Plasma VEGF-A and its soluble receptor R1 correlate with the clinical stage of colorectal cancer

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Abstract

Our aim was to assess plasma VEGF-A and its soluble receptor R1 (sVEGF-R1) in patients with colorectal cancer, in comparison with apparently healthy subjects.

Methods. Samples of plasma were collected from 26 patients with colorectal cancer before surgery and on post-operative day 3 and 10, frozen and eventually assessed, using ELISA kits.

Results. We found an increase in VEGF-A in colorectal cancer patients and a strong positive correlation between metastatic spread and postoperative VEGF-A, which also correlated with CA19.9. Soluble VEGF-R1 showed a positive correlation with lymph node involvement. Conclusions. Our findings may suggest that the patients with high postoperative VEGF-A should be scanned for metastases. In view of our finding that sVEGFR-1 correlates with lymph node involvement, precautions must be taken in studies of sVEGFR-1 as a potential therapeutic agent.

Key words: colorectal cancer, VEGF-A, sVEGF-R1

Introduction

In most countries of the world, colorectal cancer is an increasing cause of morbidity and mortality. Although early surgical treatment can be curative, many patients already have metastases at the time of operation. Moreover, many patients present in late stages of the disease. In such cases, chemotherapy is warranted. Thus, it is of crucial importance to know, whether the malignancy is localised or disseminated. Modern imaging often permits localisation of macrometastases, however, in order to detect micrometastases, more sensitive methods are needed. Molecular markers of solid cancers are of high potential value in such circumstances [1].

Neo-angiogenesis is of crucial importance for colorectal cancer growth and nutrition, with vascular endothelial growth factor (VEGF) being the most important cytokine, involved in the process [2]. VEGF plays an important role in progression, invasion and spread of colorectal cancer by influencing the proliferation and migration of endothelial cells. Because of the positive correlation between plasma concentration of VEGF and the size of tumour in patients with colorectal cancer [3], there have been attempts to inhibit VEGF synthesis in patients with advanced colorectal cancer [1].

Total VEGF is not a sufficient diagnostic tool in patients with colorectal cancer because of small sensitivity (36%) [3]. VEGF comprises a number of cytokines: VEGF-A, VEGF-C, VEGF-D and VEGF-E. VEGF-A is most abundantly expressed in colorectal cancer tissues and, therefore, it seems to be of greater value then total VEGF [2].

The group of proteins, named together as VEGF, acts on two main specific receptors, marked with number 1 (VEGFR-1), 2 (VEGFR-2) and 3 (VEGFR-3). Out of the three, VEGFR-1 has been reported to be most important for solid malignancies [2]. Also the soluble form of VEGFR-1 (sVEGFR-1) has recently been demonstrated in blood. It acts in vitro as antagonist for VEGF-A and inhibits the biological activity of VEGF-A in vivo [4]. SVEGFR-1 is probably the only known and naturally occurring endogenous antagonist to VEGFR-1 receptor. As such, it was postulated to have an important role in the treatment aiming at angiogenesis inhibition [5].

The aim of the study was to investigate sVEGFR-1 and VEGF-A in plasma of patients with colorectal cancer, before and after surgical treatment in relation to chosen clinical and laboratory parameters, such as: sex, age, BMI, tumour localisa-

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Table 1. Concentrations of VEGF-A and sVEGFR-1 in the control group ("Control") and in patients with colorectal cancer ("Pre-op" - preoperatively; "Post-op" - postoperatively). Data, given as the mean ± standard deviation; p values of comparison between the studied group and the control group are shown in brackets.

	Control [pg/ml]	Pre-op [pg/ml]	Post-op day 3 [pg/ml]	Post-op day 10 [pg/ml]
VEGF-A	38.3 ± 13.1	83.36 ± 151.61	79.53 ± 125.47	114.7 ± 198.13
p		**(p=0.0066**)**	**(p=0.0037**)**	**(p=0.0041**)**
sVEGFR-1	192 ± 245	83.4 ± 154.6	79.5 ± 125.5	114.2 ± 198.1
p		(p=0.546)	(p=0.336)	(p=0.485)

Table 2. Chosen correlations between pathological or laboratory parameters and the concentrations of VEGF-A and sVEGFR-1 in patients with colorectal cancer.

Correlation between	p
Preoperative VEGF-A and distant metastases	0.329
Postoperative VEGF-A (day 3) and distant metastases	**0.046***
Postoperative VEGF-A (day 10) and distant metastases	**0.031***
Postoperative VEGF-A (day 10) and postoperative CA19-9	**0.027***
Postoperative VEGF-A (day 3) and lymphocyte count	**0.045***
Preoperative sVEGFR1 and distant metastases	0.212
Preoperative sVEGFR1 and lymph node involvement	**0.015***
Postoperative sVEGFR1 (day 3) and distant metastases	0.798

tion within the bowel, Dukes' or clinical stage, the presence of distant metastases, lymph node involvement and albumins in serum.

Material and methods

The studies were performed on plasma samples, collected from 26 patients with colorectal cancer, treated at the 2nd Department of General and Gastroenterological Surgery, Medical University of Bialystok. The Control group consisted of 18 apparently healthy subjects: 8 men and 11 women, aged 32 to 78 (mean 65).

Among the patients with colorectal cancer, there were 11 men and 15 women. The mean age was 69.2 years (58 to 83 years). The cancer was localized in the rectum of 11 patients, in the sigmoid colon of 7, in the cecum of 3, in the ascending colon of 3, in the descending colon in 1 and in the transverse colon in 1 patient. Nineteen (19) patients underwent curative resection and 7 patients - palliative resection. In all the cases, pathological examination showed adenocarcinoma. According to Dukes' classification 11 patients were at stage B, 3 patients at C1, 5 at C2 and 7 at stage D. In all the patients with colorectal cancer, samples were collected before surgery and on the post-operative day 3 and 10. Patients and volunteers with either clinical or laboratory signs of infection were excluded from the study. All the subjects signed an informed consent.

Blood samples were collected after overnight fasting: 4.5 ml venous blood was poured into a tube, containing 0.5 ml 3.8% sodium citrate. Immediately after sampling, the specimens were centrifuged at 3.5 G for 10 minutes in 4°C. The supernatant-platelet poor plasma was transported to an Eppendorf's tube and frozen in -70°C until determination. The plasma samples were used for ELISA tests. VEGF-A was determined, according to George et al. [6] and sVEGFR-1 - as previously described [7], using a commercially available ELISA kits, purchased from the Research Diagnostics Inc., USA.

The following tests were used for statistical analysis: Shapiro-Wilk test for normality, Kruskal-Wallis test for variance analysis, Mann-Whitney test for inter-group comparison, Spearman's and Chi2 tests for correlations. A value of $p<0.05$ was regarded as significant for all the tests.

Results

We found a significant increase in VEGF-A concentrations in plasma of all the groups of patients with colorectal cancer (Tab.1), as compared to values in the control group ($p<0.01$). sVEGF-R1 (Tab.1) was detectable in 17 / 26 (65.4%) of cancer patients and in 15 / 18 (83.3%) of control subjects. The values of sVEGF-R1 tended to be lower in the studied group than those in the control group, however not significantly (p=0,336) (Tab.1). We found a positive correlation of the presence of distant metastases with VEGF-A concentration in plasma (Tab. 2), as assessed on the postoperative days 3 and 10 ($p<0.05$). There was also a negative correlation of VEGF-A in plasma on the postoperative day 10 with lymphocyte count ($p<0.05$) and a positive correlation of VEGF-A in plasma on the postoperative day 10 with CA 19.9 ($p<0.05$).

As far as preoperative sVEGF-R1 is concerned, we found its positive correlation with lymph node involvement, assessed on the basis of pathological examinations of surgical specimens (Tab.2). No correlations were observed for either the sex, the age, BMI tumour localisation within the bowel, Dukes' or clinical stage or for albumins in serum.

Discussion

VEGF-A expression has previously been studied in colorectal cancer tissues and showed an increased expression, as compared to its values in healthy subjects. An association of VEGF-A tissue expression was also reported with tumour grade and size [2]. It has been shown that VEGF-A mRNA expression in colorectal mucosa strongly correlates with liver metastases and that VEGF-A concentration is an independent predicting factor for the survival of patients with colorectal cancer [8].

Our results, concerning VEGF-A, confirm its significant correlation with the clinical stage of colorectal cancer. We demonstrated a significant increase of the cytokine level in plasma of the patients, as compared with that in healthy subjects. We found a strong positive correlation of VEGF-A plasma concentration, assessed postoperatively, with presence of distant metastases. That was in line with postoperative CA19.9 values. We also observed a moderate, though not significant, increase in VEGF-A level following a surgical procedure, performed in colorectal cancer patients. This might be attributable to the breakdown of thrombocytes and neutrophils, containing considerable amounts of VEGF, in result of surgical manipulations [9]. It was for the first time in 2002 that soluble VEGFR-1 was reported to be detectable in patients with colorectal cancer [5]. Chin et al. [8] reported initially that sVEGFR-1 in plasma could be detectable in 74% of patients with colorectal cancer. In their recent report, the detection was much lower [4]. The authors observed a suppression of sVEGFR-1 in patients with colorectal cancer. In our study, no statistical differences were observed in sVEGFR-1 plasma concentration in colorectal cancer patients, as compared to observed values in the normal controls. However, our observation of the highly significant positive correlation of sVEGFR-1 with lymph node involvement in colorectal cancer might be of importance.

In sum: the presented data may suggest that patients with high postoperative values of VEGF-A should be scanned for metastases. In view of our finding that sVEGFR-1 strongly correlates with lymph node involvement by colorectal cancer, precautions should be taken in studies of sVEGFR-1 as a potential therapeutic agent.

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