

New Approaches to the Treatment of Hepatic Malignancies

Angiogenesis and Antiangiogenic Therapy of Colon Cancer Liver Metastasis

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The fact that tumor growth and metastatic spread relies on angiogenesis has been widely proven and accepted. The understanding of cancer biology and metastasis formation has led to the development of new therapeutic approaches that target tumor biology. The survival and establishment of metastatic lesions depend on a shift in the normal balance of proangiogenic and antiangiogenic factors that favor angiogenesis. Colorectal cancer is one of the leading cancer deaths worldwide. Angiogenesis has been associated with colon cancer progression and metastatic spread, thereby significantly affecting patient survival. New experimental approaches that inhibit angiogenic processes have demonstrated promising antineoplastic effects on metastatic colorectal cancer and are partially being investigated in clinical trials. This review focuses on angiogenesis in colorectal cancer metastasis formation as a target for antiangiogenic therapy, describing the experience from experimental studies and current clinical trials. **Key Words:** Colon cancer—Angiogenesis—Liver metastases—Antiangiogenic therapy—Microenvironment.

Colon cancer is the third most common type of cancer among men and women in the United States.¹ Despite adequate surgical removal of the primary tumor, disease will recur in nearly half of these patients within 5 years.¹ The liver is the most common and critical site for the development of colorectal cancer metastases.² At present, surgical resection and/or ablation of liver metastases has been proven to be the only effective treatment to improve long-term survival and potential cure.^{2–5} However, <10% of patients with liver metastases are appropriate surgical candidates. Systemic or regional chemotherapeutic regimens have little effect on the natural history of this disease; most patients die within 2 years of diagnosis of their liver metastases. Modifica-

tions of current treatment regimens are unlikely to significantly influence the natural history of metastatic colorectal cancer.

Recently, it has been demonstrated that agents that target specific molecular defects within the tumor may improve response rates to standard chemotherapeutic regimens.⁶ Therefore, a better understanding of the biology of metastasis and the molecular events leading to the metastatic phenotype is essential for the development of innovative cancer therapies. It is well established that angiogenesis is necessary for the growth and metastasis of tumors.

This review will provide insight into the process of angiogenesis in colorectal cancer metastasis. We will discuss the experience of the effect of antiangiogenic therapy in the growth of liver metastasis in experimental studies and provide an overview of agents currently being investigated in clinical trials.

ANGIOGENESIS OF COLORECTAL CANCER

Neovascularization is essential for the growth of solid tumors beyond 1 to 2 mm in size, when oxygen diffusion

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alone is no longer sufficient to maintain an adequate tissue oxygen level.^{7,8} Angiogenesis is a complex process and depends on the local balance between various proangiogenic and antiangiogenic molecules released by tumor cells and host cells, including adjacent stromal and immune cells. This process comprises a series of interlinked steps, including separation of endothelial cells from pericytes and the basement membrane, invasion and migration across basement membranes, and subsequent extension into the tumor mass^{9–11} (Fig. 1). The importance of angiogenesis in tumor growth and metastasis has been experimentally validated in various murine tumor models.^{7,12–17}

Tumor-related neovascularization plays a critical role in colorectal cancer progression and has been well studied over the past several years. In colorectal cancer, increased angiogenesis in the primary tumor has been associated with poor prognosis and relapse of disease.^{18,19} One of the major factors that has been demonstrated to be involved in colorectal cancer angiogenesis is vascular endothelial growth factor (VEGF).^{18–22}

VEGF is a proangiogenic molecule that has been demonstrated to induce endothelial cell migration, proliferation, and invasion^{23–26} and to increase vascular perme-

ability.^{26–28} The VEGF family comprises six molecules, designated VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, and placenta growth factor. So far, the best characterized of the VEGF molecules is VEGF-A (most commonly designated VEGF), which is expressed in at least four isoforms derived by alternative splicing. (Numbers behind VEGF splice variants signify the number of amino acids in each isoform.) VEGF121 and VEGF165 are secreted by cells, whereas the larger isoforms VEGF189 and VEGF205 are cell associated.^{29,30} VEGF165 is the predominant isoform that is most commonly overexpressed in a variety of solid tumor systems. Recently, it has been hypothesized that different isoforms may have differential effects on angiogenesis. Cheung et al.³¹ described a tissue-specific expression pattern of VEGF isoforms in the malignant transformation of lung and colon, which suggests different functional roles. The predominant isoforms in these tumors were VEGF165 and VEGF121. In another study of human colorectal cancer specimens, Tokunaga et al.³² were able to demonstrate that simultaneous overexpression of all three of the isoforms VEGF121, VEGF165 and VEGF189 was associated with a significantly worse

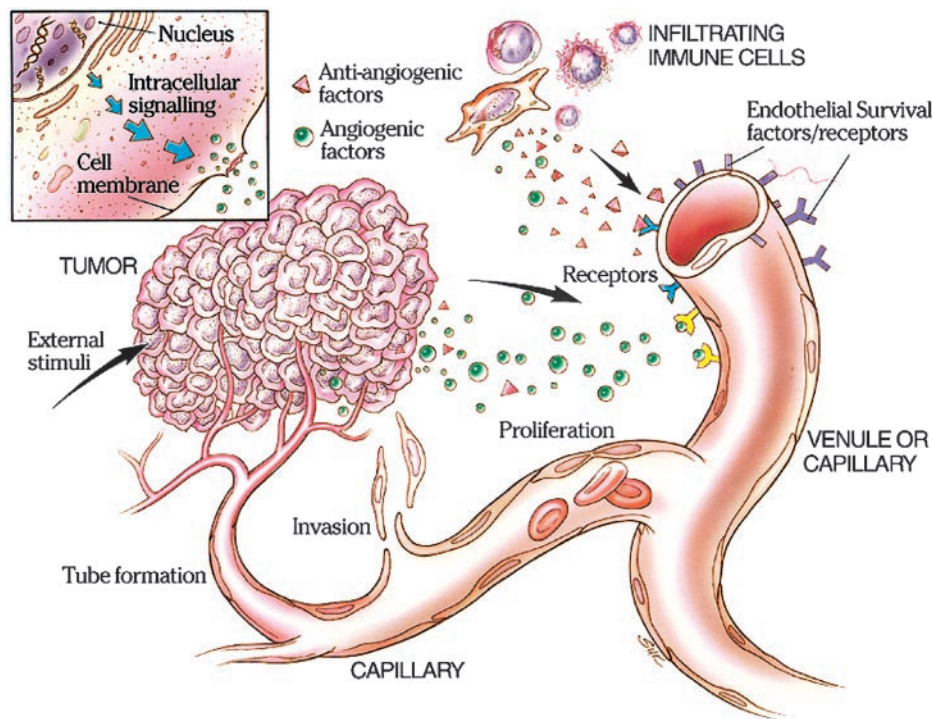


FIG. 1. The angiogenic process is balanced by the activity of numerous proangiogenic and antiangiogenic molecules that are necessary to maintain homeostasis. When the activity of proangiogenic factors exceeds that of antiangiogenic factors, new blood vessels are formed. Constitutive expression of angiogenic factors is influenced by stimuli of the cell environment, such as hypoxia, pH, cytokines, growth factors, and genetic alterations of oncogenes, tumor-suppressor genes, or both. Reprinted with permission.¹²⁸

prognosis and higher venous invasion by the tumor than overexpression of any in the predominant isoform.

The VEGFs mediate its functions by binding to one or more of three tyrosine kinase receptors—VEGF receptor (VEGFR)-1 (Flt-1), VEGFR-2 (Flk-1/KDR), and VEGFR-3 (Flt-4)³³—which, for the most part, are expressed on endothelial cells (Fig. 2). VEGFR-1 seems to be important for endothelial cell migration and differentiation,³⁴ whereas VEGFR-2 activation enhances endothelial cell survival and increases vascular permeability³⁵ and endothelial cell proliferation.³⁶ More lately, it has been shown that VEGFR-3 is involved in lymphangiogenesis and has been associated with lymphatic metastases.^{37–41} Recently, a co-receptor system for VEGF has been identified. Neuropilin-1 and neuropilin-2 are expressed on numerous cell types but do not, in and of themselves, transmit intracellular signals after ligand binding. The neuropilins bind to VEGF165 and likely enhance binding to the cell surface, perhaps serving as an anchor such that VEGF165 can bind more efficiently to VEGFR-2.^{42,43}

Two factors that have demonstrated in vivo angiogenic activity in colon cancer models are platelet-derived endothelial cell growth factor (PD-ECGF) and angiogenin. PD-ECGF, also known as thymidine phosphorylase,

is produced by stromal cells, tumor cells, and infiltrating macrophages and has angiogenic activity in vivo.⁴⁴ In an immunohistochemical analysis of human colon cancer specimens, it has been shown that PD-ECGF was expressed in infiltrating cells (macrophages) in 83% of colon cancer specimens but in only 5% in tumor epithelium, and it was associated with higher vessel counts despite low VEGF staining.⁴⁵ In these tumors, it seems that PD-ECGF was the driving force behind angiogenesis. Other studies showed a direct relationship between VEGF and PD-ECGF expression in colon cancer.⁴⁶ Saeki et al.⁴⁷ reported a statistically significant correlation between the frequency of PD-ECGF expression and microvessel density in early-stage colon cancer specimens. They concluded that microvessel density may be associated with the depth of cancer invasion and that PD-ECGF may play an important role in the early stage of colon cancer development through angiogenesis. PD-ECGF therefore seems to play an important role in colon cancer angiogenesis, although the exact mechanism by which it induces the angiogenic response is unclear.

Angiogenin is a potent angiogenic protein that was initially isolated from a colon cancer cell line.^{48,49} Expression of angiogenin in human colon cancer specimens significantly correlates with vascular involvement,

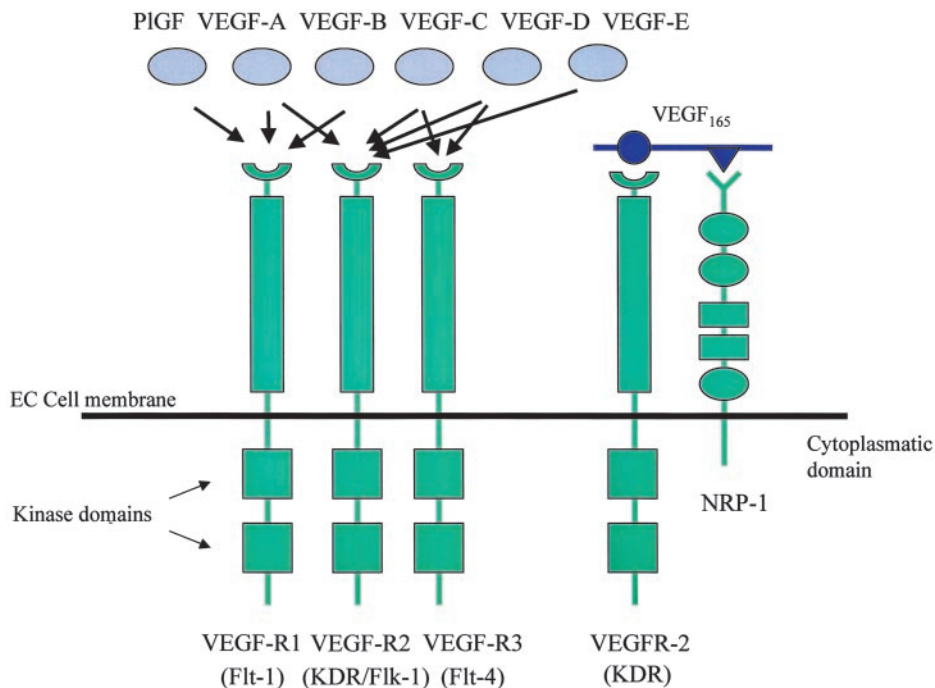


FIG. 2. Schematic vascular endothelial growth factor (VEGF) receptor (VEGFR)/ligand system including the VEGF co-receptors neuropilin (NRP)-1 and NRP-2. VEGFR-2 function can be enhanced by the simultaneous binding of a VEGF₁₆₅ molecule to NRP-1 and VEGFR-2 via different binding sites (paracrine effect). The NRP-1 receptor may also be on another cell (i.e., tumor cell) presenting bound VEGF₁₆₅ to an endothelial cell (EC) VEGFR-2 (juxtacrine effect).⁴²

lymph node metastases, liver metastases, and an advanced stage of disease.⁵⁰ Other studies have shown that VEGF in serum correlates with angiogenesis and cancer progression in patients with colon cancer.⁴⁷

Recently, the angiopoietin family of ligands has been found to play an important role in the homeostasis of the tumor vasculature. The angiopoietins are proteins involved in angiogenesis that bind to the endothelial-cell-specific tyrosine kinase receptor Tie-2. Angiopoietin-1 (Ang-1) acts as an agonist and is involved in endothelial-cell differentiation, stabilization, and survival.⁵¹ In contrast, Ang-2 binds to Tie-2 and blocks the binding of Ang-1 to this receptor.^{52,53} Few studies have examined the role of the angiopoietins in solid malignancies. We investigated the role of the angiopoietins in human colon cancer specimen and found that all specimens (11 of 11) expressed Ang-2, whereas only 54% (6 of 11) expressed Ang-1. Similarly, in colon carcinoma cell lines, most cell lines expressed Ang-2 (78%; 4 of 18), whereas Ang-1 was expressed less frequently (39%; 7 of 18). These preliminary studies suggest that an imbalance of the activity of Ang-2 over Ang-1 may play a role in angiogenesis in colon cancer, whereas there is a relatively equal frequency of expression of the angiopoietins of normal tissue.^{54,55}

Other angiogenic factors are also involved in colon cancer angiogenesis, including fibroblast growth factors-1 and -2 (acidic and basic FGF [bFGF]), transforming growth factor- α (TGF- α) and epidermal growth factor (EGF) (both of which bind to the EGF receptor), platelet-derived growth factor (PDGF), and the insulin-like growth factors (IGFs).^{45,47,56-58}

In contrast to the expression of proangiogenic molecules in colorectal malignancies, one important antiangiogenic factor has been implicated in the regulation of colon cancer angiogenesis. Thrombospondins (TSP) are high-molecular-weight glycoproteins that occur in five different subtypes (TSP-1 to TSP-5). TSP-1 and TSP-2 exhibit antiangiogenic properties and have been inversely correlated with tumor vascularity and hematogenous metastases in colon cancer.^{59,60} Maeda et al.⁶⁰ examined the correlation between expression of TSP-1 and tumor vascularity in human colon cancer specimens and determined its prognostic significance. Microvessel density was significantly higher in TSP-1-negative tumors, and a worse prognosis was associated with this reduced TSP-1 expression. Moreover, the frequency of hepatic recurrence was significantly higher in patients with tumors that were negative for TSP-1. Besides its implication as a potential prognostic marker, its antiangiogenic properties make this protein (or its analogs) potential antiangiogenic agents.

LIVER MICROENVIRONMENT AND THE ROLE OF ANGIOGENESIS IN THE DEVELOPMENT OF COLON CANCER METASTASES

The development of cancer metastases comprises a series of interlinked steps, each of which is essential for the establishment and progression of a metastatic lesion. The two major early steps in the formation of distant metastases are tumor cell proliferation of the primary lesion and creation of an extensive vascularization network from preexisting vessels. Tumor cells must also invade into the extracellular matrix (ECM) entering blood and/or lymphatic channels. The process of invasion is supported by a reduction in cell-cell adhesion, increased cell motility, and secretion of ECM-degrading enzymes, such as the matrix metalloproteinases, cathepsins, and plasminogen activators.^{61,62} After entry into the circulation, tumor cells can form aggregates with leukocytes, platelets, or both, thereby increasing the probability to form tumor emboli in distant capillary beds. Tumor cells can attach to the vascular endothelium, induce endothelial retraction, and subsequently bind to glycoproteins of the basement membrane by specific cell-surface receptors. Subsequently, some tumor cells may have the ability to extravasate into the organ parenchyma, probably by the same mechanisms that regulate the initial invasion. Further proliferation within the organ parenchyma is controlled by the interplay of growth-stimulatory and -inhibitory factors. Only metastatic cells that express adequate amounts of the corresponding receptors to the locally produced growth stimulatory factors will grow. To produce macroscopically detectable lesions, the metastases must then develop an adequate vascular network.^{12,13,63,64}

The organ preference for metastatic colonization is influenced by communication between the circulating tumor cell and the target host tissue. The effect of the organ microenvironment on tumor biology has been recognized for >100 years, since Paget's "seed and soil" hypothesis. It is now clear that the microenvironment influences tumor growth. This is supported by the fact that significant discrepancies in growth rate, metastatic potential, and efficacy of systemic treatment between ectopic and orthotopic tumors have been reported in experimental studies.^{12,65-67}

The tumor cell's microenvironment is composed of heterogeneous cell types within the liver, as well as the ECM. Each component of the microenvironment can interact with the others and with the tumor cell. Because angiogenesis is essential for metastasis formation and growth,^{12,16} site-specific microenvironmental regulation

of angiogenesis is one of the most important determinants of the organ preference of metastases. The liver microenvironment consists of not only organ-specific cells, such as hepatocytes, but also endothelial cells, pericytes, inflammatory cells, Kupffer cells, fibroblasts, and the ECM, all of which provide a favorable milieu for tumor cell implantation and initiation of angiogenic processes.⁶⁸ The observation that blood vessel growth is controlled by the microenvironment rather than by some intrinsic genetic endothelial program is also supported by transplantation studies in the quail chick system.⁶⁹ Endothelial cells lose organ-specific characteristics when they are isolated from the organ environment and cultured *in vitro*.^{70,71}

The liver is the most common and critical site for the development of colorectal cancer metastases. Most metastatic tumors in the liver are supplied by the hepatic artery; however, the portal blood flow may contribute to the tumor blood supply to varying degrees.^{72–74} Few reports on tumor vessel origin and vascularization of liver metastatic tumors are available so far.^{75–78} Terayama et al.^{75,76} studied the tumor vessel morphology in human cancers metastatic to the liver by immunohistochemical analysis of vessel diameters. Angiogenesis of liver metastases progressed stepwise as the metastases enlarged, and capillarization of sinusoidal endothelium around the liver metastases occurred. In an experimental model of metastatic liver tumors from Lewis lung carcinoma, Paku and Lapis⁷⁹ identified two types of angiogenesis in these metastases: a sinusoidal type containing convoluted vessels and lacking a basement membrane, and a portal type with a high microvessel density and positive staining for a basement membrane. In the first type, representing the dominant type, tumor cells were located between the hepatocytes and sinusoidal endothelial cells. In a rat colon cancer liver metastasis model, Gervaz et al.⁷⁸ described sinusoidal endothelial cells lining the periphery of metastases invading the tumors with increasing size. These studies clearly indicate that sinusoidal endothelial cells close to the metastatic lesion are the precursors of the tumor vessels. This has important implications for antiangiogenic strategies, because sinusoidal endothelial cells lack the classic VEGFRs VEGFR-1 and VEGFR-2.⁸⁰ “Activated” sinusoidal cells may be a potential target for antiangiogenic therapy of liver metastasis.

Our laboratory has investigated the microenvironmental influence on VEGF expression in colon cancer xenografts in nude mice. Human colon cancer cells growing in the cecum of nude mice produced more VEGF than did those in the liver (intermediate) or the subcutis (lowest) (Y.D.J. and L.M.E., unpublished data, 1999). In

another study of primary colon cancer and hepatic metastases from 17 patients who had tumors removed from both sites, we found that 11 of the 17 patients had higher VEGF levels in the primary tumor than in the liver metastases. In contrast, bFGF expression was roughly equal at the primary and metastasis sites.⁷¹

Fukumura et al.⁸¹ analyzed microvessel density and microcirculatory parameters in subcutaneously grown versus orthotopically implanted human colon adenocarcinoma. Their results showed that orthotopically grown tumors had tortuous vascular architecture and heterogeneous blood flow and that, compared with subcutaneous tumors, these tumors had a significantly lower vascular density and a significantly higher vascular permeability. Additionally, VEGF expression was lower in liver tumors than in subcutaneous tumors. However, there was higher vascular permeability in liver tumors despite lower VEGF expression. This likely can be explained by the origin of tumor vessels from sinusoidal endothelial cells, which are known to be fenestrated and lacking a basement membrane.⁸¹ Terayama et al.⁷⁵ examined tumor vessels in various human metastatic liver cancers (including colorectal cancer) and surrounding tissue by immunohistochemistry. They found that hepatocytes in the vicinity of tumors expressed higher levels of bFGF compared with tumor tissue, suggesting its relation to sinusoidal capillarization.

The liver represents a unique microenvironment for metastasis formation, not only because of its sinusoidal endothelial cell lining, but also because of the abundant expression of certain angiogenic and growth factors. In general, the expression of angiogenic factors results from complex interactions among tumor cells, smooth muscle cells, endothelial cells, pericytes, fibroblasts, and cells of the immune system. Several cytokines that are not angiogenic *in vitro*, such as interleukin (IL)-1 β , IL-6, PDGF-BB (homodimer of PDGF-B), TGF- α , IGFs, and hepatocyte growth factor, are angiogenic *in vivo*, presumably because of the co-induction of angiogenic factors such as VEGF.^{56,82}

The ability of the liver to regenerate after injury or resection plays a role in its angiogenic response. A number of studies have identified growth factors as key components of the liver regeneration process.⁸³ Microemboli to the liver caused by hematogenous tumor cell dissemination may induce a liver regenerative response secondary to perceived liver injury, thus leading to the release of certain growth factors. Growth factors and cytokines implicated in the growth of colorectal liver metastases include EGF,⁸⁴ hepatocyte growth factor,⁸⁵ TGF- α and TGF- β 1, and IGF-I.^{86,87} Further, expression of the angiogenic factors IL-8, bFGF, and PD-ECGF has

been linked to liver metastasis formation in a variety of experimental cancer models^{45,75,76,88} (Table 1). These studies highlight the importance of understanding the biological heterogeneity of tumor angiogenesis and the redundancy of angiogenic factor expression in specific organ microenvironments.

ANTIANGIOGENIC THERAPY FOR COLORECTAL CANCER AND COLON CANCER LIVER METASTASES IN EXPERIMENTAL MODELS

Angiogenesis not only is a pathologic process, but also is essential for homeostasis. Physiologic angiogenesis is important in reproduction, wound healing, and menses and is a compensatory response to ischemia in coronary artery and peripheral vascular diseases. Thus, the therapeutic efficacy of antiangiogenic therapy requires a balance where angiogenesis in tumors is inhibited without disrupting physiologic angiogenesis. The effects antiangiogenic therapy on wound healing are still controversial, but common sense dictates that if an agent does not inhibit wound healing, it may not inhibit tumor angiogenesis, because these processes are very similar.⁸⁹⁻⁹³

In general, antiangiogenic therapy targets the neovascularization of a tumor, thereby impairing growth and metastasis formation, but the strategies in the development of antiangiogenic therapies are quite diverse and distinct. However, antiangiogenic strategies can be categorized into four major target groups: those that (1) decrease the activity of specific angiogenic factors; (2) target endothelial survival factors; (3) increase the activity of naturally occurring antiangiogenic agents, i.e., angiostatin, endostatin, TSP, and so on; and (4) can indirectly downregulate the activity of angiogenic and survival factors.

TABLE 1. Pro- and antiangiogenic factors in the regulation of colorectal cancer angiogenesis and liver metastases

Proangiogenic endogenous factors
Vascular endothelial growth factor (VEGF)
Platelet-derived growth factor
Platelet-derived endothelial cell growth factor
Transforming growth factor- α , - β 1
Angiogenin
Fibroblast growth factor 1, -2
Insulin-like growth factor-1
Epidermal growth factor
Hepatocyte growth factor
Antiangiogenic endogenous factors
Thrombospondin-1, -2
Angiostatin
Endostatin
Interferon- α - β
Angiopoietin-2 (in the absence of VEGF)

It has been established that specific tumor microenvironments mediate the expression of cytokines by tumor cells,^{65,94} endothelial cells,⁹⁵ and other supporting cells.⁴⁵ Hence, the same antiangiogenic therapy may produce different responses in tumors located in different organs.⁶⁴ Support for this supposition comes from a recent study in which we injected colon cancer cells into the spleens of mice to generate liver metastases and then removed the spleens and administered therapy consisting of inhibitors to VEGF and EGF receptor activities. Although this therapy almost completely inhibited the growth and recurrence of splenic-bed tumors, it had a much less pronounced effect on liver metastases (Y.D.J. and L.M.E., unpublished data, 1999).

In colorectal cancer, the overexpression of VEGF and its tyrosine kinase receptor correlates with the development of metastases.⁷⁰ Therefore, strategies that affect the biological activity of the VEGFR/ligand system have been investigated to decrease angiogenesis, tumor growth, and metastasis formation. Anti-VEGF strategies include neutralizing antibodies to VEGF or its receptors, ribozymes to receptors, and tyrosine kinase inhibitors that block downstream signaling despite ligand binding to the VEGFR. Warren et al.⁸⁰ first reported on the effects of VEGF antibodies on the inhibition of growth of colorectal hepatic metastases. Our laboratory has used several anti-VEGF approaches in experimental models of liver metastasis. SU5416 is a small molecule that inhibits VEGFR-2 by competing at the adenosine triphosphate-binding site in the cytoplasmic domain of the protein, thus blocking the tyrosine kinase activity required for signal transduction after ligand binding.⁹⁶ SU6668 inhibits the tyrosine kinase activities of VEGFR-2, the bFGF receptor, and the PDGF receptor. Shaheen et al.⁹⁷ investigated the use of these agents in a murine model of colon cancer liver metastases. Relative to control mice, liver weights (as a gross measure of hepatic tumor burden) were less and surface liver metastases were fewer in the SU5416 and SU6668 groups. Immunohistochemical staining of the hepatic metastases for CD31 (an endothelial marker) revealed significantly fewer vessels in the SU5416 and SU6668 groups than in the controls. SU5416 and SU6668 treatment significantly reduced tumor cell proliferation and increased apoptosis in both endothelial cells and tumor cells (Fig. 3). The fact that these agents led to endothelial cell apoptosis suggests that VEGF is not only an angiogenic factor, but also a survival factor for endothelial cells.

In another experimental study, DC101, a monoclonal neutralizing antibody that targets the mouse VEGFR-2 (flk-1) and blocks ligand binding and receptor signaling, inhibited the growth and vascularity of tumors in murine

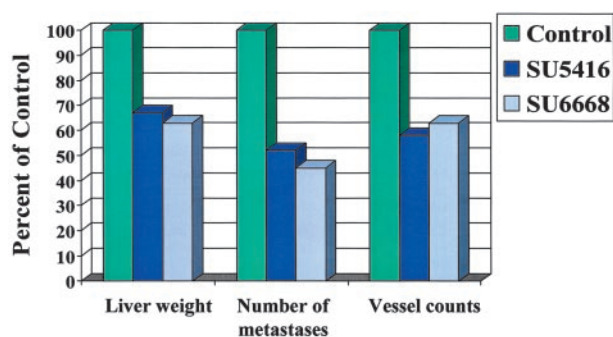


FIG. 3. Effects of SU5416 and SU6668 on colorectal cancer liver metastases in a murine model.⁹⁷ Relative to control mice, liver weights were lower and the number of surface liver metastases was fewer in the SU5416 and SU6668 groups. Immunohistochemical staining (CD31) and vessel counts of the hepatic metastases revealed significantly fewer vessels in the SU5416 and SU6668 groups than in the controls. Similar results were found with the antibody to vascular endothelial growth factor receptor (VEGFR)-2 (DC101).

models of colon cancer liver metastasis and induced apoptosis of endothelial cells and tumor cells *in vivo*.¹⁷ Besides VEGF targeting, other studies have focused on inhibiting the activity of growth factor receptors known to be associated with colorectal cancer progression. EGF is a well-recognized regulator of colon cancer proliferation *in vitro*, and overexpression of the EGF receptor correlates with the development of colon cancer metastases.^{98,99}

Another very important mechanism for endothelial cell survival is the binding of integrins on the endothelial cell surface to the ECM. At first, integrins were thought to be important only in cell-to-cell contact and binding to the ECM, but it is now known that integrins may mediate intracellular signaling, either alone or in combination with other receptors.¹⁰⁰ The integrins $\alpha v\beta 3$ and $\alpha v\beta 5$ have been shown to act as survival factors for endothelial cells, and disruption of the binding between integrins and the ECM may lead to endothelial cell death.^{100,101} Specifically, small molecules have been developed that may inhibit integrin activation, and antibodies and small peptides have been synthesized that block its binding to the ECM.¹⁰² Unpublished studies from our laboratory suggest that integrin antagonists can also inhibit the incidence, growth, and angiogenesis of liver metastases. Targeting endothelial cell survival via integrin inhibition concomitant with VEGFR antagonists may be the most promising antiangiogenic approach for colon cancer.

A cytokine that has received significant attention for its antiangiogenic activities is the interferon (IFN) family.^{14,65,103–108} These cytokines, specifically IFN- α , have been shown to be the most effective antiangiogenic agents in the therapy of life-threatening hemangiomas of

childhood.¹⁰⁴ Further studies have demonstrated that IFN- α and IFN- β can downregulate basic FGF levels.⁶⁵ In an experimental model of human colon liver metastasis (KM12L4 cell line), Ozawa et al.¹⁰⁹ demonstrated that daily subcutaneous administration of 10,000 U of IFN- α induced apoptosis in endothelial cells in liver metastases, followed by inhibition of tumor cell division and tumor cell apoptosis. IFN- α significantly inhibited tumor growth, vascularization, and expression of bFGF and metalloproteinase-9. Further studies will be necessary to determine the optimal dose for multimodality therapy regimens, although other studies from the laboratory of Fidler suggest that chronic low-dose IFN therapy is superior to high-dose bolus therapy.^{103,108}

A few natural occurring derivatives that exhibit antiangiogenic properties have been identified. Two synthetically engineered derivatives of fumagillin, TNP-470 and fr-118487, have been demonstrated to be antiangiogenic.^{78,110–113} Gervaz et al.⁷⁸ demonstrated a direct cytotoxic effect of TNP-470 on rat syngenic colon liver metastases, because microvessel density was not statistically significant compared with the control group. In contrast, Sano et al.¹¹¹ could not detect a direct antitumor effect of TNP-470 on the primary tumor (colonic injection) in a rabbit colon cancer model, but the compound was able to reduce metastatic spread by inhibition of angiogenesis. However, the tumor was kept in a dormant state when TNP-470 was used at the initial stage of the metastatic process, suggesting its potential role as adjuvant therapy for prevention of liver metastases.¹¹¹ Similar results were found by a study conducted by Takatsuka et al.,¹¹⁰ in which TNP-470 inhibited tumor growth and liver metastasis formation in a colon cancer xenograft and metastasis model. Tanaka et al.¹¹² used fr-118487 as a continuous subcutaneous infusion of an antiangiogenic agent to treat nude mice implanted with colon cancer cells into the liver and demonstrated a survival benefit in the group with surgery (resection of primary lesion) in combination with fr-118487 treatment. In this study, a short treatment trial of fr-118487 administration immediately after the early resection of the liver tumor completely inhibited both hepatic and peritoneal metastases, whereas its administration after the late resection had no effect on liver metastasis. The mice of the resection-alone group all died because of metastasis within 106 days after tumor inoculation. In contrast, half of the mice that underwent resection with subsequent fr-118487 therapy were alive at the end of an observation period of 160 days.¹¹² This study, along with data from other studies, suggests that antiangiogenic therapy may be most effective in the adjuvant setting.

Among the naturally occurring inhibitors of angiogenesis, angiostatin and endostatin, compounds discovered in the Folkman laboratory, are those that have received perhaps the most attention.^{114,115} These endogenous compounds were found to be released from primary tumors and inhibited the growth of metastatic tumors. Angiostatin, a fragment of plasminogen, was discovered in 1994.¹¹⁴ Studies^{116–118} have demonstrated that recombinant angiostatin can inhibit the growth of primary and metastatic tumors of various types. Drixler et al.¹¹⁹ investigated the effect of continuous subcutaneous infusion of angiostatin on the growth of colon cancer liver metastases after partial hepatectomy. This experimental setup was chosen to demonstrate the effect of angiostatin in the presence of local upregulation of growth factors during liver regenerative processes. Angiostatin not only suppressed xenograft tumor growth, but also significantly inhibited the outgrowth of colorectal hepatic metastases after partial hepatectomy. Again, this study suggests an optimal role of antiangiogenic therapy in the adjuvant setting.¹¹⁹

Endostatin was discovered in 1996 and was found to be a fragment of collagen XVIII.¹¹⁵ Like angiostatin, this protein fragment inhibits the growth of primary and metastatic tumors. In addition, this protein has been found to induce tumor dormancy in some tumor systems in mice.¹²⁰ In vitro studies have demonstrated that endostatin inhibits the proliferation and migration of endothelial cells but not of tumor cells. Other studies have shown that endostatin induces endothelial cell apoptosis.¹²¹ In vivo responses to endostatin vary according to the tumor system being studied; some show tumor regression, and others show growth inhibition.^{120,122,123} In a mouse model of colon cancer liver metastasis (SW620) and renal cell carcinoma lung metastasis (RenCa), cells were transfected to constitutively express a mouse endostatin protein. Endostatin expression dramatically suppressed metastasis formation compared with controls, and this demonstrated that endostatin can inhibit tumor formation in different organ microenvironments.¹²³

Lately, inhibition of angiogenesis has been demonstrated by a novel benzoic acid derivative, TAC-101, in models with liver metastasis.¹²⁴ TAC-101 can bind to retinoid acid receptors and may mediate its antiangiogenic function via retinoid acid receptor- α ; however, the exact mechanism by which this occurs is not yet known. Murakami et al.¹²⁴ examined the effect of TAC-101 on intrahepatic tumor growth of murine colon cancer cells (colon 26-L5) grown in the left lobe of the liver, on the basis of previous observations in in vitro experiments that showed inhibition of proliferation of murine hepatic sinusoidal cells by TAC-101. They were able to demon-

strate a significant reduction in liver tumor growth by TAC-101 treatment, which was potentiated by combining it with 5-fluorouracil (5-FU). Further, TAC-101-treated groups had a marked decrease of tumor VEGF expression and microvessel density.

Although some reports exist of tumor regression in experimental models of angiogenesis,^{115,125} such findings are rare; the vast majority of studies in this field demonstrate that antiangiogenic therapy leads to an inhibition of tumor growth rather than a regression of established tumors.^{80,97} The ability to interpret experimental studies appropriately is critical to ensure that extrapolations to the clinical setting are not fraught with unrealistic expectations. For example, a typical growth curve for a subcutaneously implanted tumor may demonstrate that antiangiogenic therapy significantly decreases the growth of a tumor. In this preclinical model, this “positive” result may lead to clinical trials of that same agent. In the clinic, however, inhibition of tumor growth may be interpreted as “progressive disease,” and the therapy is thus considered a failure, particularly if radiographical studies are performed at short intervals. Antiangiogenic therapy may well require a longer period of administration than chemotherapy to obtain a desirable response.

ANTIANGIOGENIC THERAPY FOR METASTATIC COLORECTAL CANCER: EARLY RESULTS FROM CLINICAL TRIALS AND REALISTIC EXPECTATIONS

Currently, several of the above-mentioned strategies are already under investigation in clinical phase I, II, or III trials in various cancer treatments. The trials that are furthest along in regard to accruing patients in phase III studies are those that target the VEGF/VEGFR system. The earliest strategies used to inhibit VEGF activity involved the use of chimeric neutralizing antibodies to VEGF. A similar strategy is used for anti-VEGFR antibodies. Another commonly used strategy for inhibiting VEGF activity is the use of tyrosine kinase inhibitors. These are small molecules that prevent kinase activation on binding of its ligand to its receptor. Although these compounds are claimed to be selective for their specific targets, in reality, these tyrosine kinase inhibitors do have some cross-reactivity with other receptors and require a much higher dose to achieve an effect. Most inhibitors must be delivered by the intravenous route, although the newer generation of tyrosine kinase inhibitors can be given orally.

Several combination treatments targeting the VEGF/VEGFR system are currently under investigation in clin-

ical trials for advanced stages of colorectal cancers; most patients have liver metastases. Preliminary results of the efficacy of a phase I/II study were reported by Miller et al.¹²⁶ In this study, SU5416 was combined with 5-FU and leucovorin (LV) and showed a potential survival benefit compared with historical controls treated with 5-FU and LV.

Currently three phase II/III clinical trials are examining the efficacy of the recombinant human VEGF (rhuVEGF) antibody bevacizumab for patients with metastatic colorectal cancer. These studies are focusing on the antineoplastic effects of combination therapies involving the standard regimens with 5-FU combined with bevacizumab, with or without irinotecan. Recently, Bergsland et al.¹²⁷ reported that a combination of bevacizumab with 5-FU and LV might improve the survival of patients with metastatic colorectal cancer who are not optimal candidates for first-line camptothecin-11 therapy.

These studies indicate that combining classic chemotherapeutic regimens with antiangiogenic compounds may alter the natural course of metastatic disease and improve survival. So far, phase I clinical trials have demonstrated that nearly all antiangiogenic agents are very well tolerated and that the maximum-tolerated dose is often not reached. However, for biologic agents, such as antiangiogenic agents, the maximum-tolerated dose may not in fact be the maximal biologic dose, as previously reported for IFN, which is most effective when administered as chronic low-dose therapy rather than as bolus high-dose therapy.¹⁰⁸

As mentioned previously, tumor regression has been reported in experimental models of antiangiogenesis, but the vast majority of studies in this field demonstrate that antiangiogenic therapy leads to an inhibition of tumor growth rather than a regression of established tumors. Therefore, it is unlikely that antiangiogenic therapy will induce tumor regression or cures, but there is a reasonable likelihood that antiangiogenic therapy will convert an acute disease (rapidly growing tumor) into a chronic disease (dormant tumor or tumor with relative slow growth). Future studies will be necessary to investigate the effects of an early combination of antiangiogenic agents with chemotherapy in an adjuvant setting (without evident metastatic disease) on survival and relapse of disease.

A comprehensive review of current antiangiogenic clinical trials is not feasible, because this area of clinical research is in constant evolution. However, the US National Cancer Institute maintains an up-to-date Web site where information on clinical trials can be accessed (<http://cancertrials.nci.nih.gov/>).

ADDENDUM IN EDITING

At the 2003 American Society of Clinical Oncology Annual Meeting, results from a phase III randomized trial in patients with metastatic colorectal cancer comparing irinotecan, 5-fluorouracil, and leucovorin (IFL) ± recombinant human monoclonal antibody to VEGF (bevacizumab; Avastin) were reported. This trial demonstrated a significant improvement in survival in those patients receiving IFL plus bevacizumab versus IFL/placebo. Median survival in the IFL/bevacizumab arm was 20.3 months vs. 15.6 months in the IFL/placebo arm ($P = .00003$). This is the first randomized phase III trial demonstrating a benefit of antiangiogenic therapy.

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