FOLIA HISTOCHEMICA ET CYTOBIOLOGICA
Vol. 47, No. 4, 2009
pp. 621-625

Serum sICAM, sVCAM and sE-selectin levels in colorectal cancer patients

M. Mantur[1], J. Snarska[2], O. Koper[1], J. Dzięcioł[3], A. Płonski[4], D. Lemancewicz[3]

[1]Department of Laboratory Diagnostics, Medical University, Bialystok, Poland
[2]Department of General and Endocrinological Surgery, Medical University, Bialystok, Poland
[3]Department of Human Anatomy, Medical University, Bialystok, Poland
[4]Department of Vascular Surgery and Transplantation, Medical University, Bialystok, Poland

Abstract: Colorectal cancer (CRC) is one of the most common cancers of the gastrointestinal tract and the fourth cause of cancers death in the world. Soluble adhesion molecules (CAMs) are thought to have an important role in host defense against carcinogenesis. They are biomarkers of inflammation and indicators of the immune response to tumors. The study included 40 CRC patients without remote metastases and 24 control subjects. Serum concentrations of sE-selectin, sICAM and sVCAM in patients with CRC were investigated by ELISA method. The level of the sCAMs decreased significantly after radical tumor resection. Preoperative serum concentrations of sICAM and sVCAM in CRC patients were significantly higher compared to the control group, whereas there were no differences regarding serum sE-selectin. Serum levels of sE-selectin, sICAM and sVCAM correlated significantly with each other. There was a significant correlation of serum levels of sICAM-1 and sVCAM-1, but not sE-selectin, with TNM stage and lymph node involvement. No significant relationship was found between serum concentrations of sICAM-1, sVCAM-1 and sE-selectin in CRC patients and patients' age or gender. Our findings suggest that an improved understanding of the mechanisms of membrane shedding of sICAM, sVCAM and sE-selectin is required to delineate their role in tumor progression.

Key words: sICAM-1, soluble intracellular adhesion molecule-1, sVCAM-1, soluble vascular cell adhesion molecule-1, sE-Selectin, soluble E-Selectin, CRC, colorectal cancer

Introduction

Intracellular adhesion molecules 1 (ICAM-1) and vascular cell adhesion molecules-1 (VCAM-1) are members of the immunoglobulin superfamily. They both participate in adhesion between cells. Soluble forms of ICAM (sICAM) and VCAM (sVCAM) have been previously identified [1]. Elevated serum levels of sICAM, sVCAM and sE-Selectin have recently been described in patients with cancers [2-8]. Several studies suggest that cell adhesion molecules (CAMs) play an important role in cancer progression and metastases [3,9,10]. Guzinska-Ustymowicz and Kemona [11] indicated that evaluation of TGF beta expression of protein in association with histological parameters can be used as a parameter of the aggressiveness of pT1 CRC. Okulczyk *et al*. indicated no significant correlations between the mutations of codon 12 K- RAS gene and clinical features. Also no correlation was observed with either Dukes or TNM clinical advancement [12]

Metastatic spread of cancer cells is a key event in tumor progression and in determining the prognosis of patients with malignant disease. Malignant cells detached from the primary tumor penetrate into blood or lymph vessels, survive in the circulation, and are then arrested in the capillary endothelium of distant organs, extravasate and grow as a secondary lesion [13]. Therefore, sICAM-1, sVCAM-1 and sE-selectin interactions between endothelial and cancer cells seem to be crucial for the successful development of metastases. These interactions are modulated by a specific cell surface receptor including selectins, integrins and immmunoglobulin like family of cell adhesion molecules [1].

It is now increasingly apparent that certain members of these families of CAMs are involved in tumor

Correspondence: M. Maria, Dept. of Laboratory Diagnostics, Medical University of Bialystok, Waszyngtona Str. 15a, 15-274 Białystok, Poland; tel./fax.: (+4885) 7468584, e- mail: mantur@umwb.edu.pl

Folia Histochem Cytobiol. 2009:47(4): 621 (621-625)
10.2478/v10042-009-0077-0

progression. Angiogenesis is mediated by the soluble forms of sE-selectin and sVCAM-1 [14]. E-selectin, also known as endothelial leukocyte adhesion molecule-1, is expressed on endothelial cells and may bind cells that express specific carbohydrate ligand containing sialyl-Lewis residue [15]. CAMs expression have been demonstrated on the endothelial cells of small vessels at the invasive margin of tumor cells involved in metastatic spread [9]. The soluble forms of CAMs have previously been recognized. They have been detected in the serum of patients with colorectal cancer, where high circulating levels are generally associated with more advanced and metastatic disease [3]. No study has documented serum levels of E-selectin, ICAM-1 and VCAM-1 in patients with colorectal cancer after radical tumor resection.

The aim of the present study was to assess the effect of tumor advancement and malignancy grade on the levels of soluble ICAM-1, VCAM-1 and E-selectin depending on surgical treatment.

Materials and methods

Patients. The study included 40 colorectal cancer patients (CRC) without remote metastases (20 men and 20 women, aged 42 – 68 years), diagnosed and operated in the Department of General Surgery at the J. Sniadecki's Hospital in Bialystok and in Department of General and Endocrinological Surgery, Medical University in Bialystok, and 24 healthy subjects (control group) (19 men and 5 women, aged 40 – 64 years) (Table 1).

The diagnosis of CRC was based on clinical symptoms and colonoscopy and abdominal ultrasound scanning. The patients underwent surgical procedure of tumor resection with subsequent regional lymphadenectomy. During surgery, samples (primary tumor, lymph node metastasis) were collected and neoplastic lesions were histopathologically verified. All patients had colorectal cancer. None of these patients received chemotherapy or radiation therapy before and after surgical intervention.

The CRC patients qualified for our study did not have any other ailment that could affect the immune response. Those patients who had a recent inflammatory or any acute infection were excluded from the study. Clinical advancement was evaluated based on TNM classification by Hutter and Sobin [14], while tumor type and malignancy grade were determined by histopathological analysis. The CRC patients were divided into two groups: A – 22 patients in stages I and II without metastases ($T_{1\text{-}2}N_0M_0$, malignancy differentiation grade G2, G3) and B – 18 patients with lymph node metastases (but not remote metastases) in stage III of clinical advancement ($T_3N_{1\text{-}2}M_0$, malignancy differentiation grade G2, G3). Demographic data of the CRC patients have been presented in Table 1.

Blood analysis. Peripheral venous blood samples in CRC patients were collected twice: three days prior to surgery and 3 months after surgery, into sterile glass tubes, in the morning after an overnight fast. The samples were allowed to coagulate at room temperature for 30 min and then were centrifuged at 2500 g for 20 min. The serum was separated and stored at -70°C until analysis. Before analysis, samples were thawed slowly and mixed gently.

The soluble forms of CAMs were measured in blood serum using a commercially available immunoenzymatic method. Serum sICAM, sVCAM and E-selectin concentrations were determined using enzyme-linked immunosorbent assay (ELISA) kits (Parameter; R&D Systems, Minneapolis, MN) according to procedures recommended by the manufacturer.

Table 1. Demographic and clinical characteristics of patients with colorectal cancer (CRC) and healthy control group. Group A – CRC patients in stage I and II of clinical advancement (T1-2N0M0, malignancy differentiation grade G2, G3). Group B – CRC patients in stage III of clinical advancement. (T3N1-2M0, malignancy differentiation grade G2, G3). Group C – control.

	Group A (without metastases)	Group B (with lymph node metastases)	Group C
n	22	18	24
Age (years)	60.6 ± 14.8	62.5 ± 12.6	54 ± 11
Gender (M/F)	10/12	10/8	19/5
Tumor stage			-
T1, T2	22	-	-
T3	-	18	-
Tumor grade			-
G2	7	8	-
G3	15	10	-

Ethical issues. The protocol was approved by the Medical University of Bialystok Bioethics Committee, according to Guidelines for Good Clinical Practice (GCP) (R-I-003/325/2004). Only patients who gave their informed written consent were enrolled in the study.

Statistical analysis. Data are presented as means and standard deviation (±SD). Nonparametric U Mann-Whitney test and Spearman correlation test were used for statistical analysis of the results. The program STATISTICA 6.0 for Windows was applied. Differences were considered statistically significant for $p<0.05$.

Results

Table 2 shows the means and standard deviation of sICAM-1, sVCAM-1 and sE-selectin in the serum of CRC patients and in the control group. Serum mean concentrations of sICAM-1 in cancer cases and controls were 366.1±114.1 and 306.4±98.2 ng/ml (p=0.037), respectively; sVCAM-1 were 974.4±214.3 and 510.1±199.4 ng/ml (p=0.001), respectively. The differences between the two groups were significant. Serum concentration of sE-selectin was not significantly higher in the cancer group compared to the control group; 88.2±34.3 and 68.2±26.3 ng/ml; p=0.074, respectively (Table 2).

To study the effect of surgical tumor removal on the levels of the circulating cell adhesion molecules in all CRC patients, we measured and compared sE-selectin, sICAM-1 and sVCAM-1 concentrations before surgical treatment and 3 months after it. Radical resection of primary tumor involved surgical removal of all or part of the colon and resection of the regional lymph

node. Only serum levels of sICAM and sVCAM, but not sE-selectin, decreased significantly after radical resection of the tumor compared with preoperative levels; sICAM 366.4±114.0 ng/ml *vs*. 247.3±68.4 ng/ml ($p<0.001$) and sVCAM 974.3±214.0 *vs*. 470.1±106.3 ng/ml ($p<0.0001$) (Table 3).

We compared the concentrations of sICAM-1, sVCAM-1 and sE-selectin in the serum of CRC patients between group A and B. We observed a significantly higher concentration of sICAM (396.2±68.9ng/ml) and sVCAM (1210.3±199.0 ng/ml) in the serum of the CRC patients with lymph node metastases. There was a significant difference between the two groups (Table 4). No significant difference was observed in sE-selectin concentration in the serum of the CRC patients before surgical intervention (group A: 82.4±23.3 group B: 88.4±29.0 (Table 4**).** There was also no significant correlation of serum sICAM-1, sVCAM-1 and sE-selectin levels in CRC cases with patients' age or gender. However, the preoperative levels of all the three adhesion molecules were significantly correlated with disease stage and lymph node involvement.

Disscusion

Adhesion molecules are thought to be essential for maintaining a normal immune defense system in a variety of important biological events including carcinogenesis and metastasis biology. It has been suggested that the process of tumor metastasis includes differential expression of adhesion molecules, with some being over-expressed, others down-regulated and some structurally changed, causing a loss of cell-cell interaction which could facilitate metastasis.

Elevated serum levels of cell adhesion molecules (CAMs) have been described in patients with cancers [17]. In our research, like in the studies of other authors [2], preoperative serum concentrations of sICAM and sVCAM in CRC patients were significantly higher compared to the control group, whereas no differences were found regarding serum sE-selectin levels. Similar results concerning sE-selectin were obtained by other investigators, who concluded that the level of sE- selectin cannot be a predictive marker in colorectal cancer, although it may be useful in the diagnosis of liver metastases [20].

We have confirmed the findings by Alexiou *et al*. [3] and Kang *et al.* [21], who found elevated levels of CAMs in CRC patients. In our study, the levels of serum sICAM-1 and sVCAM-1 in healthy controls were similar to those reported by other authors [4,9,20,22,23]. These soluble forms of sICAM, sVCAM and sE-selectin significantly correlated with tumor stage and the development of metastases of colorectal cancer [21,25]. In our study, there was a significant correlation of serum sICAM-1 and sVCAM-1, but not sE-selectin, with the disease stage, which can be explained by low neoangiogenesis in the neoplastic tissue.

As revealed by many authors, these soluble forms of CAMs significantly correlated with TNM stage and the development of metastases of gastric cancer [2,25], colorectal cancer [21], breast cancer [5,7], in urologi-

Table 2. Serum levels of sICAM and sVCAM in the colorectal cancer cases and healthy control group. sICAM-1 – soluble intracellular adhesion molecule-1, sVCAM-1 – soluble vascular cell adhesion molecule-1, sE-selectin – soluble E-selectin, both men and women are incorporated.

	Cancer cases	Controls	*P-value*
N &	40	24	
sICAM-1 (ng/ml)	366.1 ± 114.1	306.4 ± 98.2	0.037
sVCAM-1 (ng/ml)	974.4 ± 214.3	510.1 ±199.4	0.001
sE-selectin (ng/ml)	88.2 ± 34.3	68.2 ± 26.3	0.074

Table 3. Serum levels of sICAM, sVCAM and sE-selectin in the colorectal cancer cases before and after surgical intervention. sICAM-1 – soluble intracellular adhesion molecule-1, sVCAM-1 – soluble vascular cell adhesion molecule-1, sE-selectin – soluble E-selectin, both men and women are incorporated.

	CRC cases		
	before radical tumor resection	after radical tumor resection	
N &	40	40	P-value
sICAM-1 (ng/ml)	366.4±114.0	247.3±68.4	<0.001
sVCAM-1 (ng/ml)	974.3±214.0	470.1±106.3	<0.0001
sE-selectin (ng/ml)	88.2±34.3	68.9±28.1	>0.05

Table 4. Serum levels of sICAM, sVCAM and sE-selectin in CRC patients before surgical intervention: group A – without metastases and group B – with lymph node metastases. sICAM-1 – soluble intracellular adhesion molecule-1, sVCAM-1 – soluble vascular cell adhesion molecule-1, sE-selectin – soluble E-selectin. Values were expressed as the means SD, both men and women are incorporated.

	Group A	Group B	*P*-value
N &	22	18	
sICAM-1 (ng/ml)	354.2±94.0	396.2±68.9	<0.05
sVCAM-1 (ng/ml)	774.4±214.1	1210.3±199.0	<0.001
sE-selectin (ng/ml)	82.4±23.3	88.4±29.0	>0.05

Folia Histochem Cytobiol. 2009:47(4): 623 (621-625)
10.2478/v10042-009-0077-0

cal malignancies [6] and in chronic B-lymphocytic leukemia [4]. Numerous investigations in clinical diagnosis have been focused on the evaluation of the adhesion molecule expression to identify the interaction between tumor cells and host immune response. The preoperative levels of sICAM-1, sVCAM-1, elevated in our study, showed relatively high specificity, but had low sensitivity for diagnosis of colorectal cancer. There was a significant correlation of serum sICAM-1 and sVCAM-1 levels, but not sE-selectin, with TNM stage and lymph node involvement. No significant relationship was found between the concentrations and patients' age or gender. The factors regulating CAMs in colorectal cancer are complex. The association among immunohistochemical CAM expression, tumor vascularity and leukocyte infiltration suggests an important role for these molecules in host immune response and in tumor progression [2].

We observed significantly higher concentrations of sICAM and sVCAM in the serum of the CRC patients with lymph node metastases. Metastatic spread of cancer cells is a key event in tumor progression and in determining the prognosis of patients with malignant disease. CAM expressions have been demonstrated on endothelial cells of small vessels at the invasive margin of tumor cells involved in metastatic spread [9]. In various vascular disorders, activated lymphocytes and macrophages produce cytokines, of which interleukin-1(IL-1), tumor necrosis factor (TNF-α) and interferon-γ are the main inducers of adhesion molecule expression on endothelial cells [26].

We believe that the circulating sCAMs may have a regulatory role, although their biological role has not been completely elucidated. The migration of lymphocytes from intravascular system to tissue is a fundamental event in the immune response to an inflammatory process and it is the result of specific interactions of adhesion molecules on endothelium and their ligands on lymphocytes. Since LFA-1 is the ligand for ICAM-1 and it is involved in the initial steps of adhesion of cytotoxic T lymphocytes to the target tumor cells [27], we suggest that ICAM-1 shedding from tumor cells could block binding of cytotoxic T lymphocytes to cancer. Additional loss of adhesion molecules from activated endothelium could inhibit counter ligands on the tumor cells themselves, preventing their adhesion to endothelium sites and thereby promoting metastases. We have confirmed this assumption, showing a correlation of the level of adhesion molecules with tumor differentiation grade and the process of metastasis. In gastric cancers, serum sICAM-1 and sVCAM-1 levels decreased significantly after radical tumor resection [3]. In our study, serum levels of sICAM and sVCAM, but not sE-selectin, were found to be significantly higher in patients before surgical treatment for CRC compared to those after radical resection. *In vitro* studies have shown that at least ICAM-1 expressed by tumor cells is shed in the cell culture supernatants [28], which may explain our observations.

These findings seem to suggest that serum concentrations of sCAMs can reflect tumor progression and metastases, and may be clinically useful. We think that an improved understanding of the mechanisms of membrane shedding of sICAM, sVCAM and sE-selectin, their effect on the host immune response, their relationship to disease stage and lymph node involvement is required to delineate their role in tumor progression.

Acknowledgments: I would like to thank Krzysztof Kanigowski MD, for collecting samples of patient's blood at the Department of General Surgery Sniadecki's Hospital of Bialystok. This work was supported by the grant 4 09 660F from Medical University of Białystok.

References

[1] Etzioni A. Adhesion molecules: their role in health and disease. *Pediatr Res*. 1996;39:191-198.
[2] Alexiou D, Karayiannakis AJ, Syrigos KN *et al*. Clinical significant of serum levels of E-selectin, intercellular adhesion molecule-1, and vascular adhesion molecule-1 in gastric cancer patients. *Am J Gastroenterol*. 2003;98:478-485.
[3] Alexiou D, Karayiannakis AJ, Syrigos KN *et al*. Serum levels of E-selectin, ICAM-1 and VCAM-1 in colorectal cancer patients: correlations with clinicopathological features, patients survival and tumor surgery. *Eur J Cancer*. 2001;37: 2392-2397.
[4] Christiansen I, Sundstrom C, Totterman TH. Elevated serum levels of soluble vascular cell adhesion molecule-1 (sVCAM-1) closely reflect tumor burden in chronic B-lymphocytic leukaemia. *Br J Haematol*. 1998;103:1129-1137.
[5] O'Hanlon DM, Fitzsimons H, Lynch J, Tormey S, Malone C, Given HF. Soluble adhesion molecules (E-selectin, ICAM-1 and VCAM-1) in breast carcinoma. *Eur J Cancer*. 2002;38: 2252-2257.
[6] Perabo F, Sharma S, Gierer R. Circulating intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and E-selectin in urological malignancies. *Indian J Cancer.* 2001;38:1-7.
[7] Silva HC, Garcao EC, Coutinho CF, De Oliviera CF, Regateiro FJ. Soluble VCAM-1 and E-selectin in breast cancer: relationship with staging and with the detection of circulating cancer cells. *Neoplasma*. 2006;53:538-543.
[8] Pasieka Z, Kuzdak K, Czyz W, Stępień H, Komorowski J. Soluble intracellular adhesion molecules (sICAM, sVCAM-1) in peripheral blood of patients with thyroid cancer. *Neoplasma.* 2004;51:34-37.
[9] Benoliel AM, Pirro N, Marin V, Consentino B, Pierres A, Vitte J. Correlation between invasiveness of colorectal tumor cells and adhesive potential under flow. *Anticancer Res*. 2003;23:4891-4896.
[10] Zhang GJ, Adachi I. Serum levels of soluble intercellular adhesion molecule-1 and E-selectin in metastatic breast carcinoma: correlations with clinicopathological features and prognosis. *Int J Onocol.* 1999;14:71-77
[11] Guzinska-Ustymowicz K, Kemona A. Transforming growth factor beta can be a parameter of aggressiveness of pT1 colorectal cancer. *World J Gastroenterol*. 2005;28;11:1193-5.

[12] Okulczyk B, Kovalchuk O, Piotrowski Z, Mysliwiec P, Chyczewski L. Clinical usefulness of K-RAS mutation detection on colorectal cancer and surgical margines of colon. *Adv Med Sci*. 2004;49:52-54.
[13] Tremblay PL, Auger FA, Huot J. Regulation of transendothelial migration of colon cancer cells by E-selectin-mediated activation of p38 and ERK MAP kinases. *Oncogene*. 2006; 25:6563-73.
[14] Koch AE, Halloran MM, Haskell CJ, Shah MR, Polverini PJ. Angiogenesis mediated by soluble forms of E-selectin and vascular cell adhesion molecule-1. *Nature*. 1995;376:517-519.
[15] Byrne GJ, Ghellal A, Iddon J *et al.* Serum soluble vascular cell adhesion molecule-1: role as a surrogate marker of angiogenesis. *J Natl Cancer Inst*. 2000;92:1329-1336.
[16] Hutter RVP, Sobin LH. A universal staging system for cancer of the colon and rectum. *Arch Path Lab Med*. 1986;110:367-368.
[17] Mantur M, Kemona H, Kozłowski R, Kemona-Chetnik I. Effect of tumor stage and nephrectomy on CD62P expression and sP- selectin concentration in renal cancer. *Neoplasma*. 2003;50:262-265.
[18] Mantur M, Koper O, Snarska J, Sidorska A, Kruszewska-Wnorowska K. Evaluation of PDGF- AB and sP- selectin concentration in relation to platelet count in patients with colorectal cancer before and after surgical treatment. *Pol Arch Med Wewn*. 2008;118:345-349.
[19] Dymicka-Piekarska V, Matowicka- Karna J, Osada J, Kemona H, Butkiewicz AM. Changes in platelet CD 62P expression and soluble P-selectin concentration in surgically treated colorectal carcinoma. *Adv Med Sci*. 2006;51:304-308.
[20] Uner A, Akcali Z, Unsal D. Serum levels of soluble E-selectin in colorectal cancer. *Neoplasma*. 2004;51:269-274.
[21] Kang X, Wang F, Xie JD, Cao J, Xian PZ. Clinical evaluation of serum concentrations of intercellular adhesion molecule-1 in patients with colorectal cancer. *World J Gastroenterol.* 2005;11:4250-4253.
[22] Basoglu M, Yildirgan MI, Taysi S, Yilmaz I, Kiziltunc A, Balik AA. Levels of soluble intercellular adhesion molecule-1 and total sialic acid in serum of patients with colorectal cancer. *J Surg Oncol.* 2003;83:180-184.
[23] Mantur M, Snarska J, Sidorska A, Ostrowska H, Kruszewska-Wnorowska K, Wojszel J. Changes in PDGF concentration in surgically treated colorectal carcinoma. *Adv Med Sci*. 2008; 53:37-41.
[24] Mantur M, Koper O. Platelet-derived growth factor (PDGF)-the construction, role and it's receptors. *Pol Merk Lek*. 2008; 140:173-176.
[25] Nakata B, Hori T, Sunami T *et al*. Clinical significance of serum soluble intercellular adhesion molecule-1 in gastric cancer. *Clin Cancer*. 2000;6:1175-1179.
[26] Lieuw-a-Fa M, Schalkwijk C, van Hinsbergh VW. Distinct accumulation patterns of soluble forms of E-selectin, VCAM-1 and ICAM-1 upon infusion of TNFα in tumor patients. *Thromb and Haemost.* 2003;89:1052-1058.
[27] Sanchez-Rovira P, Jimenez E, Carracedo J, Barneto IC, Ramirez R, Aranda E. Serum levels of intercellular adhesion molecule 1 (ICAM-1) in patients with colorectal cancer: inhibitory effect on cytotoxicity. *Eur J Cancer*. 1998;34:394-398.
[28] Franzke A, Probst-Kepper M, Buer J *et al.* Elevated pretreatment serum levels of soluble vascular cell adhesion molecule 1 and lactate dehydrogenase as predictors of survival in cutaneous metastatic malignant melanoma. *Br J Cancer.* 1998;78: 40-45.

Submitted: 12 January, 2009
Accepted after reviews: 10 October, 2009