Clinical significance of Vascular Endothelial Growth Factor in Egyptian colorectal cancer patients

Mie Afify 1,*, Nirvana Samy 1, Maha Hashim 1, Tarek Essam 2

1 Biochemistry Department, National Research Center, Cairo
2 Surgical Department, National Cancer Institute, Cairo University

Submitted: 1 Nov. 2008; Accepted: 1 Dec. 2008

Abstract
Angiogenesis represents an essential step in tumor proliferation, expansion, and metastasis. Among angiogenic molecules, vascular endothelial growth factor (VEGF) appears to have a central role. The objective of the current study was to assess the prognostic significance of VEGF plasma level as an indicator of recurrent disease in patients with colorectal carcinoma scheduled for surgery with curative intent, and to determine its relationship with disease stage at presentation. Also, we studied the protein expression pattern of VEGF isoforms in colorectal tumors in comparison to the corresponding adjacent normal tissues. Preoperative and postoperative plasma VEGF, Serum CEA, CA19-9 levels were determined by ELISA and vascular endothelial growth factor Protein expression was detected in 78 patients with colorectal cancer scheduled for surgery. Fifty healthy individuals served as a control group. This study showed that preoperative VEGF, carbohydrate antigen 19.9 (CA 19-9) and carcinoembryonic antigen (CEA) levels were significantly high in patients with colorectal carcinoma compared to control group. Moreover, VEGF plasma levels were significantly lowered in patients who underwent curative surgery, with a further downward trend until the 6 month postoperative (P<0.05). Multiple regression analysis demonstrated significant positive correlation (r) between preoperative VEGF plasma levels and Dukes stages, TNM stages, T classes, CEA levels, and CA 19-9 (P<0.05). Combining of VEGF and CEA markers result in significant increase in the specificity, positive predictive value (PPV) and accuracy (P<0.05) in comparison with CEA alone. Over-expression of VEGF in colorectal cancer tissue was confirmed by western blot (WB); a main protein band was detected with molecular weight of 23 kDa (VEGF165), which was expressed in both normal and tumor tissues of colorectal, but the expression of VEGF165 was detected at higher levels in tumor tissues and the intensities of the bands varied according to plasma VEGF concentrations. This study revealed that preoperative plasma VEGF concentrations might be valuable parameter for tumor burden and predicting the outcome of colorectal cancer patients. Also, post-operative VEGF plasma level can be considered as indicator for recurrent disease. VEGF165, considered as a predominant form of VEGF expression secreted by a variety of normal and transformed cells.

Keywords: colorectal cancer, vascular endothelial growth factor (VEGF), carcinoembryonic antigen (CEA), carbohydrate antigen, VEGF isoforms.

INTRODUCTION
Colorectal carcinoma (CRC) is one of the leading causes of cancer death in both developed and developing nations. Worldwide, 900,000 new cases of colorectal carcinoma are diagnosed each year and colorectal carcinoma accounts for nearly 500,000 cancer deaths annually (Parkin et al., 2001). Globally, colorectal carcinoma is the third most common cancer in the world, contributing 8.9% of all cancers. The disease is more common in developed than in developing countries and this is attributed to differences in diet. Thus, in developed countries, the high consumption of fat in diet is associated with increased risk of colorectal cancer, whereas, in developing countries the mostly vegetarian diet plays a protective role (El-Bolkainy et al., 2006). In USA colorectal cancer constitutes 9.5% of all cancers (Jemal et al., 2008). In Egypt it contributes 6.5% of all cancers. The site of distribution of colorectal cancer also varies in developed and developing countries, in USA rectal carcinoma constitutes 25% of all colorectal cancer but in Africa it is 50% (El-Bolkainy et al., 2006).

In Egypt, CRC has unique characteristics that differ from that reported in other countries of the western society. It was estimated that 35.6% of the Egyptian...
CRC cases are below 40 years of age (Soliman et al., 1997). Colorectal cancer in Egypt has no age predilection and more than one-third of tumors affect a young population. The high prevalence in young people can neither be explained on a hereditary basis nor can it be attributed to Bilharziasis (Abou-Zeid et al., 2002).

Currently, the most important factor predictive for survival is regional lymph node status at the time of initial surgery. However, this is not sufficient to predict outcomes accurately. Approximately 20% of patients with Stage II disease (i.e., without regional lymph node involvement) and approximately 50% of patients with Stage III disease will not be alive 5 years after curative resection (Midgley et al., 1999). Although much has been learned regarding the molecular pathogenesis of colon carcinoma in the past 20 years, the elucidation of markers of prognosis that also could serve as therapeutic targets is necessary to better understand and improve outcomes (Karayiannakis et al., 2002).

Tumor stage is still the most important prognostic factor in patients with CRC. Although there have been a lot of reports showing the significance of many other prognostic parameters including histological grade, age, serum carcinoembryonic antigen (CEA) levels, gender, location of the primary tumor, histological subtypes and flow cytometric DNA analysis, none of those has been widely used in the clinic (Skibber et al., 2001). The fact that solid tumor growth and metastasis is angiogenesis-dependent suggests a potential value of blood and tissue angiogenic markers as prognostic and survival determinants. Neo-angiogenesis which is of great importance for tumor growth and nutrition, is preferentially mediated by the cytokine vascular endothelial growth factor (VEGF), which is a dimeric, heparin-binding glycoprotein that functions as the most potent angiogenic protein known, and is elevated in serum of colorectal cancer patients. It has a direct effect on vascular endothelial cell proliferation and migration (Papetti et al., 2002).

As a result of alternative splicing, 6 VEGF isoforms of 121, 145, 165, 183,189 and 206 amino acids are produced from a single gene. Due to differential incorporation of basic residues encoded by exon 6 and 7, VEGF isoforms differ in their heparin-binding properties, membrane association, and secretion. VEGF121, which lacks the basic residues of both exons, does not bind heparin-containing cell surface proteoglycan, and is freely soluble. VEGF165 is also secreted; however, cationic residues in exon 7 enable VEGF165 to bind heparin, thus, some remains bound to the cell surface or to extracellular matrix. VEGF189 which retains both exons has the highest affinity for heparin and therefore, remains tightly cell associated (Cressey et al., 2005).

A clear understanding of the clinical utility of plasma and serum tumor markers remains a major goal in the diagnostic area of clinical oncology. Therefore, the aim of this study was to determine: 1) the efficacy of serum markers; CEA, carbohydrate antigen 19-9 (CA 19-9) and plasma VEGF as "decision making" clinical parameters used in combination with accepted diagnostic procedures in colorectal carcinoma patients; 2) the prognostic significance of the post-operative VEGF plasma level as an indicator of recurrent disease after curative surgery; 3) the protein expression pattern of VEGF isoforms in colorectal tumors in comparison to the corresponding adjacent normal tissues.

SUBJECTS AND METHODS

After approval of the medical and ethical committee of the National Cancer Institute, Cairo University, this study was conducted on 78 patients (51 males, 27 females, mean age 54 years, with a range of 26–63 years) with a histologically proven colorectal carcinoma {colon: n = 32 (41.1%), rectum: n = 46 (58.9%)}. They were scheduled for curative resection of the tumor, from the Surgical Department at National Cancer Institute, Cairo University. Clinical staging was expressed according to the TNM and Dukes’ classification system based on evaluation of findings of physical examination, routine laboratory tests, radiological reports and pathological records. All the patients had their primary tumors resected and none had been given chemotherapy or radiotherapy before or after the operation. A group (n=50) of healthy volunteers (29 males, 21 females; mean age 52.1 years, range: 25–56 years) served as a control group. Both groups (patients and controls) were comparable in mean age, age distribution and gender. Informed consent was obtained from all patients and healthy subjects. The exclusion criteria are: recent trauma, surgery, pregnancy and concomitant diseases suspected of raising VEGF plasma levels (i.e., chronic inflammatory disorders, diabetes mellitus, and ischemic heart disease).

Sampling

Preoperative peripheral blood samples were drawn from the patients on the day of their operation and another two post-operative blood samples at day 7 and 6 month. Ten ml of fasting venous blood samples were collected from each subject. Five ml blood put in tubes containing EDTA to separate the plasma after centrifuging for 10 minutes. The other five ml blood was left to clot at room temperature to separate sera after centrifuging for 10 minutes at 3000 r.p.m. Sera were separated, divided into several aliquots and stored at – 70°C until assay.
Colorectal tissue samples were taken from partial colon resection for carcinoma. Samples were obtained by taking biopsies of the fresh specimen from a non-necrotic central portion of the tumor and from a peripheral part of the macroscopically normal colonic epithelium. Samples were divided into two aliquots: the first was formalin-fixed and paraffin embedded for histopathological diagnosis; the second was frozen at −80°C for performing VEGF protein expression assays.

All subjects were subjected to careful history taking, thorough clinical examination, chest x-ray, abdominal sonography, abdominal computed tomography [CT] scans; endoscopy, laparoscopy followed by biopsy to define the diagnosis.

**Laboratory investigations**

**vascular endothelial growth factor (VEGF)**

Plasma vascular endothelial growth factor (VEGF) level was detected by use of a solid phase sandwich Enzyme Linked-Immunosorbent Assay (ELISA) using kit supplied from (Biosource International, California, USA). A polyclonal antibody specific for human VEGV has been coated onto the wells of the microtitre strips to capture VEGF in samples. VEGF activity is detected with a substrate solution (Biotin-Steptavidin-HRP detection). The intensity of this colored product is directly proportional to the concentration of human VEGF present in original samples (Yoshikawa et al., 2000).

**Serum Carcinoembryonic antigen (CEA)**

Serum Carcinoembryonic antigen (CEA) level was detected by use of (ELISA) using kit supplied from (Quorum diagnostics). A monoclonal antibody specific for human CEA has been coated onto the wells of the microtitre strips to capture CEA in samples. VEGF activity is detected with a substrate solution (biotin-steptavidin-HRP detection). The intensity of this colored product is directly proportional to the concentration of human CEA present in original samples (Chieregatti, 1990).

**Serum CA 19-9**

Serum CA 19-9 level was detected by use of ELISA using kit supplied by Panomics, Redwoodcity, USA. The assay system utilizes a polyclonal anti-CA 19-9 antibody directed against intact CA 19-9 for solid phase immobilization on the microtiter wells. A monoclonal anti-CA 19-9 antibody conjugated to horseradish peroxidase (HRP) is in the antibody-enzyme conjugate solution. The test sample is allowed to react first with the immobilized polyclonal antibody. The wells are washed to remove any unbound antigen. The monoclonal-HRP conjugate is then reacted with the captured antigen, resulting in the CA 19-9 molecules being sandwiched between the solid phase and enzyme-linked antibodies. The wells are washed with water to remove unbound labeled antibodies. A TMB Reagent solution is added, resulting in the development of a blue color. The color development is stopped with the addition of Stop Solution, which changes the color to yellow, and the absorbance is measured using a spectrophotometer at 450 nm. The concentration of CA 19-9 is directly proportional to the color intensity of the test sample (Magnani, 2004).

**serum aspartate transaminase (AST)**

Determination of serum aspartate transaminase (AST) and serum alanine transaminase (ALT) by using the method recommended by the Committee on Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology (Committee on enzymes, 1974), the test was performed using already commercially available kit from Boehringer-Mannhiem Company, Germany.

**Serum gamma glutamyle transferase (γGT)**

Serum gamma glutamyle transferase (γGT) was detected by using the method recommended by the Committee on Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology (Committee on enzymes, 1976), the test was performed using already commercially available kit from Boehringer-Mannhiem Company, Germany.

**Total protein**

Total protein was determined by using photometric colorimetric test by Biuret method according to Weichselbaum (Weichselbaum, 1946), cupric ions react with serum protein in alkaline medium to form a purple complex. The absorbance of this complex is proportional to the protein concentration in the sample. The reaction was performed by using commercially available kit from Bio Systems Company, Spain.

**albumin**

Determination of albumin by immunodiffusion technique according to Mancini (Mancini et al., 1965) and the recommendations of Ingild (Ingild, 1983), and A/G ratio were calculated.

**serum urea and creatinine**

Determination of serum urea and creatinine by use of Synchron CX7 autoanalyzer of Beckman (Beckman Instruments, INC.Fullerton, CA92834-3100).

**Vascular endothelial growth factor Protein expression**

Vascular endothelial growth factor Protein expression was done by Western Blotting (WB) technique.
Western blot was performed according to Sambrook (Sambrook et al., 1998) and adapted by Eissa (Eissa et al., 2004). Briefly, proteins from 60 µg cell lysates were separated by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The gels were trans-blotted to nitrocellulose (NC) filters in Tris-glycine buffer (25 mmol/l Tris, 192 mmol/l glycine, 20% methanol, and pH 7.4) for 5 h at 60 volt. The NC sheets were washed and the unoccupied binding sites were saturated with blocking solution (Chromogenic Western blotting kit, Biorad-Roche Diagnostics, GmbH, Germany) for 1 h at 37°C and then were incubated with anti-VEGF antibodies overnight at 4°C (VEGF165 protein standard (R&D System, USA). The membranes were washed with phosphate-buffered saline containing 0.3% Tween. The antibodies bound to the NC membrane were visualized by incubation with anti-mouse IgG-alkaline phosphatase conjugate for 90 minutes at room temperature. Finally, the filters were incubated with alkaline phosphatase substrate solution (5-bromo-4chloro-3-indoyl phosphate/nitroblue tetrazolium in 0.1mol/l Tris-buffer) at room temperature till the developed bands reached the desired intensity, and then the reaction was stopped by adding 200 µl of 0.5 mol/l EDTA (pH 8.0) and 50ml of phosphate buffered saline. Comparison of the NC results with others where normal mouse IgG serum was substituted for VEGF Ab permitted identification of the VEGF band.

Statistical analysis

Statistical analysis was performed using the SPSS software package for Windows [SPSS (UK) Ltd., Surrey, United Kingdom]. ANOVA was used to determine the difference between the means of the groups. Further analysis was carried out using a nonparametric test for two independent samples (Mann-Whitney U test), whereas t-test was used for continuous variables. The predictive value of the tumor markers for the detection of CRC stages was expressed in terms of sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and efficacy. The optimal cut-off value was estimated from Receiver operating characteristic (ROC) curve (Henderson et al., 1993), P-value < 0.05 is considered significant.

RESULTS

The study included 78 (51 males and 27 females) consecutive patients who had undergone curative resection of primary CRC. The mean age of the patients at the time of surgery was 54 ± 8 years. All patients had histologically verified carcinoma localized in the colon or in the rectum and were staged according to the TNM and Dukes’ classifications (Wiley- Liss; Am. Joint Committee on Cancer [AJCC]). The clinical data and biochemical profile of the subjects enrolled in this study is presented in Table 1 [Supplementary data] liver function and kidney function were done to assess the clinical state of patients.

No significant differences were found between plasma VEGF, serum CA 19-9 and CEA preoperative levels and disease site, gender, or histological types of the tumor. While as regard the tumor differentiation the poor differentiation tumor showed a significant increase in the levels of these tumor markers as compared to the well differentiated tumor Table 2 [Supplementary data].

Plasma VEGF, serum CA 19-9 and CEA levels were detectable in all control subjects. Their mean levels were 86.92 pg/mL, 3.03 U/mL and 1.065 ng/mL respectively. Preoperative VEGF, CA 19-9 and CEA levels were significantly higher in patients with CRC carcinoma (mean ± SD, 411.95 ± 128.41 pg/mL, 40.55 ± 22.11U/mL and 26.5 ± 12.53 ng/mL respectively) compared with the levels of the control group. Plasma level of VEGF, serum CA 19-9 and CEA in all patients dropped significantly after 7 days of surgery, but still higher than the upper normal levels and demonstrated a further downward drop after 6 month postoperative to reach the normal levels in the 66 patients (84.6%) who did not develop local recurrence (Table 3 [Supplementary data]).

The data demonstrated significant difference between each determination and the previous determination (P< 0.05). We noticed that, among the 78 patients who underwent intended curative surgery, 10 patients experienced tumor recurrence after six month of surgery; these patients showed persistent high tumor markers (VEGF, CEA, and CA 19-9) post-operatively. These patients developed local recurrence as evident by radiological study and tumor biopsy, their tumor markers level were higher compared with the values of the 66 patients who did not develop tumor recurrence (P<0.05). They received adjuvant treatment in the form of chemotherapy. There were 2 patients died within the first week post operatively Table 3.

The plasma levels of VEGF variation according to the character of the tumor showed that in:

**T Classes.** T1 tumors with mean plasma VEGF of 136.5 pg/mL were not significantly elevated compared to controls (P >0.05). However, T2 (mean VEGF, 225.9 pg/mL), T3 (mean VEGF, 445 pg/mL), had significantly elevated VEGF levels over controls (P < 0.05). VEGF levels in T3 class of colorectal cancer were significantly higher (p<0.05) compared to plasma VEGF levels in T1 and T2 classes.

**TNM Stages:** Patients at all TNM stages had elevated plasma VEGF compared to controls. The significance
Clinical Significance of VGEF

of difference from controls increased with the increasing stage. There were 7 patients (8.9%) with TNM stage I, 43 patients (55.1%) with stage II and 28 patients (35.8%) with stage III, colorectal cancer. TNM I (mean VEGF, 216 pg/mL), TNM II (mean VEGF, 380.2 pg/mL) and TNM III (mean VEGF, 526.0 pg/mL) had significant elevation of plasma VEGF compared to controls (P<0.05). In addition, TNM stage III VEGF level were significantly elevated compared to TNM I and II stages (P <0.05; Table 5 [Supplementary data]).

Dukes’ Stages: Plasma VEGF levels in Dukes’ A (mean VEGF, 283 pg/mL), Dukes’ B (mean VEGF, 429.8 pg/mL) and Dukes’ C (mean VEGF, 531.9 pg/mL) were significantly higher than the control group (P<0.05; table 5).

The results of the current study showed significant difference (P<0.05) between the different Dukes’ stages, T classes, and TNM stages. There was a trend toward increasing VEGF levels with increasing TNM and Dukes’ stages and increasing T classes. There was a significant correlation between TNM stages (P < 0.05), Dukes’ stages (P < 0.05), T classes (P < 0.05), and plasma VEGF (Table 4 [Supplementary data]).

The receiver operating characteristic (ROC) curve showed that, the sensitivity of preoperative VEGF plasma in detecting any colorectal cancer, using mean VEGF of controls (86.92 pg/mL) at cut off 96.27 pg/mL, was 93.22% with 75% specificity. The positive predictive value of this level of plasma VEGF in detecting any colorectal cancer was 85.94%, negative predictive value was 87.09% with efficacy of 86.315%. While sensitivity of serum CEA in detecting any colorectal cancer, using mean CEA of controls (1.065ng/mL) at cut off 2.26 ng/mL, was 98.3% with 89.5% specificity. The positive predictive value of serum CEA level in detecting any colorectal cancer was 90.55%, negative predictive value was 90.89% and with efficacy of 91.73%. Sensitivity of serum CA 19-9 in detecting any colorectal cancer was 91.52% with 83.3% specificity. The positive predictive value of serum CA 19-9 level in detecting any colorectal cancer was 90%, negative predictive value was 85.71% and with efficacy of 88.42%.

Combining of VEGF and CEA markers, the specificity, PPV and accuracy increased significantly (P<0.05) in comparison with CEA alone, while combining both VEGF and CA 19-9 markers, the sensitivity, specificity and NPV increased but not significantly (P>0.05) in
The expression of VEGF165 was confirmed by Western Blotting as shown in Fig (4), a protein band was detected in colorectal tumor tissues with molecular weight of 23 kDa (VEGF165), where as the VEGF165 was expressed in both normal and tumor tissues of colorectal, but the expression of VEGF165 was detected at higher levels in tumor tissues of colorectal cancer tissues and the intensities varied according to VEGF concentration. Expression of the VEGF165 isoform was observed in 65.3% (51 of 78 patients) of colorectal tumors.

**DISCUSSION**

It has been demonstrated that most of human tumors express elevated levels of the angiogenic factor VEGF, the biologic effects of which include regulation of pathologic angiogenesis through enhanced endothelial cell mitogenesis and migration, remodeling of the extracellular matrix, and increased vascular permeability. Plasma and serum VEGF have been demonstrated to increase in human patients with malignant tumors, but because of the platelets role as one of the sources of VEGF in serum, the number of platelets per ml blood has to be determined to calculate platelet corrected VEGF concentrations in serum (Ellis et al., 2000).

This study was designed to elucidate the possible relationship between tumor burden and angiogenesis marker in colorectal cancer. The results showed, high plasma levels of VEGF in patients with colorectal carcinoma, and this elevation increased with the advance of the tumor, thus suggesting an association with poor outcome. This result was in agreement with Broll (Broll et al., 2001) who stated that VEGF is an important regulator of tumor angiogenesis and it is as an index of angiogenesis. Also, the plasma levels of VEGF in cancer colon patients were generally reported to be related with tumor burden. The influence of neoangiogenesis as well as VEGF expression have been related to relapse and poor prognosis in colorectal cancer, thus adding important information for the prognostic evaluation of this type of cancer (Iervolino et al., 2002).

In one study, VEGF was not found to demonstrate any correlation with disease recurrence in patients with stages Dukes' B2 or Dukes' C disease (Andre et al., 2000), but was found to be associated with an increased risk for disease recurrence in patients with TNM stage II colon carcinoma in another study (Cascinu et al., 2000). Also, in a large study of the Danish Colorectal Cancer Study Group (Werther et al., 2002; Tsai et al., 2007), they suggested a biologically relevant role for circulating VEGF concentrations in patients with colorectal carcinoma; because they found that high preoperative VEGF concentrations were associated with a reduced overall survival in contrast to patients who had lower VEGF values. Also, Kim (Kim et al., 2003) suggested that high preoperative serum and plasma vascular endothelial growth factor (VEGF) concentrations may predict poor prognosis in patients with CRC.

In this study, post-operative plasma levels of VEGF dropped significantly after surgery, with a further downward trend until the 6 months postoperative, supporting the notion of a relevant role for carcinoma in the generation of elevated VEGF levels. While there were persistent elevation of VEGF, CEA and CA 19-9 in the patients who developed local recurrence later. These results were in accordance with the data from other study showing that mean VEGF levels tend to decrease post-operatively compared with preoperative concentration; they stated that VEGF levels may be helpful in identifying patients who are eligible for potentially curative surgery. Elevated VEGF levels after surgery may indicate significant residual disease, even if it is not evident macroscopically (Nakayama et al., 2002).

Cubo (Cubo et al., 2004) found that, pre and postoperative VEGF determination has prognostic significance, regardless of tumor stage, in patients with colorectal cancer. In survival methods, postoperative VEGF levels >343 pg/ml correlated with tumor relapse and mortality. These results suggested the use of serum VEGF levels as a prognostic and monitoring factor besides CEA. While on the other hand, Bossi (Bossi et al., 1995) and Pietra (Pietra et al., 2000) reported that angiogenesis and VEGF levels did not provide any significant prognostic information for colorectal cancer patients.

In this study, combining both markers VEGF and CEA, the specificity, PPV and accuracy increased significantly in comparison with CEA alone, while combining both VEGF and CA 19-9 markers, the sensitivity, specificity and NPV increased but not significantly in comparison with CA 19-9 alone, these results were in agreement with Tsai (Tsai et al., 2007) who stated that preoperative plasma VEGF and serum CEA levels as the only good prognostic indicators of curative and non-curative surgery. Also, this finding is in accordance with Broll (Broll et al., 2001) who indicated that the combination of preoperative serum CEA and plasma VEGF significantly increases the preoperative diagnostic sensitivity.

It has been suggested that, high serum CEA significantly correlated to poor prognosis. In multivariate analyses, the combination of high serum CEA and high plasma VEGF was significantly
associated with poor survival compared to high serum CEA and low plasma VEGF. It was concluded that 6 months postoperatively serum CEA was a better prognostic marker than corresponding serum and plasma VEGF alone. However, high serum VEGF with high serum CEA was an even better predictor of overall survival than high serum CEA alone (Werther et al., 2003).

It has become clear that the growth of solid tumors is dependent on the process of angiogenesis and that VEGF is a central positive regulator of this process. Most VEGF-producing cells appear preferentially to express VEGF121, VEGF165 and VEGF189 (Ratchada et al., 2005). In our Western Blot study of VEGF expression in CRC and normal tissues, a major protein band with molecular weight 23-kDa was detected in both colorectal tumors and normal tissues, whereas the 23-kDa protein bands (VEGF165) was detected at higher level in colorectal tumors and the intensity of the band increased according to the VEGF concentration as in advanced stage of the tumor. Confirming previous reports (Takahashi et al., 1997; Tokunaga et al., 1998) detected a correlation between tissue VEGF expression and tumor aggressiveness in colon carcinoma. It was found that VEGF expression was higher in patients who had metastatic tumors compared with patients who had non-metastatic tumors. Whereas Cascinu (Cascinu et al., 2000) confirmed that positive VEGF status was associated with a significant reduction in the 5-year disease free survival rate (DFS), and VEGF165 has also been demonstrated to play an important role in tumor angiogenesis.

VEGF exists in at least four isoforms resulting from alternative exon splicing of its ribonucleic acid (RNA) transcript. As a at least four transcripts encoding mature monomeric VEGF containing 121, 165, 189, and 206 amino acid residues (VEGF121, VEGF165, VEGF189, and VEGF206) have been detected. The secretion pattern of the four isoforms differs markedly. VEGF121 is a weak acidic polypeptide that does not bind to heparin, and is freely soluble. VEGF165, the most abundant form secreted by a variety of normal and transformed cells (Mark et al., 2001).

When different isoforms of VEGF were transfected into the VEGF-null cells in isolation and the transfected cells were implanted into nude mice, it was found that VEGF165 was the most prominent isoform that can fully rescue expansion of the angiogenesis-deficient tumor, while VEGF121 and VEGF189 only partially or failed completely to rescue tumor growth, respectively. However, these authors suggested that VEGF isoforms work in a coordinated fashion to recruit and expand tumor vasculature (Grunstein et al., 2000).

Khorana (Khorana et al., 2003) have demonstrated that angiogenesis is essential for tumor growth and metastasis. Vascular endothelial growth factor (VEGF) is an important proangiogenic cytokine and is required to initiate the formation of immature vessels by vasculogenesis or angiogenic sprouting. Tumor cells are the predominant source of VEGF; however, tumor-associated stroma also has been shown to produce VEGF.

In conclusion, assessment of circulating plasma VEGF concentration might be a valuable parameter for detecting the tumor burden and predicting the outcome of colorectal cancer patients who undergo surgery. Persistent elevation of VEGF plasma level might be considered as an indicator for local recurrent disease. Combing VEGF with CEA can be considered more specific and sensitive marker for tumor staging and provides prognostic information. VEGF165, might be considered as a predominant form of VEGF expression secreted by a variety of normal and transformed cells. VEGF165 expression has been associated with colonic neoplastic progression. Further studies with larger patient populations are required for clear evaluation of the plasma VEGF values that are useful for these purposes.

References


Clinical Significance of VEGF


