Digestive Diseases and Sciences, Vol. 50, No. 6 (June 2005), pp. 1019–1024 (© 2005)
DOI: 10.1007/s10620-005-2697-3

Stem Cell Factor (SCF) and Interleukin 3 (IL-3) in the Sera of Patients with Colorectal Cancer

BARBARA MROCZKO,* MACIEJ SZMITKOWSKI,* URSZULA WERESZCZYŃSKA-SIEMIĄTKOWSKA,† and BOGNA OKULCZYK‡

For a long time markers that can detect a malignant cell transformation as early as possible have been sought. Substances which have been discovered are known as tumor markers. Stem cell factor (SCF) and interleukin 3 (IL-3) are members of a group of glycoprotein growth factors called hematopoietic cytokines (HCs). These factors take part in the regulation of developmental processes of hematopoietic progenitor cells and it was proved that HCs can be produced by different cancer cells, including colorectal cancer. The aim of this study was to investigate a potential role for SCF and IL-3 as tumor markers for colorectal cancer. We compared the serum levels of SCF and IL-3 in colorectal cancer patients with those in healthy subjects (control group) and commonly accepted tumor markers, such as carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA 19-9). We defined the diagnostic sensitivity, specificity, positive predictive value, negative predictive value, and receiver-operating characteristics (ROC) curve of tested substances. SCF and IL-3 were determined using enzyme-linked immunosorbent assay (ELISA). CEA and CA 19-9 were measured by microparticle enzyme immunoassay. The serum levels of HCs and tumor markers were investigated in 75 patients with colorectal cancer and in 40 healthy subjects. There were significant differences in the level of circulating SCF and IL-3 in the colorectal cancer patients compared to the control group. Moreover, the diagnostic sensitivity of SCF was higher than the sensitivity of CEA and CA 19-9. The SCF area under the ROC curve was larger than the IL-3 area but smaller than the CEA and CA 19-9 areas. The diagnostic specificities of cytokines were lower than those of tumor markers, but the combined use of cytokines and tumor markers increased the diagnostic values. The highest values of diagnostic parameters were observed for the combined use of SCF and CA 19-9. These results suggest a potential role for SCF and IL-3 as tumor markers for colorectal cancer, especially in combination with CEA or CA 19-9.

KEY WORDS: stem cell factor; interleukin 3; colorectal cancer.

Colorectal cancer is one of the most common forms of neoplasmas. The prognosis for patients with cancer of colon or rectum is strongly correlated with the pathologic stage at the time of diagnosis (1) and it is very important to find markers which would detect a malignant cell transformation as early as it is possible (2). Stem cell factor (SCF) and interleukin 3 (IL-3) are hematopoietic cytokines (HCs), which induce proliferation of hematopoietic progenitor cells (3). The effect of these factors is not limited to bone marrow cells (4). Hematopoietic cytokine receptor have been found on nonhematopoietic tumor cell lines including colorectal cancer (5, 6). A number of studies have shown autologous production of hematopoietic cytokines in various human cell lines derived from cancer and demonstrated that these factors can stimulate tumor progression (7, 8). Moreover, the enhanced production of extracellular matrix-degrading proteinases by cancer cells

Manuscript received September 3, 2004; accepted October 22, 2004.
From the *Department of Biochemical Diagnostics, †Department of Gastroenterology, and ‡Second Department of General Surgery, Medical Academy, Bialystok, Poland.

Address for reprint requests: M. Szmitkowski, MD, PhD, Department of Biochemical Diagnostics, Medical Academy, M. Skłodowska-Curie 24A, 15-276 Białystok, Poland; zdb@amb.edu.pl.

in response to treatment with HCs may represent a biochemical mechanism which promotes the invasive behavior of cancer cells (9). The ability of colon carcinoma cell lines to synthetize HCs has been documented, but little is known about the in vivo production of SCF and IL-3 by colorectal cancer and their serum levels in cancer patients (10, 11). We found increased concentrations of IL-3, granulocyte–macrophage colony stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), and macrophage colony stimulating factor (M-CSF) in the sera of colorectal cancer patients (12, 13) and increased serum levels of M-CSF in pancreatic cancer patients (14), but we did not define the diagnostic criteria for SCF and IL-3 in these patients.

In the current investigation, which is a continuation of our previous studies, we tested the serum level of hematopoietic cytokines, such as SCF and IL-3, in colorectal cancer patients in relation to a control group and to commonly accepted tumor markers, such as carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA 19-9). We defined the diagnostic criteria, such as diagnostic sensitivity, specificity, positive predictive value, negative predictive value, and receiver-operating curve characteristics (ROC) curve of tested cytokines and markers.

METHODS

Patients

The protocol was approved by the Human Care Committee of the Medical Academy in Bialystok, Poland (Approval No. R-I-0003/213/2001). All patients gave on informed consent for the examination.

The study included 75 colorectal cancer patients (43 males and 32 females, aged 34–86 years) diagnosed by the Oncology Group and operated on in the Second Department of General Surgery at the Medical Academy Hospital in Bialystok and 40 healthy people (control group, 20 males and 20 females; aged 21–66 years). All of the colorectal cancer patients underwent surgical resection. Tumors were localized in the colon ($n = 36$) and in the rectum ($n = 39$). None of the patients had received chemo- or radiotherapy before blood sample collection.

Pretreatment staging procedures included physical and blood examinations, chest roentgenogram, computed tomography (CT), abdominal ultrasound scanning, and colonoscopy. In addition, radioisotopic scans of bones, examination of bone marrow aspirates and abdominal, and brain CT scans were performed when necessary. During the operations, radical lymph node dissection was uniformly performed. Postoperative, pathological staging (primary tumor, regional lymph node involvement, occurrence of distant metastasis) was performed by correlating the operative and histological findings. The American Joint Committee on Cancer Classification and stage grouping was used to classify the tumors (15). Patients were divided into three groups: 32 patients in stage II, 28 patients in stage III, and 15 patients in stage IV. Each tumor was histopathologically classified according to its morphology, using the World Health Organization criteria presented by Jass and Sobin (16).

Material

Venous blood samples were collected from every patient before surgery, centrifuged to obtain serum samples, and stored at -80°C until assayed.

Biochemical Assays

Determination of SCF and IL-3. SCF and IL-3 were measured by enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Abingdon, UK), according to the manufacturer's instructions. The manufacturer claims a sensitivity of <9 ng/L, an intra-assay variability of 2.0% at a SCF concentration of 655 and <7.4 ng/L, and an intra-assay variability of 5.7% at a IL-3 concentration of 115 ng/L. The cutoff point was 1285 ng/L for SCF and 0.10 ng/L for IL-3.

Determination of CEA and CA 19-9. CEA and CA 19-9 were measured by microparticle enzyme immunoassay (MEIA) kits (Abbott, Chicago, IL, USA) using cutoff values of 4 μg/L for CEA and 30×10^3 U/L for CA 19-9.

All cutoff values were obtained from a study of a healthy population (the 95th percentile) in our department.

Statistical Analysis

A preliminary statistical analysis (chi-square test) revealed that the distribution of cytokines and tumor marker levels does not follow a normal distribution. Consequently, the Mann–Whitney U test was used for statistical analysis. Data are presented as median and range. Statistically significant differences were defined as comparisons resulting in $P < 0.05$. The diagnostic criteria, such as the diagnostic sensitivity, specificity, predictive value, and ROC curve, were determined using the GraphRoc Program for Windows (University of Turku, Turku, Finland) (17).

$$\text{Sensitivity} = \frac{\text{number of true-positive results} \times 100\%}{\text{number of true-positive results} + \text{number of false-negative results}}$$

$$\text{Specificity} = \frac{\text{number of true-negative results} \times 100\%}{\text{number of true-negative results} + \text{number of false-positive results}}$$

$$\text{Positive predictive value} = \frac{\text{number of true - positive results} \times 100\%}{\text{number of true-positive results} + \text{number of false-positive results}}$$

$$\text{Negative predictive value} = \frac{\text{number of true - negative results} \times 100\%}{\text{number of true-negative results} + \text{number of false-negative results}}$$

RESULTS

Table 1 shows the median and range of cytokine and tumor markers levels in the sera of colorectal cancer patients and healthy subjects (control group). The median IL-3, CEA, and CA 19-9 levels in the colorectal cancer patients were significantly higher, but the median SCF level significantly lower, than those in the control group. The

TABLE 1. SERUM LEVELS OF HEMATOPOIETIC CYTOKINES AND TUMOR MARKERS IN COLORECTAL CANCER PATIENTS

	SCF (ng/L)		*IL–3 (ng/L)*		*CEA (μg/L)*		*CA 19–9 (U/L)*	
Tested group	*Median*	*Range*	*Median*	*Range*	*Median*	*Range*	*Median*	*Range*
Cancer patients group ($n = 75$)	891*	473–1706	3.67*	0.00–48.5	2.80*	0.10–426	$6.11^* \times 10^3$	$0.00–411 \times 10^3$
Stage II ($n = 32$)	959*	599–1495	2.25*	0.00–27.7	1.75*	0.20–41.1	$4.67^* \times 10^3$	$0.00–336 \times 10^3$
Stage III ($n = 28$)	807*	473–1706	5.18*	0.00–45.0	2.90*	0.10–426	$7.28^* \times 10^3$	$0.00–265 \times 10^3$
Stage IV ($n = 15$)	920*	493–1307	5.30*	0.00–48.5	17.6*	0.80–180	$13.8^* \times 10^3$	$0.00–411 \times 10^3$
Control group ($n = 40$)	1092	642–1812	0.00	0.00–36.5	0.95	0.00–2.60	1.12×10^3	$0.00–19.0 \times 10^3$

*Statistical significance compared to the control group.

median IL-3 level in the control group was 0 ng/L, because the serum levels of more than 50% of the patients in this group were not detectable (0 ng/L). The IL-3, CEA, and CA 19-9 levels were higher in the more advanced tumor stage (stage IV) than in stage II, but the SCF levels were the highest in stage II. The cytokine and tumor marker serum levels in all stages were significantly higher (IL-3, CEA, CA 19-9) or lower (SCF) than those in the control group.

The diagnostic criteria for cytokines and tumor markers are shown in Table 2. The sensitivity of SCF was higher than the sensitivity of IL-3 and tumor markers. The specificities and positive predictive values of CEA and CA 19-9 were higher than those of cytokines. The highest negative predictive values were observed for IL-3 and SCF.

Table 3 presents the diagnostic criteria of combined use of cytokines and tumor markers. The diagnostic sensitivity and the negative predictive values were the highest for the combined use of SCF with CEA (90 and 85%) or CA 19-9 (92 and 87%) and the lowest for the combination of CEA and CA 19-9 (45 and 49%, respectively). Other diagnostic parameters, such as the diagnostic specificity and the positive predictive value, were 100%, except for the combination of SCF with IL-3.

The relationship between diagnostic sensitivity and specificity was illustrated with ROC curves (Figures 1 and 2). They show that the area under the ROC curve for SCF (0.7232; Figure 1) was larger than the ROC area for IL-3 (0.6840; Figure 2) and smaller than the areas for CEA (0.7963) and CA 19-9 (0.7362) (Table 2).

DISCUSSION

Colorectal cancer is a leading cause of cancer, its incidence being highest in industrialized countries (18). Each year in the United States, colon cancer is diagnosed in nearly 140,000 men and women and leads to the death of nearly 60,000 (19). Therefore, this cancer has been the focus of intense research for many years.

Tumor markers are usually proteins associated with a malignancy and might be clinically useful in patients with cancer (20). A tumor marker can be detected in a solid tumor, in peripheral blood, in lymph nodes, in bone morrow, or in other body fluids (ascites, urine, and stool). These markers are synthesized and excreted by tumor tissue, released on tumor disintegration, or formed by normal tissue as a reaction of the organism to a tumor. Tumor markers can be measured quantitatively in biological fluids to indicate the presence, location, or extent of malignant tumors (20). The ideal tumor marker should possess a high diagnostic specificity, i.e., not detectable in benign cases and healthy subjects, a high diagnostic sensitivity, i.e., detectable very early when only a few cancer cells are present, organ specificity, correlation with the tumor stage or tumor mass, correlation with prognosis, and reliable prediction value. Although there has been a rapid expansion of the number of proposed tumor markers during the last few years, none has fulfilled all of the above criteria.

Colon cancer cells are capable of producing hematopoietic cytokines constitutively (10, 11). These cytokines may act on cancer cells in an autocrine manner or on supporting tissues such as fibroblasts and blood vessels to produce an environment conductive to cancer growth. The cytokines may also induce normal cells, such as tumor-associated macrophages (TAM) and endothelial cells, to produce additional cytokines that support the malignant process (7). Several cell lines of malignant tumors have been demonstrated to secrete large amounts of HCs, but

TABLE 2. DIAGNOSTIC CRITERIA FOR HEMATOPOIETIC CYTOKINES AND TUMOR MARKERS FOR COLORECTAL CANCER

Tested marker	*Cutoff*	*Area under ROC curve*	*Diagnostic sensitivity (%)*	*Diagnostic specificity (%)*	*Positive predictive value (%)*	*Negative predictive value (%)*
SCF	1285 ng/L	0.7232	89	17	67	47
IL–3	0,10 ng/L	0.6840	55	80	84	48
CEA	4,0 μg/L	0.7963	37	100	100	46
CA 19–9	30×10^3 U/L	0.7362	20	100	100	40

TABLE 3. DIAGNOSTIC CRITERIA FOR COMBINED USE OF HEMATOPOIETIC CYTOKINES AND TUMOR MARKERS FOR COLORECTAL CANCER

Tested marker	*Diagnostic sensitivity (%)*	*Diagnostic specificity (%)*	*Positive predictive value (%)*	*Negative predictive value (%)*
SCF + IL–3	92	82	90	85
SCF + CEA	90	100	100	85
SCF + CA 19–9	92	100	100	87
IL–3 + CEA	75	100	100	68
IL–3 + CA 19–9	60	100	100	57
CEA + CA 19–9	45	100	100	49

the question is whether the serum levels of these factors are higher in colon cancer patients than in healthy subjects and whether hematopoietic cytokines might be useful as tumor markers.

To our knowledge, this is the first study showing the all diagnostic criteria for SCF and IL-3 in colorectal cancer patients. Previously, we found increased concentrations of IL-3, GM-CSF, G-CSF, and M-CSF in the sera of colorectal cancer patients (12,13) and increased serum levels of M-CSF in pancreatic cancer patients (14), but we did not define the diagnostic criteria for SCF and IL-3 in these patients. In the current study, the serum IL-3, CEA, and CA 19-9 levels in colorectal cancer patients were significantly higher, but the serum SCF level significantly lower, than those of the control group. The results of the current study support our previous findings. Interestingly, the serum SCF levels were lower in cancer compared with the control group, which suggests that the alterations in the levels of tested cytokines may be a result of changes in the immune system. Moreover, the SCF-c-kit system may have a growth-regulating role in the normal tissues, which is altered during malignant transformation (21).

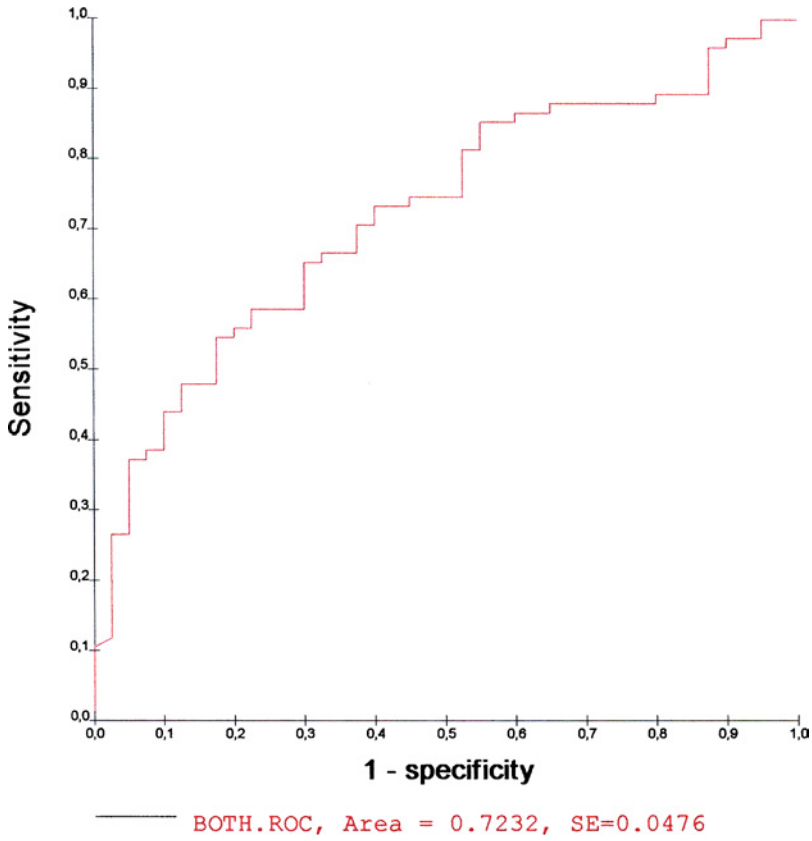

Fig 1. The receiver-operating characteristics (ROC) curve for the relationship between diagnostic sensitivity and specificity of stem cell factor in colorectal cancer patients.

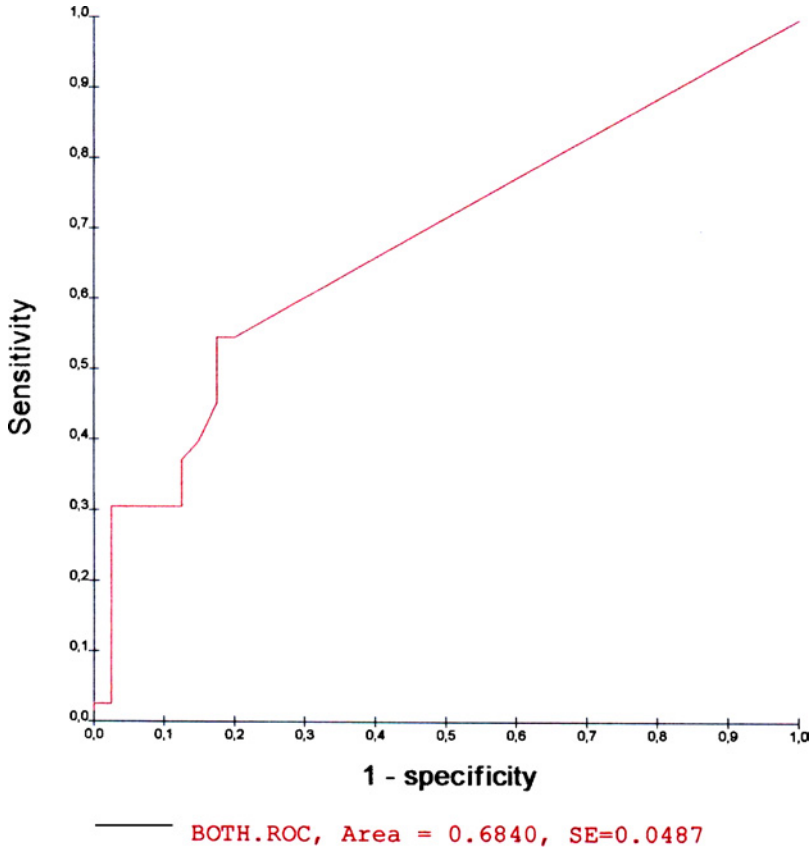

Fig 2. The receiver-operating characteristics (ROC) curve for relationship between diagnostic sensitivity and specificity of interleukin 3 in colorectal cancer patients.

The diagnostic criteria for tumor markers are sensitivity, specificity, and predictive values. In our previous study, in lung cancer patients the sensitivity of M-CSF was lower (38%), but the sensitivity of G-CSF was higher (56%), than the sensitivity of the commonly accepted tumor markers such as CYFRA 21-1 (51%) and not as high as that of CEA (62%) (22, 23). In colorectal cancer patients, the M-CSF sensitivity was higher (65%) than the sensitivity of G-CSF (31%) and the sensitivity of the other markers (12). In our current study, the CEA and CA 19-9 specificities were 100%, but the sensitivities of these markers were low. The combination of CEA and CA 19-9 did not significantly improve this diagnostic parameter, but the diagnostic sensitivity was the highest for the combined use of SCF with CEA or CA 19-9. This suggests that the combination of SCF with these markers may be useful for diagnosis of colorectal cancer.

The positive predictive value represents the probability that a tumor exists in the case of positive test results. The negative predictive value indicates the probability that there is no tumor in the case of negative test results. In this investigation, the combined use of SCF with CEA or CA 19-9 proved to have the highest predictive values. The most important criterion for tumor markers is sensitivity/specificity diagram ROC curves. The area under the ROC curve indicates the clinical usefulness of a tumor marker. A larger area under the ROC curve corresponds to a better tumor marker. In this study, the SCF area under the ROC curve was larger than the IL-3 ROC area and smaller than the CEA and CA 19-9 areas. In lung cancer patients, the ROC area of GM-CSF (0.72) was larger than the ROC area of other tested cytokines (SCF, 0.67; G-CSF, 0.65; M-CSF, 0.56) (22, 23). In colorectal cancer, according to our other studies, the ROC area of M-CSF (0.86) was larger than those of SCF (0.72), GM-CSF (0.71), G-CSF (0.69), IL-3 (0.68), and tumor markers (CEA, 0.78; CA 19-9, 0.75) (13). These results suggest, compared with our previous studies, that hematopoietic cytokines may be good candidates for tumor markers in colorectal cancer. However, at present it is difficult to say whether the levels of these cytokines can be used as diagnostic tests for colorectal cancer or whether these findings may have therapeutic implications. Further investigations and confirmation by a prospective study are necessary.

In conclusion, this is the first study showing all the diagnostic criteria for SCF and IL-3 in colorectal cancer patients. These results suggest a potential role for SCF and IL-3 as tumor markers for colorectal cancer, especially in combination with CEA or CA 19-9.

ACKNOWLEDGMENT

This work was supported by The State Committee for Scientific Research (KBN) (Grant 3 PO5B 02325).

REFERENCES

1. Ahmad NA, Kochman Ml, Ginsberg GG: Endoscopic ultrasound and endoscopic mucosal resection for rectal cancers and villous adenomas. Hematol Oncol Clin N Am 16:897–906, 2002
2. Negm RS, Verma M, Srivastava S: The promise of biomarkers in cancer screening and detection. Trends Mol Med 8:288–293, 2002
3. Smith MA, Court El, Smith JG: Stem cell factor: laboratory and clinical aspects. Blood Rev 15:191–197, 2001
4. Berdel WE, Denhauser-Riedl S, Steinhauser G, Winton: Various human hematopoietic growth factors (interleukin-3, GM-CSF, G-CSF) stimulate clonal growth of nonhematopoietic tumor cells. Blood 73:80-83, 1989
5. Trutmann M, Terracciano l, Noppen C, Kloth J, Kaspar M, Peterli R, Tondelli P, Schaefer Ch, Zajac P, Heberer M, Spagnoli GC: GM-CSF gene expression and protein production in human colorectal cell lines and clinical tumor specimens. Int J Cancer 77:378–385, 1998
6. Turner AM, Zsebo KM, Martin F, Jacobsen FW, Bennett lG, Broudy VC: Nonhematopoietic tumor cell lines express stem cell factor and display c-kit receptors. Blood 80:374–380, 1992
7. Dunlop RJ, Campbell CW: Cytokines and advanced cancer. J. Pain Symptom Manage 20:214–232, 2000
8. McDermott RS, Deneux l, Mosseri V, Vedrenne J, Clough K, Fourquet A, Rodriguez J, Cosset JM, Sastre X, Beuzeboc Ph, Pouillart P, Scholl SM: Circulating macrophage colony stimulating factor as a marker of tumor progression. E Cytokine Network 13:121–127, 2002
9. Pei XH, Nakanishi Y, Takayama K, Bai F, Hara N Granulocyte, granulocyte-macrophage, and macrophage colony-stimulating factors can stimulate the invasive capacity of human lung cancer cells. Br J Cancer 79:40–46, 1999
10. Lahm H, Amstad P, Yilmaz A, Borbenyi Z, Wyniger J, Fischer JR, Suardet l, Givel JC, Odartchenko N: Interleukin 4 down-regulates expression of c-kit and autocrine stem cell factor in human colorectal carcinoma cells. Cell Growth Diff 6:1111–1118, 1995
11. Lahm H, Wyniger J, Hertig S, Yilmaz A, Fischer J, Givel JC, Odartchenko N: Secretion of bioactive granulocyte-macrophage colony-stimulating factor by human colorectal carcinoma cells. Cancer Res 54:3700–3702, 1994
12. Mroczko B., Szmitkowski M., Okulczyk B: Granulocyte-colony stimulating factor (G-CSF) and macrophage-colony stimulating factor (M-CSF) in colorectal cancer patients. Clin Chem Lab Med 40:351–355, 2002
13. Mroczko B, Szmitkowski M, Okulczyk B: Hematopoietic growth factors in colorectal cancer patients. Clin Chem lab Med 41:646–651, 2003
14. Mroczko B, Szmitkowski M, Wereszczynska-Siemiatkowska U, Jurkowska G: Stem cell factor and macrophage-colony stimulating factor in patients with pancreatic cancer. Clin Chem Lab Med 42:256–260, 2004
15. Fleming ID, Cooper JS, Henson DE, Hutter VP, Kennedy BJ, Murphy GP, O'Sullivan B, Sobin lH, Yarbro JW: Colon and Rectum. AJCC Cancer Staging Manual. New York, Lippincott–Raven, 1997
16. Jass JR, Sobin LH: Definition and explanatory notes. Large intestine. Histological Typing of Intestinal Tumors. New York, Springer-Verlag, 1989
17. Kairisto V, Virtanen A, Uusipaikka E, Voipio-Pulkki lM, Nanto V, Peltola O, Irjala K: Method for determining reference changes from patients serial data: example of cardiac enzymes. Clin Chem 39:2298–2304, 1993
18. Gongoll S, Peters G, Mengel M, Piso P, Klempnauer J, Kreipe H, Wasielewski R: Prognostic significance of calcium-binding protein S100A4 in colorectal cancer. Gastroenterology 123:1478–1484, 2002
19. Lynch JP, Hoops TC: The genetic pathogenesis of colorectal cancer. Hematol Oncol Clin N Am 16:775–810, 2002
20. Lindblom A, liljegren A: Tumour markers in malignancies. BMJ 320:424–427, 2000
21. Esposito I, Kleeff J, Bischoff SC, Fischer l, Collecchi P, Iorio M, Bevilacqua G, Buchler MW, Friess H: The stem cell factor-c-kit system and mast cells in human pancreatic cancer. Lab Invest 82:1481–1492, 2002
22. Mroczko B, Szmitkowski M, Niklinski J: Stem cell factor and granulocyte-macrophage–colony stimulating factor as candidates for tumour markers for non-small-cell lung cancer. Clin Chem lab Med 37:959–962, 1999
23. Mroczko B, Szmitkowski M, Niklinski J: Granulocyte-colony stimulating factor and macrophage-colony stimulating factor in patients with non-small-cell lung cancer. Clin Chem Lab Med 39:374–379, 2001