 Prague Medical Report / Vol. 108 (2007) No. 4, p. 348–357

Serum Erythropoietin and Angiogenetic Factors in Human Colorectal Cancer

Chabowska A.[1], Sulkowska M.[1], Wincewicz A.[1], Chabowski A.[2], Mysliwiec M.[3], Pawlak K.[3], Koda M.[1], Kedra B.[4], Sulkowski S.[1]

[1]Departments of Medical and General Pathomorphology, Medical University of Bialystok, Poland;
[2]Department of Physiology, Medical University of Bialystok, Poland;
[3]Department of Nephrology and Transplantology, Medical University of Bialystok, Poland;
[4]Department of General and Gastrointestinal Surgery, Medical University of Bialystok, Poland

Received November 5, 2007; Accepted December 14, 2007.

Key words: Erythropoietin – VEGF – VE-cadherin – Anemia – Colorectal cancer

Mailing Address: Andrzej Wincewicz, Department of Pathomorphology, Collegium Pathologicum, Medical University of Bialystok, Waszyngtona 13, 15-269 Bialystok, Poland; Phone: +48-85-7485945; Fax: +48-85-7485944; e-mail: ruahpolin@yahoo.com

Abstract

Aims and background: Erythropoietin, VEGF, VE-cadherin are involved in angiogenesis. Besides that erythropoietin stimulates erythropoiesis and increases haemoglobin and hematocrit levels as well. Moreover, erythropoietin could directly stimulate colorectal cancer cell growth due to the presence of both erythropoietin receptor and erythropoietin production in malignant cells of this neoplasm. Therefore we aimed at measurement and comparison of serum erythropoietin with VEGF, VE-cadherin levels, blood haemoglobin and hematocrit in colorectal cancer patients of different clinicopathological profiles.

Methods: We applied ELISA kits to evaluate preoperative serum levels of endogenous erythropoietin, VEGF and VE-cadherin in samples from 92 colorectal cancer patients and control group of 16 healthy volunteers.

Results: Endogenous erythropoietin was significantly elevated in preoperative sera in colorectal cancer patients ($p=0.013$) compared with healthy volunteers, however, erythropoietin levels were not significantly higher with the advancement of colorectal cancer. There were significantly higher levels of erythropoietin in the group of anaemic men in comparison to men with normal haemoglobin levels ($p<0.0001$). VEGF and VE-cadherin did not correlate with erythropoietin. Erythropoietin levels negatively correlated with haemoglobin and hematocrit levels in all cancer patients; particularly in node positive cancers (N+), moderately differentiated tumours (G2) and deeply invading neoplasms (pT3+pT4).

Conclusions: Erythropoietin levels increase in colorectal cancer but circulating erythropoietin does not associate with progression of the disease. Thus, the use of recombinant erythropoietin seems to be safe. Our results suggest that negative feedback regulation persists between haemoglobin and erythropoietin in colorectal cancer. Production of erythropoietin remains therefore anaemia-associated, hypoxia-dependent and doesn't seem to be autonomic despite abundant expression of erythropoietin by colorectal cancers.

Introduction

The effective tissue blood supply requires sufficient erythropoiesis and angiogenesis. VEGF (vascular endothelial growth factor) induces formation of vascular network during tumourgenesis [1]. Physiologically, it is obvious that erythropoietin (EPO) stimulates erythropoiesis as a result of the decreased kidney oxygen supply [2]. Moreover, EPO has also been suggested to induce angiogenesis in various cancers [3, 4]. It is firmly established that haemoglobin and hematocrit levels are associated with serum EPO in the mechanism of negative feedback. In colorectal cancer, it is regularly observed that there is a decline in haemoglobin levels and subsequent induction of endogenous EPO levels as a consequence of chemotherapy [5]. Similarly, VEGF induces maturation of human embryonic stem cells (hESCs) in the same signalling pathway of erythropoiesis [6]. As one can expect, EPO in vitro induces proliferation of endothelial cells that are organized

in capillary network of human myocardium samples, as it has been shown for VEGF [7]. However, rhuEPO was also considered as antiangiogenic agent because it reduced HIF-1 transcriptional activity and expression of VEGF in ovarian cancer cells. Nevertheless, these alterations of expression did not affect tumour growth [8]. In fact HIF-1 turns on transcription of both EPO and VEGF proteins in hypoxic environment [9, 10]. Vascular endothelial cadherin (VE-cadherin) is a component of adherens junctions among endothelial cells and preserves the tightness and stability of developed intercellular bonds in the structure of vascular network [11]. VE-cadherin and EPO can be mutually expressed in tissues of foetal blood vessels and trophoblasts, but their levels have never been compared with each other in colorectal cancer and in any other cancer as well [12]. Apart from the growth phase of organism, there are a few pathological conditions, in which the enhanced neovascularization and erythropoiesis is commonly present. The later includes diabetes mellitus and various neoplasms. However, recent studies have showed that in diabetic sera VEGF and EPO do not correlate [13]. It is apparent that EPO and VEGF perform their angiogenic functions separately through different signalling pathways. There are almost no reports on serum levels of VEGF and EPO in cancer. Similarly to the lack of relation between EPO and VEGF in sera of diabetic patients, these factors are not associated with each other at any statistical level in blood plasma of multiple myeloma patients and in tissues of central nervous system hemangioblastomas [14, 15]. However, in Hep3B cells, a cultured human hepatocellular carcinoma cell line, hypoxia led to the increased co-production of VEGF and EPO mRNA [16]. Besides angiogenic function, VEGF is thought to be an inducer of proliferation of colorectal cancer cells [17]. Similarly, EPO is frequently involved as a growth factor in progression of several neoplasms. EPO receptors (EPOR) were found in HCT-116 human colon cancers [18]. EPO certainly improves the efficiency of tissue oxygenation and, by means of it, it increases survival rates of weaken oncological patients, but proangiogenic properties of EPO have never been estimated in epithelial malignancies of large intestine. So far no comparison of endogenous EPO with VEGF, VE-cadherin haemoglobin, hematocrit has been done in sera of colorectal cancer patients. Thereby, we aimed at evaluation of their preoperative levels together with eventual correlation between them in epithelial malignancies of large intestine.

Patients and methods

Group of patients

Preoperative blood samples were obtained from 92 colorectal cancer subjects. Neither radiotherapy nor chemotherapy was applied before blood sampling. None of the patients was treated with EPO before the blood collection. In case of 7 colorectal cancer patients we failed to collect detailed pathological data because their tumours were excluded from surgical operation. All the patients were divided into two groups: the first one constituted patients older than 60 years

and the second group patients under the age of 60. Standard histopathological parameters of the tumours were determined by two independent pathologists including AJCC/UICC TNM stage (American Joint Committee on Cancer/ International Union Against Cancer Tumor Node Metastasis stage), tumour type and grade of histological differentiation (G). According to guidelines of World Health Organization (WHO), primary tumours of patients were classified as histological grade G2 (moderately differentiated) and G3 (poorly differentiated) cancers. The histological type of presented cases was diagnosed to be adenocarcinoma and mucinous adenocarcinomas. We formed two groups of patients according to the site of the tumour: rectal and colon cancers. As for the pT classification, we divided patients into two groups: the first included tumours of pT1 or pT2 (pT1+pT2), which were shallowly invading tumours confined to submucosa or muscularis propria). The second group consisted of primary cancers that were advanced at the stage of pT3 or pT4 (pT3+pT4), which were deeply invading cancers that went beyond the muscularis propria. We distinguished groups of cancers that metastasized to local lymph nodes (pN+) and tumours which did not spread to lymph nodes (pN-). We also distinguished female and male groups of anaemic and non-anaemic patients according to WHO standards, which defined anaemia to be male haemoglobin level below 13.0 g/dL and female haemoglobin level below 12.0 g/dL without reference to age or menopausal status. Control blood samples were taken from 16 healthy volunteers. Our study was performed in agreement with the ethical standards laid down in the latest revision of Declaration of Helsinki from 2004 (the project was approved by the local ethics committee of Medical University of Bialystok). All the subjects gave their informed consent before they were included in the study.

Method of detection of serum factors

We applied commercially available ELISA kits to evaluate preoperative serum levels of VEGF, VE-cadherin and EPO in samples from 92 colorectal cancer patients and control group of 16 healthy volunteers.

Determination of EPO serum levels

EPO levels were examined in the quantikine IVD erythropoietin ELISA kit as described by supplier (R&D Systems Inc.). Blood samples were warmed up to 21C and underwent coagulation, centrifugation at 760 x g* for a quarter and supernatant was collected within 30 minutes in case of each sample. After filling with 100 μL of Erythropoietin Assay Diluent, 100 μL of every serum sample was separately added in each microwell. Next, during one hour incubation at room temperature and 500 rpm of microplate shaker, EPO molecules joined their specific monoclonal murine antibodies that were attached to microwell walls. After immobilization of erythropoietin at the interior surfaces, 200 μL of erythropoietin conjugate were added in every well and incubated for one hour at room

temperature at a plate shaker. EPO conjugate was a complex of rabbit polyclonal antibody to recombinant human EPO and horseradish peroxidase. The solutions of unbound reagents were aspirated and plates were washed for 4 times in the recommended solution of the wash buffer concentrate. Then substrate solution (mixture of tetramethylbezidine and hydrogen peroxide) developed colour reaction during 25 minutes of incubation. The absorbencies were estimated at 450 nm in next 15 minutes with correction of wavelength done at 600 nm. Obtained results were transposed to serum levels of Epo (mIU/ml) with the help of the standard curve (as recommended by producer).

Determination of serum VEGF and VE-cadherin levels

The procedures of evaluation of preoperative serum VEGF (VEGF ELISA kit, PromoCell GmbH, Heidelberg, Germany) and VE-cadherin (human VE-cadherin BMS253, MedSystems Diagnostics GmbH Vienna Austria) levels were described in our previous paper [19]. It can be assumed that serum levels of VEGF do not reflect accurately level of circulating VEGF, as activated platelets release VEGF during serum sampling. Despite the difference between plasma and serum levels the proportion between them was sustained and they correlated with each other both in patients' and control groups [20, 21].

Determination of haemoglobin and hematocrit levels was performed with routine protocol that is used in everyday diagnostics.

Statistical analysis

EPO levels were compared between control group and groups of various clinicopathological features by means of Mann-Whitney test. Statistical analysis was conducted for correlation between EPO with VEGF, VE-cadherin haemoglobin and hematocrit with Spearman's rank test among different clinico-pathological groups of colorectal cancer patients. The level of statistical significance was set at 0.05.

Results

Statistical differences between serum levels of EPO from cancer patients and healthy volunteers

EPO serum levels were significantly increased in cancer patients in comparison to healthy subjects ($p=0.013$). Preoperative serum values of VE-cadherin and VEGF were compared between all colorectal cancer patients and healthy volunteers and appeared to be significantly higher in neoplasmatic sera versus controls as presented in our previous report [19-22].

Comparison of EPO levels between selected groups of colorectal cancer patients

EPO levels were significantly higher in rectal cancers (mean level of serumEPO 49,97 mIU/ml) in comparison to colon cancers (mean 35,87 mIU/ml) ($p=0.008$). Anaemia affects significantly levels of serum EPO in male populations. There were

significantly higher levels of EPO (32,53 mIU/ml on average) in anaemic men (all 35 male patients with haemoglobin below 13g/dl) in comparison to men (16,25 mIU/ml on average) with normal levels of haemoglobin (all 18 male patients with haemoglobin above 13 g/dl) ($p<0.0001$). Nevertheless, there was no statistical significance between EPO levels in anaemic and non-anaemic female colorectal patients ($p=0.562$). Besides that EPO levels did not significantly differ with respect to sex, age, histological types, grading, pT staging and nodal status, either. No significant correlations were observed for haemoglobin and hematocrit regarding to comparison of all clinico-pathological groups (data not shown).

Comparison of EPO and VEGF or VE-cadherin in selected groups of colorectal cancer patients

Levels of EPO did not show any relationship with VEGF or VE-cadherin in preoperative sera in all cases of colorectal cancer (Table 1).

Comparison of EPO with haemoglobin and hematocrit in selected groups of colorectal cancer patients

EPO preoperative serum levels negatively correlated with haemoglobin and hematocrit. Namely, decrease in the EPO levels was significantly associated with

Table 1 – Comparisons between preoperative serum levels of EPO, VEGF and VE-cadherin in CRC patients and control healthy volunteers Spearman's correlation rank test

			Comparison of serum protein values			
		Number	EPO – VEGF		EPO – VE-cadherin	
Groups of patients		of patients	p	r	p	r
All of CRC patients		92	0.840	0.021	0.576	–0.059
N	(–)	39	0.226	0.198	0.579	0.092
	(+)	46	0.367	–0.136	0.428	–0.120
G	2	54	0.719	–0.050	0.437	0.108
	3	30	0.411	–0.156	0.895	–0.025
pT	pT1+pT2	7	0.071	0.714	0.939	0.036
	pT3+pT4	78	0.800	0.029	0.735	–0.039
HP Type	Adenoca	70	0.723	0.043	0.371	–0.109
	Ad. muc	15	0.611	0.143	0.726	–0.099
Sex	Males	53	0.314	0.141	0.981	0.003
	Females	39	0.413	–0.135	0.576	–0.092
Age	≤60 years	26	0.104	0.340	0.674	0.090
	>60 years	66	0.594	–0.070	0.605	–0.068
Site	Rectum	42	0.770	–0.046	0.886	–0.023
	Colon	43	0.497	–0.106	0.882	–0.023

HP – type – Histopathologic type, pT – tumour size, N – lymph node involvement, G – grading of histological differentiation; Ad. – adenocarcinoma, Ad muc. – mucinous adenocarcionoma

In case of 7 colorectal cancer patients we failed to collect detailed, pathological data because their tumors were excluded from surgical resection, so in regard to different subgroups total sum of patients is 85.

the increase in haemoglobin or hematocrit levels. This negative correlation was especially evident in case of further advancement of colorectal cancer (Table 2)

Discussion

Regarding to our study, malignant growth appears to be a cause for increase of EPO levels in preoperative blood samples from colorectal cancer patients. However preoperative level of EPO is useless to indicate progression of cancer development as there are no significant differences among levels of EPO in groups of various stage, grade, histopathological type of tumours and clinical features of patients. EPO is produced in response to hypoxia which is associated with anaemia [23]. Anaemia was reported to cause significant increase in serum EPO. However, colorectal cancer associated anaemia is probably not the cause of EPO elevation in sera of studied patients because we demonstrated serum levels of EPO being influenced by the level of haemoglobin in male patients only. Moreover, female levels of EPO did not differ significantly in anaemic and non-anaemic women. Such lack of statistical significance suggested that female patients of our study were better adaptable to anaemia; probably because women are naturally adapted to deal with regular blood loss during menstrual cycle. EPO measurement might be advantageous at least because of its predictive value in case of cancer patients' response to recombinant EPO. Endogenous serum EPO levels were reported to be significantly lower in the responders versus the non-responders to rHuEPO [24].

Table 2 – Correlations between preoperative serum levels of EPO, Hb and Ht in CRC patients and control healthy volunteers Spearman's correlation rank test

		Comparison of given levels			
		EPO – Hb		EPO – hematocrite	
Groups of patients		p	r	p	r
All of CRC patients		<0.0001	–0.404	0.001	–0.341
N	(–)	0.106	–0.263	0.197	–0.211
	(+)	0.001	–0.456	0.005	–0.404
G	2	0.001	–0.424	0.005	–0.377
	3	0.253	–0.215	0.494	–0.130
pT	pT1+pT2	0.879	–0.071	0.879	–0.071
	pT3+pT4	<0.0001	–0.412	0.003	–0.337
HP Type	Adenoca	<0.0001	–0.424	0.001	–0.382
	Ad. muc	0.907	–0.033	0.636	0.133
Sex	Males	<0.0001	–0.563	<0.0001	–0.510
	Females	0.165	–0.227	0.296	–0.172
Age	≤60 years	0.082	–0.362	0.198	–0.272
	>60 years	0.002	–0.386	0.012	–0.318
Site	Rectum	0.028	–0.339	0.012	–0.386
	Colon	0.032	–0.328	0.227	–0.188

Endogenous erythropoietin has recently been reported not to stimulate growth of tumour cell lines which express EPO receptor [25]. Such a receptor is also present in colorectal cancers [26]. Based on our present work it can be assumed that, serum erythropoietin levels play rather minor role in the stimulation of cancer growth, since there was no association between EPO serum levels and histopathological features of the colorectal cancers. However, we did not refer serum levels of EPO to presence of distant metastases. Therefore, our results might imply that EPO administration to overcome anaemia is beneficial in colorectal cancer as it was proved in various disorders [24, 27–29]. In the present work we also demonstrated the significant increase in serum cancer EPO levels in comparison to healthy subject levels as well as the relationship of EPO, haemoglobin and hematocrit in patients' preoperative blood samples. Particularly, negative correlations between EPO and haemoglobin or hematocrit levels showed that the lower EPO levels were accompanied with a higher haemoglobin or hematocrit levels, especially in greater advancement of colorectal cancer. Such a coincidence of serum EPO decline and increase in haemoglobin or hematocrit levels may suggest that negative feedback regulation persists between haemoglobin and EPO in colorectal cancer. Our results could also negate autonomy of production EPO by cancer cells and our study may imply that EPO generation remains anaemia-associated and hypoxia-dependent in colorectal cancer. It seems that more sufficient blood supply and better oxygenation (resulted from high haemoglobin or hematocrit levels) of tumours favours faster growth and spread of malignancy and causes decrease in endogenous EPO level in patients with colorectal cancers. Therefore we suggest that the use of recombinant EPO is probably devoid of that particular side-effect of essential stimulation of cancer growth and it can be applied to combat anaemia safely in colorectal cancer [30].

The fact that serum levels of VEGF and EPO did not correlate with each other suggest that they do not coordinate their actions in development of vascular bed during cancer disease. The lack of correlations between VE-cadherin and EPO probably results from completely different pathway of VE-cahderin induction, which – unlike EPO and VEGF – was not reported to be dependent on HIF-1 [31]. The vascular effect of VE cadherin is also different from that one of VEGF as VE-cadherin binds tightly endothelial cells to shape vascular tubes in contrast to VEGF which loosens their junctions to migrate and settle in new areas for blood supply [19]. The lack of correlations between EPO and VE-cadherin serum levels might indicate diverse functions of those proteins as well.

Conclusion

Erythropoietin levels are up-regulated in colorectal cancer but serum erythropoietin doesn't associate with progression of the disease. Therefore, therapeutic administration of recombinant erythropoietin seems to be safe if it is needed to combat cancer related anaemia. Our results suggest that negative

feedback regulation persists between haemoglobin, as a marker of anaemia and erythropoietin in colorectal cancer. In the light of our findings, production of erythropoietin remains anaemia-associated, hypoxia-dependent and doesn't seem to be autonomic despite abundant expression of erythropoietin by colorectal cancers.

References

1. TERLIKOWSKI S., LENCZEWSKI A., SULKOWSKA M., FAMULSKI W., SULKOWSKI S., KULIKOWSKI M.: Tissue expression of VEGF as a prognostic factor in early cervical squamous cell carcinoma. *Folia Histochem Cytobiol.* 39: 112–113C, 2001.
2. SCHMIDT E. K., FICHELSON S., FELLER S. M.: PI3 kinase is important for Ras, MEK and Erk activation of Epo-stimulated human erythroid progenitors. *BMC Biol.* 2: 7, 2004.
3. RIBATTI D., POLIANI P.L., LONGO V., MANGIERI D., NICO B., VACCA A.: Erythropoietin/ erythropoietin receptor system is involved in angiogenesis in human neuroblastoma. *Histopathology.* 50: 636–641, 2007.
4. RIBATTI D., MARZULLO A., GENTILE A., LONGO V., NICO B., VACCA A., DAMMACCO F.: Erythropoietin/erythropoietin-receptor system is involved in angiogenesis in human hepatocellular carcinoma. *Histopathology.* 50: 591–596, 2007.
5. CANAPARO R., CASALE F., MUNTONI E., ZARA G. P., DELLA PEPA C., BERNO E., PONS N., FORNARI G., EANDI M.: Plasma erythropoietin concentrations in patients receiving intensive platinum or nonplatinum chemotherapy. *Br. J. Clin. Pharmacol.* 50: 146–153, 2000.
6. CERDAN C., ROULEAU A., BHATIA M.: VEGF-A165 augments erythropoietic development from human embryonic stem cells. *Blood* 103: 2504–2512, 2004.
7. JAQUET K., KRAUSE K., TAWAKOL-KHODAI M., GEIDEL S., KUCK K. H.: Erythropoietin and VEGF exhibit equal angiogenic potential. *Microvasc. Res.* 64: 326–333, 2002.
8. LENZ T., GAUER S., WEICH H. A., HAAK T., BERGNER R., GOSSMANN J.: Vascular endothelial growth factor and its soluble receptor, Flt-1, are not correlated to erythropoietin in diabetics with normal or reduced renal function. *Nephrology.* 10: 84–89, 2005.
9. MICHEL G., MINET E., MOTTET D., REMACLE J., MICHIELS C.: Site-directed mutagenesis studies of the hypoxia-inducible factor-1alpha DNA-binding domain. *Biochim. Biophys. Acta* 1578: 73–83, 2002.
10. MINET E., MICHEL G., REMACLE J., MICHIELS C.: Role of HIF-1 as a transcription factor involved in embryonic development, cancer progression and apoptosis. *Int. J. Mol. Med.* 5: 253–259, 2000.
11. HALE S. A., WONG C., LOUNSBURY K. M.: Erythropoietin disrupts hypoxia-inducible factor signaling in ovarian cancer cells. *Gynecol. Oncol*. 100: 14–19, 2006.
12. CROSBY C. V., FLEMING P. A., ARGRAVES W. S., CORADA M., ZANETTA L., DEJANA E., DRAKE C. J.: VE-cadherin is not required for the formation of nascent blood vessels but acts to prevent their disassembly. *Blood* 105: 2771–2776, 2005.
13. PAVLOV N., HATZI E., BASSAGLIA Y., FRENDO J. L., BRION D. E., BADET J.: Angiogenin distribution in human term placenta, and expression by cultured trophoblastic cells. *Angiogenesis* 6: 317–330, 2003.
14. DI RAIMONDO F., AZZARO M. P., PALUMBO G., BAGNATO S., GIUSTOLISI G., FLORIDIA P., SORTINO G., GIUSTOLISI R.: Angiogenic factors in multiple myeloma: higher levels in bone marrow than in peripheral blood. *Haematologica* 85: 800–805, 2000.
15. KRIEG M., MARTI H. H., PLATE K. H.: Coexpression of erythropoietin and vascular endothelial growth factor in nervous system tumours associated with von Hippel-Lindau tumour suppressor gene loss of function. *Blood* 92: 3388–3393, 1998.

16. SUGIMACHI K., TANAKA S., TAGUCHI K., AISHIMA S., SHIMADA M., TSUNEYOSHI M.: Angiopoietin switching regulates angiogenesis and progression of human hepatocellular carcinoma. *J. Clin. Pathol.* 56: 854–860, 2003.
17. CALVIELLO G., DI NICUOLO F., GRAGNOLI S., PICCIONI E., SERINI S., MAGGIANO N. TRINGALI G., NAVARRA P., RANELLETTI F.O., PALOZZA P.: n-3 PUFAs reduce VEGF expression in human colon cancer cells modulating the COX-2/PGE2 induced ERK-1 and -2 and HIF-1alpha induction pathway. *Carcinogenesis* 25: 2303–2310, 2004.
18. HARDEE M.E., KIRKPATRICK J.P., SHAN S., SNYDER S.A., VUJASKOVIC Z., RABBANI Z.N., DEWHIRST M.W., BLACKWELL K.L.: Human recombinant erythropoietin (rEpo) has no effect on tumour growth or angiogenesis *Br. J. Cancer* 93:1350–1355, 2005.
19. SULKOWSKA M., FAMULSKI W., WINCEWICZ A., MONIUSZKO T., KEDRA B., KODA M., ZALEWSKI B., BALTAZIAK M., SULKOWSKI S.: Levels of VE-cadherin increase independently of VEGF in preoperative sera of patients with colorectal cancer. *Tumouri* 92: 67–71, 2006.
20. ANTHONY F. W., EVANS P. W., WHEELER T., WOOD P. J.: Variation in detection of VEGF in maternal serum by immunoassay and the possible influence of binding proteins. *Ann. Clin. Biochem.* 34: 276–280, 1997.
21. GEORGE M. L., ECCLES S. A., TUTTON M. G., ABULAFI A. M., SWIFT R. I.: Correlation of plasma and serum vascular endothelial growth factor levels with platelet count in colorectal cancer: clinical evidence of platelet scavenging? *Clin. Cancer Res.* 6: 3147–3152, 2000.
22. FAMULSKI W., SULKOWSKA M., WINCEWICZ A., KEDRA B., PAWLAK K., ZALEWSKI B., SULKOWSKI S., KODA M., BALTAZIAK M.: P53 correlates positively with VEGF in preoperative sera of colorectal cancer patients. *Neoplasma* 53: 43–48, 2006.
23. STASI R., BRUNETTI M., BUSSA S., CONFORTI M., DI GIULIO C., CRESCENZI A. TERZOLI E., VECCHIONE A., PAGANO A.: Response to recombinant human erythropoietin in patients with myelodysplastic syndromes. *Clin. Cancer Res.* 3733–3739, 1997.
24. FJORNES T., WIEDEMANN G. J., SACK K., JELKMANN W.: Serum erythropoietin and creatinine concentrations as predictive factors for response to recombinant human erythropoietin treatment in anaemic tumour patients on chemotherapy. *Oncol. Rep*. 5: 81–86, 1998.
25. WESTPHAL G., NIEDERBERGER E., BLUM C., WOLLMAN Y., KNOCH T. A., REBEL W.: Erythropoietin and G-CSF receptors in human tumour cells: expression and aspects regarding functionality. *Tumouri* 88: 150–159, 2002.
26. CHABOWSKA A., SULKOWSKA M., CHABOWSKI A., WINCEWICZ A., KODA M., SULKOWSKI S.: Erythropoeitin and Erythropoeitin Receptor expression in Colorectal Cancer. *Int. J. Surg. Pathol.* 2008 in printing.
27. SULKOWSKA M., WINCEWICZ A., CHABOWSKA A., KANCZUGA-KODA L., SZYMANSKA M., KODA M., WITKOWSKA E., SULKOWSKI S.: To Give or Not to Give Recombinant EPO to Anemia Endangered Cancer Patients. *Prague Med Rep*. 107: 281–289, 2006.
28. ARDIZZONI A., CAFFERATA M. A., ROSSO R.: Epoietin alfa in lung cancer. *Tumouri* 84: S20–26, 1998.
29. MILANO M., SCHNEIDER M.: EPO in cancer anemia: benefits and potential risks. Crit Rev *Oncol. Hematol.* 62: 119–125, 2007.
30. CHRISTODOULAKIS M., TSIFTSIS D. D.; Hellenic Surgical Oncology Perioperative EPO Study Group. Preoperative epoetin alfa in colorectal surgery: a randomized, controlled study. *Ann. Surg. Oncol.* 12: 718–725, 2005.
31. MALOYAN A., ELI-BERCHOER L., SEMENZA G.L., GERSTENBLITH G., STERN M. D., HOROWITZ M.: HIF-1alpha-targeted pathways are activated by heat acclimation and contribute to acclimation-ischemic cross-tolerance in the heart. *Physiol Genomics.* 23: 79–88, 2005.