

Vascular Endothelial Growth Factor in Human Colon Cancer: Biology and Therapeutic Implications

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ABSTRACT

Tumor growth and metastasis are dependent on angiogenesis. Vascular endothelial growth factor (VEGF) plays an important role in the angiogenesis of numerous solid malignancies including colon cancer. Evidence from preclinical and clinical studies indicates VEGF is the predominant angiogenic factor in human colon cancer and is associated with formation of metastases and poor prognosis. Based on these results, it was hypothesized that inhibition of VEGF receptor activity could inhibit colon cancer liver metastasis. To test this hypothesis, the authors evaluated the ability of a small

molecule inhibitor specific for the tyrosine kinase VEGF receptor Flk-1/KDR (SU5416) or multiple tyrosine kinase receptors (SU6668) to inhibit tumor angiogenesis and metastasis in a model of colon cancer hepatic metastasis. Both SU5416 and SU6668 inhibited metastases, microvessel formation, and cell proliferation while increasing tumor cell and endothelial cell apoptosis. These results showed that targeting the VEGF receptor/ligand system is a rational approach to inhibiting tumor growth and prolonging survival. *The Oncologist* 2000;5(suppl 1):11-15

INTRODUCTION

In vivo experimental studies have demonstrated that tumor growth and metastasis are dependent on angiogenesis [1-5]. Increased vascularity may allow not only an increase in tumor growth but also a greater chance of hematogenous tumor embolization. Thus, inhibiting tumor angiogenesis may halt tumor growth and decrease metastatic potential of tumors. Identification of specific angiogenic factors in individual tumor types provides a rational target for antineoplastic therapy that may be used alone or in combination with other modalities.

Tumor angiogenesis depends on the expression of specific factors that initiate the cascade of events leading to formation of the microvasculature. Numerous growth factors that promote angiogenesis have been identified. Of these, vascular endothelial growth factor (VEGF) is most commonly associated with tumor angiogenesis. One distinguishing characteristic of VEGF, in addition to its ability to induce angiogenesis, is its ability to induce vascular permeability [6]. VEGF induces vascular permeability 50,000-fold greater than that induced by histamine, the gold standard for inducing

permeability. Permeability induced by VEGF allows diffusion of proteins into the interstitium, the lattice network on which endothelial cells migrate.

Several high-affinity VEGF receptor tyrosine kinases have been identified, including VEGFR-1 (Flt-1), VEGFR-2 (Flk-1/KDR), and VEGFR-3 (Flt-4). VEGF receptors are expressed almost exclusively on endothelial cells; however, expression of the various VEGF receptors has been demonstrated on cells of neural origin, Kaposi's sarcoma cells, hematopoietic precursor cells, and other rare tumor cell types [7-10]. Different VEGF receptors may mediate distinct functions within the endothelial cell. For example, VEGFR-1 may function in cellular migration, whereas VEGFR-2 may function in induction of permeability and endothelial cell proliferation.

VEGF expression is upregulated in numerous solid malignancies [11], including primary and metastatic carcinomas of the gastrointestinal tract [12, 13]. Several studies have implicated VEGF in human colon cancer angiogenesis [14]. A series of studies have attempted to determine the role of VEGF in the angiogenesis, metastasis, and proliferation of human colon cancer. On the basis of the results of these studies,

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SU5416, a small molecule inhibitor of the VEGF-Flk-1/KDR signaling pathway, has been evaluated in a model of colon cancer liver metastasis. The results of these studies are summarized below.

ROLE OF VEGF IN PRIMARY COLON CANCERS

To determine the role of VEGF in the angiogenesis and malignant potential of primary colon cancers, immunohistochemical techniques were used to assess VEGF expression and vessel counts in 52 human colon carcinoma specimens [15]. This study aimed to determine if vessel counts correlate with the development of metastasis in human colon carcinoma specimens and if a specific angiogenic factor correlates with vessel counts and/or metastasis formation. Immunohistochemical analysis of tumor specimens using antibodies against VEGF and its receptor (Flk-1/KDR) demonstrated that increasing vessel density correlated with the likelihood that a patient would develop metastatic disease ($p < 0.001$) (Fig. 1) [15]. A direct correlation was also seen between VEGF expression and the development of metastatic disease ($p < 0.001$). VEGF expression at the invasive edge and in the tumor also correlated with vessel count. In contrast, no correlation was observed between basic fibroblast growth factor (bFGF) and prevalence of metastatic disease or vessel count.

Studies were then performed to determine whether VEGF and vessel counts could serve as prognostic markers in node-negative colon cancer [16]. Patients in this study had not received adjuvant chemotherapy and were followed for a

minimum of five years. Tumor specimens from patients were immunostained for VEGF, factor VIII, bFGF, and proliferating cell nuclear antigen (PCNA). Slides were reviewed for differentiation, mucin production, and the presence of vascular, lymphatic, or perineural invasion. The results of this study demonstrated that relatively low vessel counts were associated with favorable prognosis, whereas high vessel counts were associated with recurrence (Fig. 2) [16]. Similarly, patients with low VEGF expression had a significantly better survival than patients with high VEGF expression. The results of these studies further support the hypothesis that VEGF is an important angiogenic factor in colon cancer and indicate that vessel count and the expression of VEGF may be useful in predicting metastasis from colon cancer.

REGULATION OF VEGF EXPRESSION BY COLON CANCER IN VITRO

To determine the overall prevalence of VEGF expression in human colon cancer cell lines and tissues, Northern blot analysis was used to evaluate VEGF mRNA expression in these tissues [17]. VEGF expression was observed in all surgical specimens, including normal mucosa, primary colon cancers, and metastatic tumors (>20 specimens). VEGF expression was also found in all human cell line studies (12/12). To determine if alteration of VEGF levels in human colon cancer cells would affect a surrogate marker of angiogenesis, a cell line with

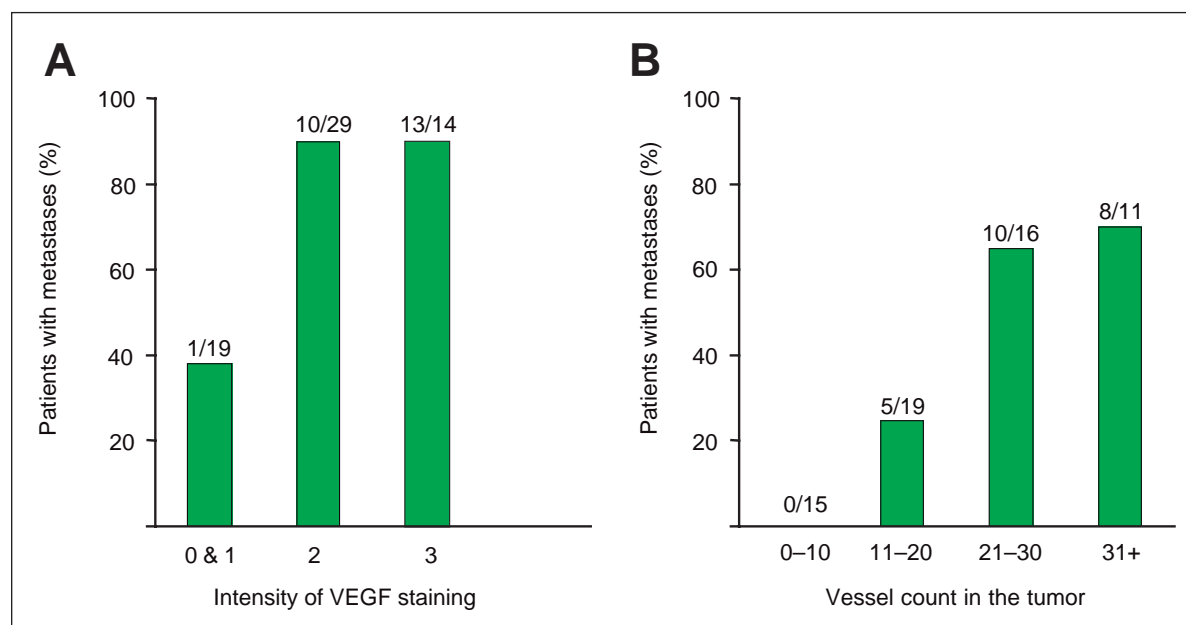


Figure 1. Relationship between vascular endothelial growth factor (VEGF) expression and metastasis (A) and vessel count and metastasis (B). The prevalence of metastatic disease increases as the intensity of VEGF expression or vessel count increases. Reproduced with permission [15].

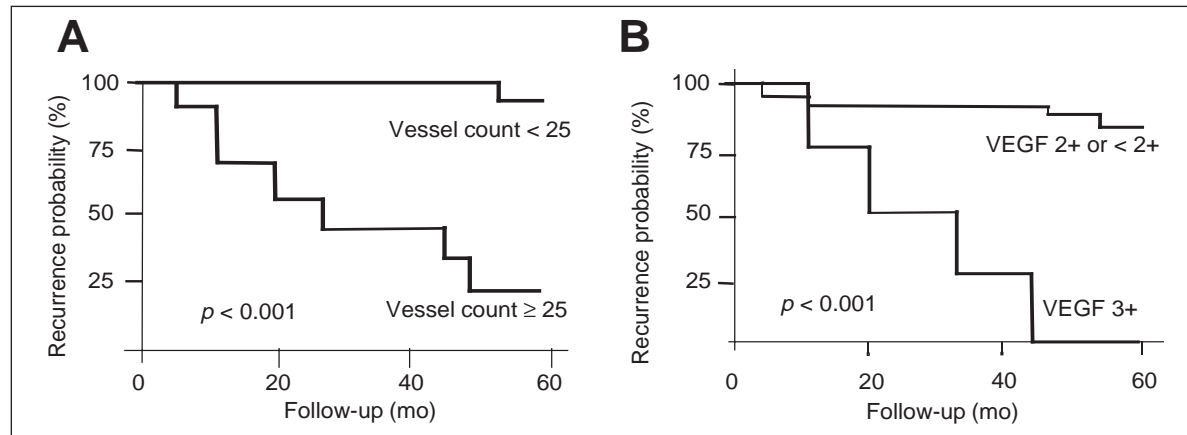


Figure 2. Kaplan-Meier survival curves for vessel count and vascular endothelial growth factor (VEGF) expression in node-negative colon cancers. Patients with high vessel counts (A) or high VEGF expression (B) had a significantly worse prognosis than patients with low vessel counts or low VEGF expression. Reproduced with permission [16].

relatively low VEGF expression (SW480) was selected for transfection of a vector containing the full-length VEGF-121 cDNA. Conversely, a cell line with relatively high VEGF expression (SW620) was chosen for transfection of a vector containing the full-length antisense VEGF-121 cDNA. Cells were transfected with the vector alone as control. SW480-VEGF sense transfectants exhibited a sixfold increase in VEGF secretion. SW620-VEGF antisense transfectants exhibited a 50% reduction in VEGF secretion. Endothelial cells were grown in conditioned, serum-free media produced from experimental cells, and proliferation was determined. Endothelial cells grown in conditioned media from SW480-VEGF sense transfectants demonstrated a 1.5- to 2-fold increase in proliferation. Addition of conditioned media from SW620-VEGF antisense transfectants resulted in a 25% to 50% decrease in endothelial cell proliferation. Thus, alteration of VEGF expression by colon cancer cells may affect the proliferative activity of the target endothelial cell.

ANTIANGIOGENIC THERAPY IN MURINE MODEL OF COLON CANCER HEPATIC METASTASES

Because VEGF expression was associated with metastases in human colon cancer, strategies that affect the biological activity of the VEGF receptor/ligand system were implemented. One strategy to inhibit tumor angiogenesis was to use tyrosine kinase inhibitors of the VEGF receptor. The small molecule SU5416 inhibits the tyrosine kinase activity of VEGFR-2, whereas SU6668 inhibits the tyrosine kinase activity of VEGFR-2, bFGF receptor, and platelet-derived growth factor receptor. In the model used, mice are injected with colon cancer cells beneath the splenic capsule, creating liver metastases that are visible in four to seven days [18]. This is a relevant model for colon cancer since

most patients who die of colon cancer die with hepatic involvement. In this study, Balb/c mice underwent splenic injection of syngeneic CT26 colon carcinoma cells. Four days after tumor cell injection, mice were randomized to receive either control solvent, SU5416 (12 mg/kg/d, i.p. injection), or SU6668 (60 mg/kg/d, i.p. injection). All mice were sacrificed when the mice in the control group became moribund secondary to tumor burden (day 22); their livers were then weighed and processed for further analyses.

No significant differences were found in body weight among the three groups at the end of the experiment. Toxic drug reactions were not observed. Harvested livers were weighed as a gross measure of tumor burden. Relative to control mice, liver weights decreased in the SU5416 ($p = 0.002$) and SU6668 ($p < 0.001$) groups (Fig. 3A) [18]. Fewer surface liver metastases were present in the SU5416 ($p < 0.001$) and SU6668 ($p < 0.001$) groups than in the control group (Fig. 3B).

Immunohistochemical staining for CD31 to detect vessels in hepatic metastases revealed a significant decrease in tumor vessel counts in the SU5416 ($p < 0.001$) and SU6668 ($p < 0.001$) groups compared with the control group (Fig. 3C) [18]. Additionally, no significant differences were observed in tumor vessel counts between the SU5416 group and the SU6668 group.

Immunohistochemical staining for PCNA and immunofluorescent terminal deoxynucleotidyl transferase-mediated nick end labeling (TUNEL) staining, with and without concurrent staining for CD31, was performed in tumor-bearing liver sections to evaluate tumor cell proliferation, endothelial cell apoptosis, and tumor cell apoptosis, respectively. SU5416 and SU6668 treatment significantly reduced tumor cell proliferation ($p < 0.001$) and significantly increased apoptosis in both endothelial cells ($p < 0.001$) and tumor cells ($p < 0.001$)

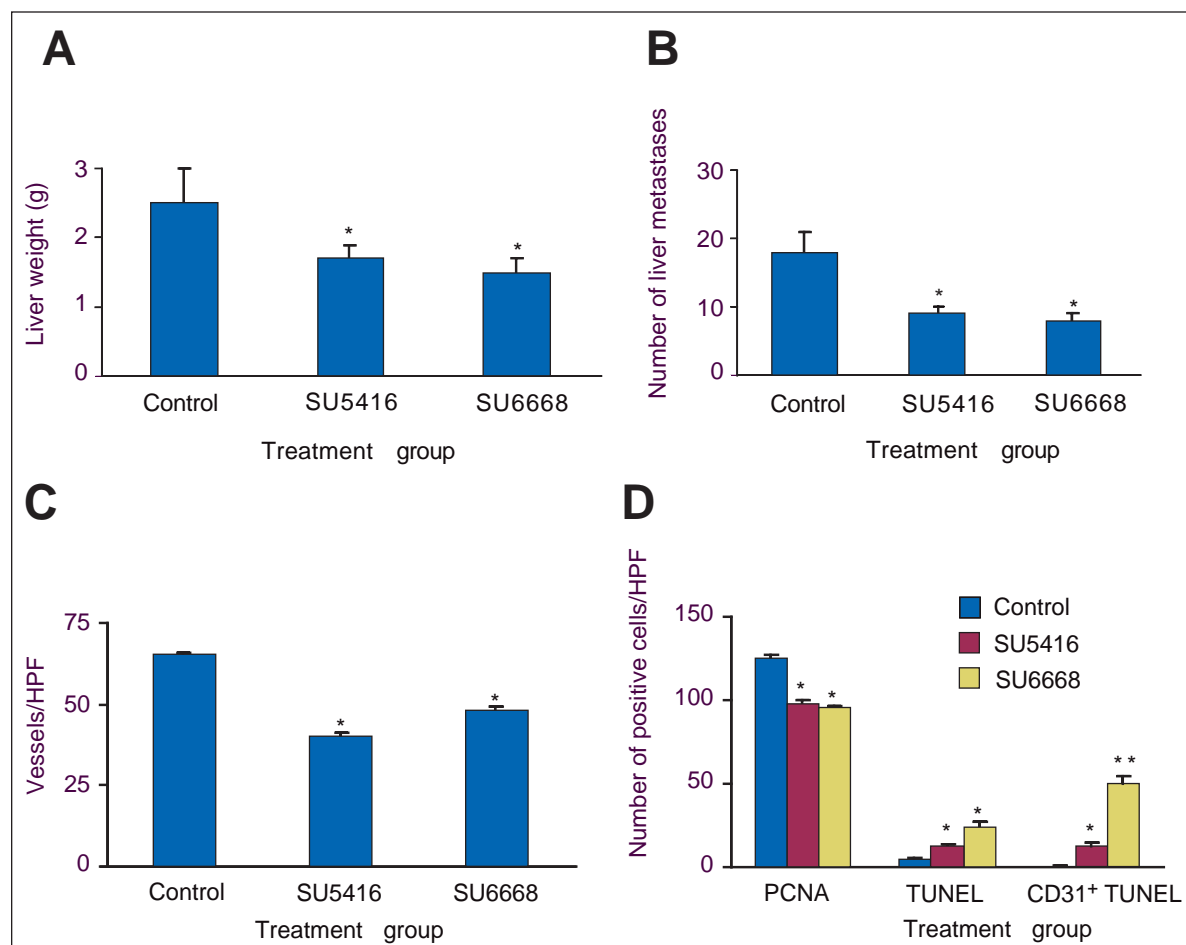


Figure 3. Effect of vascular endothelial growth factor (VEGF) receptor antagonist on various indices in mice with colon cancer liver metastases. Therapy targeting VEGF decreased liver weight, a gross measure of tumor burden (A), number of metastases (B), vessel count (C), and proliferative index (D). The number of cells undergoing apoptosis was highest in the group receiving anti-VEGF therapy. HPF = hepatic plasma flow; PCNA = proliferating cell nuclear antigen; TUNEL = terminal deoxynucleotidyl transferase-mediated nick end labeling. Bars = Standard error. * $p < 0.001$ versus control; ** $p < 0.048$ versus SU5416. Reproduced with permission [18].

(Fig. 3D) [18]. No differences were found in PCNA expression between the SU5416 and SU6668 groups. However, SU6668 treatment produced 4.4-fold higher endothelial cell apoptosis ($p < 0.001$) and 1.7-fold higher tumor cell apoptosis ($p < 0.048$) than treatment with SU5416.

Because inhibiting the tyrosine kinase activity of VEGF led to a decrease in tumor burden, it was hypothesized that this therapeutic strategy would improve survival in mice with liver metastases. Balb/c mice were again injected with CT26 colon cancer cells beneath the splenic capsule. After four days, the mice were randomized to receive either solvent alone or SU6668 (60 mg/kg/d, i.p. injection). Mice were sacrificed when moribund. A significant improvement in survival (40% increase in median survival) was seen in mice receiving SU6668 ($p < 0.05$) [18]. In fact, many of the mice that died in the SU6668 group succumbed to splenic tumor burden rather than hepatic tumor burden. There was also an

increase in both tumor cell and endothelial cell apoptosis in the mice treated with SU6668 ($p < 0.05$ [unpublished data]). These data suggest that VEGF may be an *in vivo* survival factor for tumor endothelium.

CONCLUSION

VEGF appears to be the predominant angiogenic factor in human colon cancer and is associated with metastases formation and poor prognosis. These studies show that antiangiogenic therapy targeting the tyrosine kinase receptor for the VEGF receptor inhibits the vascularity, proliferation, and growth of colon cancer liver metastasis and significantly increases endothelial and tumor cell apoptosis. These results suggest an important role for VEGF as a survival factor for tumor endothelium. Survival studies demonstrated that the growth inhibition achieved with these tyrosine kinase inhibitors led to a survival advantage. There is a need for

effective systemic therapies that significantly improve survival in patients with metastatic colon cancer. Our findings indicate that targeting the VEGF receptor/ligand system is a rational approach to inhibiting tumor growth and may have clinical benefit in the management of colon cancer liver metastasis.

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