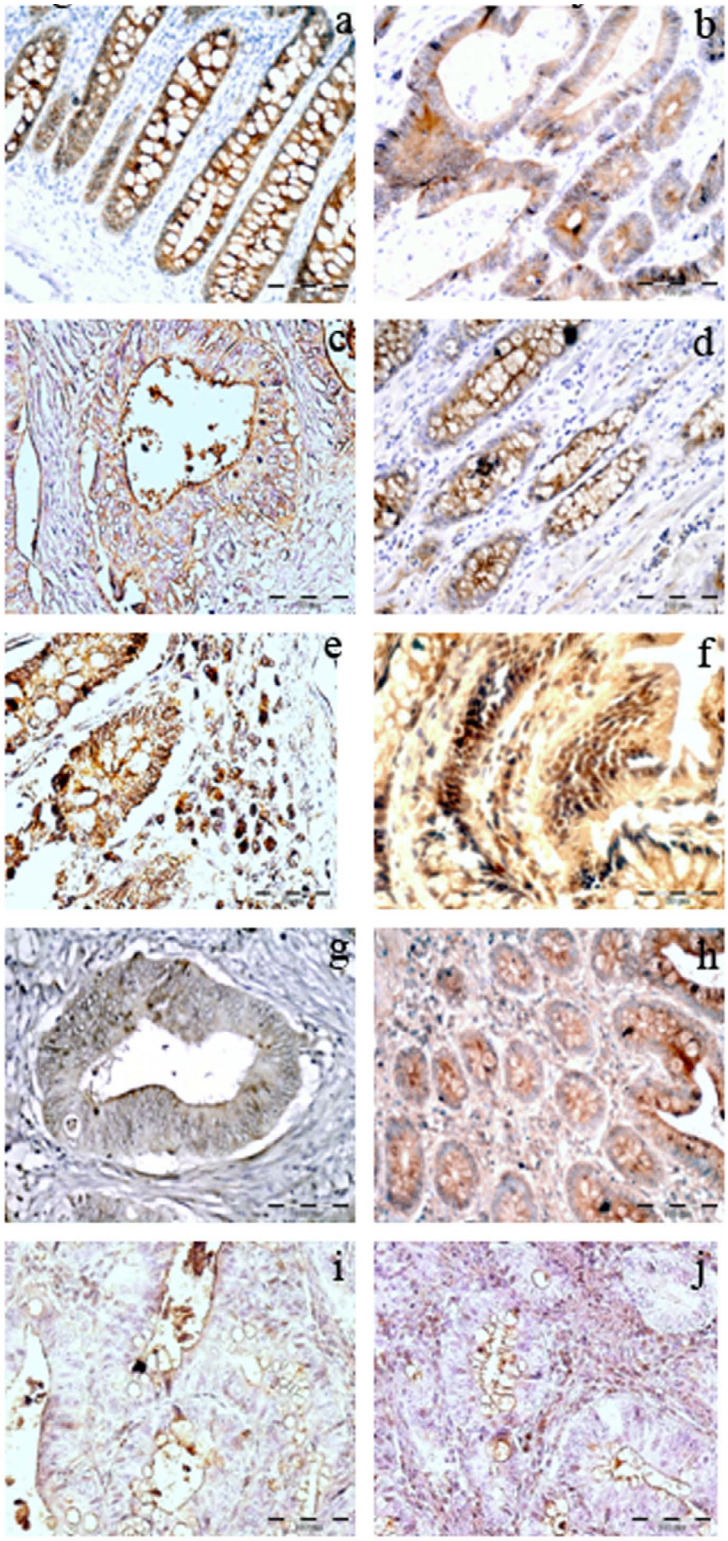

Figure 2. Specific pattern of immunohistochemical expression of E-cadherin, mCEA, cyclin B1, VEGF, and EPOR in colorectal adenocarcinoma and controls. Note the strong membranous deposition of E-cadherin in control cells (a) in contrast to colorectal cancer cells (b). mCEA showed strong immunoreactivity in colorectal cancer cells (d) in comparison with control cells (c). Cyclin B1 expressed significantly higher in colorectal cancer cells (f) in comparison with control cells (e). EPOR showed moderate membranous and partly perinuclear staining in colorectal cancer cells (h) in comparison with its decreased expression in control cells (g). VEGF immunoreactivity was significantly higher in tumor cells (j) in comparison with control tissue sections (i). Magnification: 20× (a, b, c, d, g, h, i, and j); 40× (e and f).

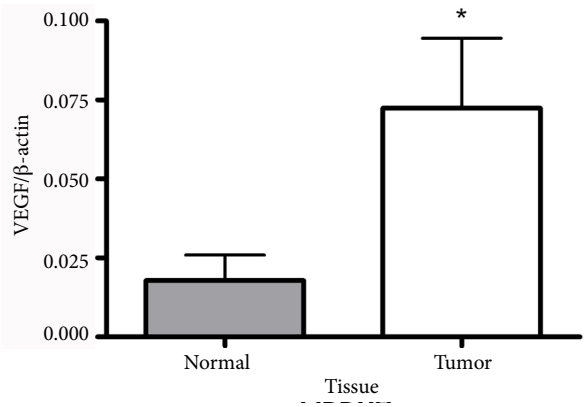

Figure 3. VEGF gene expression in colorectal adenocarcinoma tissue samples and neighboring healthy tissues (controls). Tumor samples vs. controls: * $P < 0.05$.

Acknowledgment

This work was supported by a grant from the Serbian Ministry of Education, Science, and Technological Development, project no. 175053.

References

1. Zavoral M, Suchanek S, Zavada F, Dusek L, Muzik J, Seifert B, Fric P. Colorectal cancer screening in Europe. World J Gastroentero 2009; 15: 5907-5915.
2. Antić V. Colorectal cancer incidence variation in Serbian districts. Med J (Krag) 2014; 48: 48-53.
3. Vrabie CD, Ceauşu M, Petrescu A, Waller M, Dina I. The usefulness of immunohistochemistry in sporadic colorectal cancer. Rom J Morphol Embryo 2008; 49: 525-535.
4. Fernandes LC, Matos D. Marcadores tumorais no câncer colorretal. Rev Col Bras Cir 2002; 29: 106-111 (in Portuguese).
5. Dorudi S, Sheffield JR, Poulsom R, Northover JM, Hart IR. E-cadherin expression in colorectal cancer. An immunocytochemical and in situ hybridization study. Am J Pathol 1993; 142: 981-986.
6. Takeichi M. Cadherin cell adhesion receptors as a morphogenetic regulator. Science 1991; 251: 1451-1455.
7. Frixen UH, Behrens J, Sachs M, Eberle G, Voss B, Warda A, Löchner D, Birchmeier W. E-cadherin mediated cell-cell adhesion prevents invasiveness of human carcinoma cells. J Cell Biol 1991; 113: 173-185.
8. Pignatelli M, Ansari TW, Gunter P, Liu D, Hirano S, Takeichi M, Klöppel G, Lemoine NR. Loss of membranous E-cadherin expression in pancreatic cancer: correlation with lymph node metastasis, high grade, and advanced stage. J Pathol 1994; 174: 243-248.
9. Schipper JH, Frixen UH, Behrens, Unger A, Jahnke K, Birchmeier W. E-cadherin expression in squamous cell carcinomas of the head and neck: inverse correlation with tumor dedifferentiation and lymph node metastasis. Cancer Res 1991; 51: 6328-6337.
10. Farrell F, Lee A. The erythropoietin receptor and its expression in tumor cells and other tissues. Oncologist 2004; 9: 18-30.
11. Acs G, Acs P, Beckwith SM, Pitts RL, Clements E, Wong K, Verma A. Erythropoietin and erythropoietin receptor expression in human cancer. Cancer Res 2001; 61: 3561-3565.
12. Hardee ME, Cao Y, Fu P, Jiang X, Zhao Y, Rabbani ZN, Vujaskovic Z, Dewhirst MW, Arcasoy MO. Erythropoietin blockade inhibits the induction of tumor angiogenesis and progression. PLoS ONE 2007; 2: e549.
13. Chabowska AM, Sulkowska M, Chabowski A, Wincewicz A, Koda M, Sulkowski S. Erythropoietin and erythropoietin receptor in colorectal cancer. Int J Surg Pathol 2008; 16: 269.
14. Hashim AF, Al-Janabi AA, Mahdi LH, Al-Toriahi KM, Yasseen AA. Vascular endothelial growth factor (VEGF) receptor expression correlates with histologic grade and stage of colorectal cancer. Libyan J Med 2010; 5: 5059-5062.
15. Galizia G, Lieto E, Ferraraccio F, Orditura M, De Vita F, Castellano P, Imperatore V, Romano C, Ciardiello F, Agostini B et al. Determination of molecular marker expression can predict clinical outcome in colon carcinomas. Clin Cancer Res 2004; 10: 3490-3499.

16. Grabsch H, Lickvers K, Hansen O, Takeno S, Willers R, Stock W, Gabbert HE, Mueller W. Prognostic value of cyclin B1 protein expression in colorectal cancer. Am J Clin Pathol 2004; 122: 511-516.

17. Nurse P. Universal control mechanism regulating onset of M phase. Nature 1990; 344: 503-508.

18. Keyomarsi K, Pardee AB. Redundant cyclin overexpression and gene amplification in breast cancer cells. P Natl Acad Sci USA 1993; 90: 1112-1116.

19. Paterlini P, Suberville AM, Zindy F, Melle J, Sonnier M, Marie JP, Dreyfus F, Bréchot C. Cyclin A expression in human hematological malignancies: a new marker of cell proliferation. Cancer Res 1993; 53: 235-238.

20. Syrigos KN, Karayiannakis AJ, Charalabopoulos K, Katirtzoglou N, Pignatelli M. Abnormal expression of E-cadherin in dysplastic Barrett's oesophagus. Annals of Gastroenterology 2000; 13: 92-108.

21. Mialhe A, Louis J, Montlevier S, Peoch M, Pasquier D, Bosson JL, Rambeaud JJ, Seigneurin D. Expression of E-cadherin and alpha-, beta- and gamma-catenins in human bladder carcinomas: are they good prognostic factors? Invas Metast 1997; 17: 124-137.

22. Moll R, Mitze M, Frixen UH, Birchmeier W. Differential loss of E-cadherin expression in infiltrating ductal and lobular breast carcinomas. Am J Pathol 1993; 143: 1731-1742.

23. Duffy M. Carcinoembryonic antigen as a marker for colorectal cancer: is it clinically useful? Clin Chem 2001; 47: 624-630.

24. Goslin R, O'Brien MJ, Steele G, Mayer R, Wilson R, Corson JM, Zamcheck N. Correlation of plasma CEA and CEA tissue staining in poorly differentiated colorectal cancer. Am J Med 1981; 71: 246-253.

25. Ferrara N. VEGF and the quest for tumor angiogenesis factors. Nat Rev Cancer 2002; 2: 795-803.

26. Yodavudh S, Tangjitgamol S, Puangsa-art S. Prognostic significance of microvessel density and mast cell density for the survival of Thai patients with primary colorectal cancer. J Med Assoc Thailand 2008; 91: 723-732.

27. Wang Y, Yao X, Ge J, Hu F, Zhao Y. Can vascular endothelial growth factor and microvessel density be used as prognostic biomarkers for colorectal cancer? A systematic review and meta-analysis. Sci World J 2014; Article ID 102736.

28. Cao D, Hou M, Guan YS, Jiang M, Yang Y, H Gou. Expression of HIF-1alpha and VEGF in colorectal cancer: association with clinical outcomes and prognostic implications. BMC Cancer 2009; 9: 432.

29. Lee WY, Huang SC, Hsu KF, Tzeng CC, Shen WL. Roles for hypoxia-regulated genes during cervical carcinogenesis: somatic evolution during the hypoxia-glycolysis-acidosis sequence. Gynecol Oncol 2008; 108: 377-384.