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Educational Review

Role of Angiogenesis in the Development and Growth of Liver Metastasis

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Abstract: Cancer metastasis is a highly complex process that involves aberrations in gene expression by cancer cells leading to transformation, growth, angiogenesis, invasion, dissemination, survival in the circulation, and subsequent attachment and growth in the organ of metastasis. Angiogenesis facilitates metastasis formation by providing a mechanism to (1) increase the likelihood of tumor cells entering the blood circulation and (2) provide nutrients and oxygen for growth at the metastatic site. The formation and establishment of metastatic lesions depend on the activation of multiple angiogenic pathways at both primary and metastatic sites. A variety of factors involved in the angiogenesis of liver metastasis have been identified and may serve as prognostic markers and targets for therapy. Vascular endothelial growth factor, interleukin-8, and platelet-derived endothelial cell growth factor are all proangiogenic factors that have been associated with liver metastasis from various primary tumor types. Inhibition of the activity of these factors is a promising therapeutic approach for patients with liver metastases. In addition, inhibition of integrins that mediate endothelial cell survival may also serve as a component of therapeutic regimens for liver metastases. This review focuses on the biology of angiogenesis in liver metastasis formation and growth. Because colorectal carcinoma is the most common tumor to metastasize to the liver, this disease will serve as a paradigm for the study of angiogenesis in liver metastases.

Key Words: Angiogenesis—Liver metastasis—Angiogenic factors—Microenvironment.

Folkman and colleagues have established that tumor growth is angiogenesis dependent.[1] Rapid exponential growth of tumors does not begin until neovascularization occurs, and tumor growth in organs where blood vessels do not proliferate is limited to the distance that oxygen can diffuse (1–2 mm). Further evidence of the dependence of tumor growth on angiogenesis is the fact that the proliferative index of tumor cells decreases with increasing distance from the nearest capillary blood vessel. In addition, the proliferation of tumor cells is directly proportional to the labeling index of vascular endothelial cells in the tumor. These principles have provided the foundation for our understanding of the biology of tumor angiogenesis.

Angiogenesis is an essential step, not only in the growth of primary tumors but also in the formation of metastases.[2] Once tumor cells are established in the organ of metastasis, the metastatic tumor must develop its own blood supply to grow. The purpose of this review is to provide an overview of the biology of the angiogenesis of liver metastasis to identify potential targets for antiangiogenic therapy.

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METASTASIS AND ANGIOGENESIS

Metastasis of cancer is a highly selective, nonrandom process consisting of a series of linked, sequential steps favoring the survival of a subpopulation of metastatic cells pre-existing within the primary tumor mass[3] (Fig. 1). For a tumor cell to be able to form a metastasis, it must express a complex phenotype that begins with the invasion of the surrounding normal stroma, either by a single tumor cell with increased motility or by groups of cells from the primary tumor. Once the invading cells penetrate the vascular or lymphatic channels, cells may detach and be transported within the circulatory system. Tumor emboli must survive the host's immune defenses and the turbulence of the circulation, arrest in the capillary bed of compatible organs, extravasate into the organ parenchyma, proliferate, and establish a micrometastasis. Growth of these small tumors requires the development of a vascular supply (angiogenesis) and continuous evasion of host defense cells. Failure to complete one or more steps of the process (e.g., inability to grow in a distant organ's parenchyma) eliminates the cells. To produce clinically relevant metastases, the successful metastatic cell must therefore exhibit a complex phenotype that is regulated by transient or permanent changes in different genes at the DNA and/or messenger RNA level(s).[2,3]

An essential step in the metastatic cascade is angiogenesis. Early work in the field of angiogenesis was based on a simple model in which a tumor cell would release a soluble factor that would then bind to an endothelial cell and induce endothelial cell proliferation, leading to neovascularization. Bouck[4] refined this model, proposing that angiogenesis is actually the outcome of the balance between stimulatory and inhibitory factors. Further studies demonstrated that the delicate balance of these stimulatory and inhibitory angiogenic factors can be regulated by oncogenes and tumor suppressor genes.[5] Pathologic angiogenesis occurs when the effect of stimulatory factors outweighs the effect of inhibitory factors (Table 1). A better understanding of the process of angiogenesis led to the realization that the process involves more than simply endothelial cell proliferation but rather discrete steps in which endothelial cells divide, invade the basement membrane, migrate, and eventually undergo differentiation and capillary tube formation. More recently, Holash and associates[6] proposed that newly formed metastases survive by co-opting pre-existing blood vessels within an organ. The metastases then induce neovascularization from these pre-existing blood vessels to support further growth. This theory is especially relevant to liver metastasis because this is the primary site of gastrointestinal cancer metastases.

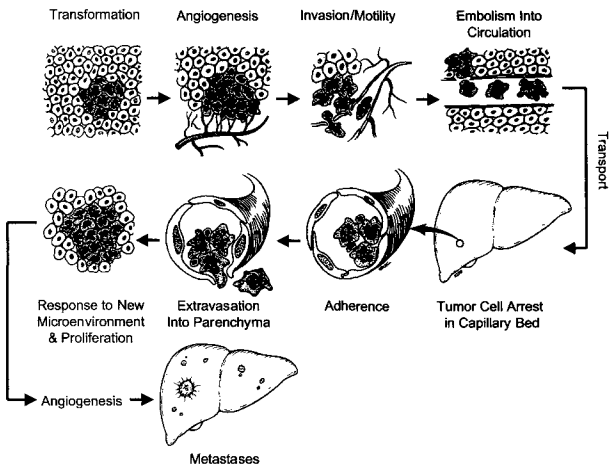

FIG. 1. The metastatic cascade. Angiogenesis is critical in the growth of the primary tumor, the release of tumor cells into the circulation, and the growth of tumors at metastatic sites. (Reproduced with permission from Fidler et al. Biology of Cancer Angiogenesis. In: DeVita JR, Hellman S, Rosenberg SA, eds. *Cancer: Principles and Practice of Oncology, 6th Edition.* Philadelphia: Lippincott Williams & Wilkins Publishers, 2001:137–47; Copyrighted 2001, Lippincott Williams & Wilkins).

LIVER MICROENVIRONMENT AND ANGIOGENESIS

Successful metastasis depends in part on the interaction of favored tumor cells with a compatible milieu provided by a particular organ environment.[7] In humans and in experimental rodent systems, numerous examples exist in which malignant tumors metastasize to specific organs.[3] The microenvironment of each organ can influence the implantation, invasion, survival, growth, and angiogenesis of tumors. A number of studies have shown that endothelial cells in different organs are phenotypically distinct and express different levels of receptors for specific angiogenic factors.[8] In addition, tumors themselves can alter the endothelial cell phenotype independent of the organ of endothelial cell origin. This obviously has important implications for antiangiogenic therapy.

The liver is the most common site of distant metastasis from colorectal cancer for two main reasons. First, the liver filters the venous drainage from the intra-abdominal viscera, including the distal esophagus, stomach, spleen, small bowel, colon, rectum, adrenals, pancreas, gallbladder, and biliary tree. Furthermore, the liver receives 30% of the cardiac output. Thus, the volume of blood filtered by the liver is second only to that filtered by the lungs. Second, physiologically, the liver is occupied by numer-

TABLE 1. *Endogenous proangiogenic and antiangiogenic factors*

Proangiogenic factors	Antiangiogenic factors
Acidic and basic fibroblast growth factor	Angiostatin
Angiogenin	Endostatin
Hepatocyte growth factor	Interferon-α, -β
Interleukin-8	Interferon inducible protein-10
Placenta growth factor	Platelet factor 4
Platelet-derived endothelial cell growth factor	Prolactin fragment
Transforming growth factor-α, -β	Thrombospondin
Tumor necrosis factor-α	Tissue inhibitor of metalloproteinase
Vascular endothelial growth factor/vascular permeability factor	Tumstatin
Others	Vasculostatin
	Others

ous cell types capable of providing a rich milieu for tumor cell growth. Tumor cells that survive the systemic circulation may eventually reach the liver. If the tumor cells express the appropriate phenotype allowing progression through all stages of the metastatic cascade, then the result is a metastasis.

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 extracellular matrix, all of which provide a favorable milieu for tumor cell implantation and initiation of angiogenesis.[9] Angiogenesis of liver metastases progresses stepwise as the metastases enlarge and capillarization of the sinusoidal endothelium around the liver metastases occurs.[10,11] In an experimental model of metastatic liver tumors from Lewis lung carcinoma, Paku and Lapis 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, which was the dominant type, tumor cells were located between the hepatocytes and sinusoidal endothelial cells.[12] Others have also demonstrated that the sinusoidal endothelial cells eventually comprise the vasculature of metastasis.[13]

ANGIOGENIC FACTORS RELATED TO LIVER METASTASIS

Vascular Endothelial Growth Factor

Vascular endothelial growth factor (VEGF) plays a pivotal role in vasculogenesis and angiogenesis; of all the angiogenic factors identified, VEGF is the one most frequently associated with tumor progression and metastasis.[14] VEGF is expressed as at least five isoforms that produce alternative splicing of messenger RNA: VEGF-121, VEGF-145, VEGF-165, VEGF-189, and VEGF-206.[15] Preliminary evidence suggests that overexpression of various isoforms of VEGF may have differential effects on tumor angiogenesis.[16]

Tokunaga et al.[16] specifically studied the expression of various VEGF isoforms in 61 colon cancer specimens. Patients whose tumors expressed the three isoforms VEGF-121, VEGF-165, and VEGF-189 had a greater incidence of liver metastasis and a poorer prognosis than did patients whose tumors expressed only VEGF-121 or VEGF-121 and VEGF-165. Relatively high VEGF expression was associated with metastasis in colon cancer patients, whereas low VEGF expression was associated with a favorable prognosis. This is similar to findings from our own laboratory[17] (Fig. 2), as well as others (Table 2). Therefore, the expression of VEGF may be useful as a prognostic marker in colon cancers.

Receptors for VEGF are expressed predominantly on endothelial cells, although recently VEGF receptors have

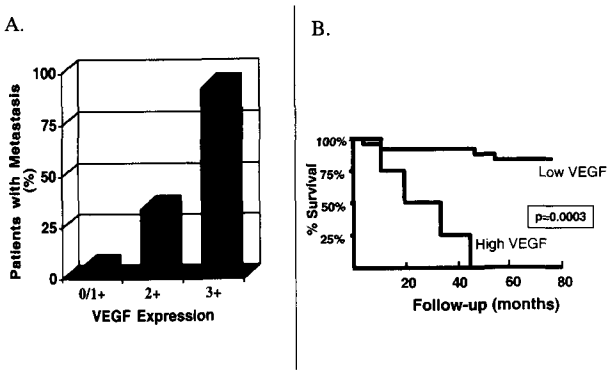

FIG. 2. Effect of vascular endothelial growth factor (VEGF) expression by the primary tumor on metastasis formation and survival. **(A)** Primary colon cancer specimens were immunohistochemically stained for VEGF. As the expression of VEGF increased in the primary tumor, the probability of metastasis increased. **(B)** VEGF expression was assessed in the primary tumors of node-negative colon cancer patients. Patients with high VEGF expression in their primary tumor had a lower survival rate. (Reproduced with permission from Takahashi et al. *Cancer Res* 1995;55:3964–8; Copyrighted 1995, *Cancer Research*, and Takahashi et al., *Arch Surg* 1997;132:541–6; Copyrighted 1997, American Medical Association).

TABLE 2. *Select studies of angiogenic factors and their role in colon cancer progression and metastasis*

Factor	No. Colon cancer patients	Associations	Study (y)
Angiogenin	65	MVD	Etoh et al.[18] (2000)
Angiomodulin	89	Poor prognosis	Adachi et al.[19] (2001)
Interleukin-10	53	Thrombospondin expression, less MVD	Kawakami et al.[20] (2001)
PD-ECGF	96	MVD, PD-ECGF predominantly in infiltrating cells	Takahashi et al.[21] (1996)
Thrombospondin-1	150	Smaller MVD, fewer hepatic recurrence	Maeda et al.[22] (2001)
Thrombospondin-2	61	Fewer liver metastases, better prognosis	Tokunaga et al.[48] (1999)
UPAR	44	VEGF, vessel count	Nakata et al.[23] (1998)
VEGF	614	Poor prognosis	Werther et al.[24] (2000)
VEGF	121	Poor prognosis	Cascinu et al.[25] (2000)
VEGF	145 + 30 polyps	Progression	Lee et al.[24] (2000)
VEGF	52	MVD, VEGFR-2 positivity, metastasis	Takahashi et al.[17] (1995)
VEGF	28	MVD, survival in node-negative patients	Takahashi et al.[27] (1997)

MVD, microvessel density; PD-ECGF, platelet-derived endothelial cell growth factor; uPAR, urokinase-like plasminogen activator receptor; VEGF, vascular endothelial growth factor; VEGFR-2, vascular endothelial growth factor receptor 2.

been found on numerous other cell types, both malignant and nonmalignant. The current nomenclature for the VEGF receptors lists three receptors: VEGF-R1 (Flt-1), VEGF-R2 (KDR/Flk-1), and VEGF-R3 (Flt-4). These tyrosine kinase receptors require dimerization to induce intracellular signaling; they bind to specific ligands as shown in Fig. 3. Different VEGF receptors may mediate distinct functions within the endothelial cells. For example, VEGFR-1 may function in cellular migration, whereas VEGFR-2 may function in induction of permeability and endothelial cell proliferation.

Interleukin-8

The chemokine interleukin-8 (IL-8), originally discovered as a chemotactic factor for leukocytes, has been shown to contribute to human cancer progression through its potential function as a mitogenic, angiogenic, and motogenic factor.[28] IL-8 expression is regulated by the tumor microenvironment; tumor hypoxia and acidosis increase expression of IL-8. IL-8 not only may directly stimulate tumor cell proliferation but also may support tumor growth by direct or indirect induction of angiogenesis. In one study, IL-8 expression in vitro directly correlated with the extent of local growth and the development of spontaneous liver metastasis after orthotopic implantation of human pancreatic carcinoma cells into the pancreases of nude mice.[29] In a study of colon cancer cell lines, there was a strong correlation between constitutive expression of IL-8 and its receptors, CXCR1 and CXCR2, and increasing metastatic potential.[30] In clinical studies, serum levels of IL-8 were significantly higher in colorectal cancer patients with liver metastasis than in those without liver metastasis.[31] Overexpression of IL-8 has also been associated with tumor aggressiveness in gastric cancer.[32] Thus, IL-8 may contribute to tumor progression and angiogenesis in several gastrointestinal tumor types.

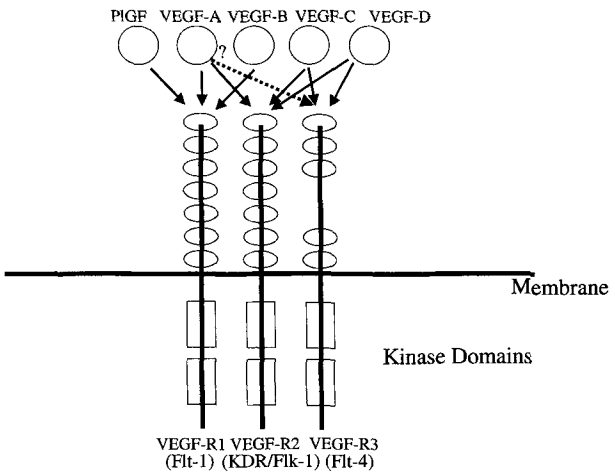

FIG. 3. Vascular endothelial growth factor (VEGF) family members and receptors. PlGF, placenta growth factor.

Integrins and Extracellular Matrix

The integrins are a family of cell adhesion receptors, each of which is a heterodimer complex of two transmembrane subunits, α and β. Thus far, 16 different α and eight different β subunits with 22 different combinations have been identified. Integrins bind to extracellular matrix adhesion proteins. Many studies have demonstrated that integrins, as important transducers of extracellular matrix signals, maintain endothelial cell survival. If the integrins cannot interact with the extracellular matrix, the endothelial cells will no longer receive the survival sig-

nal from the extracellular matrix and will rapidly undergo apoptosis.

Integrins are implicated in the development and growth of hepatic metastasis. In 110 resected human gastric cancers, $\alpha_{2\beta1}$ and $\alpha_{3\beta1}$ integrins were associated with liver metastasis and found to be independent prognostic factors related to liver metastasis in a multivariate analysis.[33] Blocking cell-surface $\alpha_{v\beta3}$ molecules with specific anti-β_3 monoclonal antibodies resulted in significant decreases in the adhesion of highly liver-colonizing H10 cells and significant inhibition of the formation of experimental liver metastases of murine RAW117 large cell lymphoma in the liver.[34] Recently, our laboratory demonstrated that the integrin $\alpha_{5\beta1}$ antagonist ATN-161 in combination with a low-dose continuous infusion of 5-fluorouracil reduced liver metastasis formation and improved survival in a murine colon cancer model.[35] In other studies, antagonists to the integrins $\alpha_{v\beta3}$ and $\alpha_{v\beta5}$ have also led to a decrease in colon cancer liver metastasis formation and angiogenesis.[36]

Platelet-Derived Endothelial Cell Growth Factor

Platelet-derived endothelial cell growth factor (PD-ECGF), also known as thymidine phosphorylase, is another tumor angiogenic factor; in several cancer systems, PD-ECGF has chemotactic activity for endothelial cells in vitro and angiogenic activity in vivo.[37] PD-ECGF strongly induces neovascularization in the rat sponge model, and PD-ECGF–transfected breast carcinoma cells exhibit accelerated growth in xenografts in mice.[38] In one study, Maeda et al.[39] immunostained 120 gastric cancer specimens for PD-ECGF and microvessels and found a significantly higher microvessel density in tumors that expressed PD-ECGF. Moreover, the frequency of liver metastasis was significantly higher in patients with PD-ECGF–positive tumors than in those with PD-ECGF–negative tumors. Studies from our laboratory have shown that PD-ECGF expression in gastric cancer was greater in intestinal-type tumors that metastasize to the liver than in diffuse-type tumors that typically metastasize to the peritoneal cavity.[40] Others have shown, in a liver metastasis model using a PD-ECGF–transfected cell line, that a novel inhibitor of PD-ECGF can inhibit liver metastasis formation.[41]

Thrombospondin-2

Thrombospondin (TSP) is a high-molecular-weight, multifunctional glycoprotein first described as a product of platelets released in response to thrombin activation.[42] TSP is synthesized and secreted by fibroblasts, vascular smooth muscle cells, monocytes, and macrophages, as well as neoplastic cells.[43] Two of five subtypes of TSP, TSP-1 and TSP-2, have been implicated in the inhibition of angiogenesis. Tokunaga et al.[44] investigated the significance of TSP-2 in colon cancer. Among 61 colon cancer specimens, 38 were positive for TSP-2 expression; the incidence of liver metastasis in these patients was much lower than in patients whose tumors did not express TSP-2.

ANGIOGENIC FACTORS AS PROGNOSTIC FACTORS AND THERAPY TARGETS

Antiangiogenic therapy is perhaps the most active field of anticancer research. However, the mechanism of action of many agents being investigated in preclinical and clinical trials is not known. A more effective approach to antiangiogenic therapy might be to target a specific angiogenic factor and to develop therapies to inhibit the activity of this factor. VEGF is one potential target for antiangiogenic therapy. Currently, potential therapies to inhibit VEGF expression include VEGF antibodies or antibodies to its receptors, specific tyrosine kinase inhibitors of the VEGF receptors, VEGF antisense DNA, ribozymes, or soluble VEGF receptors. Preclinical studies with agents that inhibit VEGF activity have demonstrated decreased growth of metastasis, which is associated with a decrease in angiogenesis.[45–48] However, it must be recognized that a decrease in growth is, in reality, slowly "progressive disease" by standard oncologic perspectives. Thus, one should not expect antiangiogenic therapy alone to lead to tumor regressions, but a realistic expectation is to anticipate a delayed time-to-progression and improved survival.

It is unlikely that single agent antiangiogenic therapy will be of benefit to patients with established metastatic disease. It is more likely that antiangiogenic therapy will be most efficacious when combined with standard chemotherapeutic regimens, thus targeting the tumor cells in general and the endothelium specifically. There are several phase III clinical trials either completed or in progress examining the effect of chemotherapy with or without anti-VEGF therapy for patients with metastatic colorectal cancer. For a detailed list of current antiangiogenic agents in clinical trials, access the Web site http://www.cancer.gov/clinical_trials/.

CONCLUSIONS

Angiogenesis is essential for the growth of primary tumors, the development of metastasis, and the continued growth of liver metastasis. The unique vascular architecture of the liver enables a tumor to acquire adequate nutrients and oxygen through various mechanisms, such

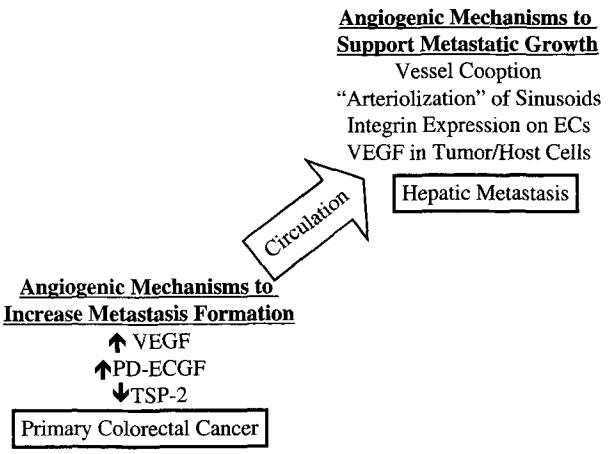

FIG. 4. Importance of the angiogenic process in primary and metastatic tumor growth. The process of metastasis is dependent on alterations in the angiogenic phenotype at both the primary site and the organ of metastasis. ECs, endothelial cells; VEGF, vascular endothelial growth factor; PD-ECGF, platelet-derived endothelial cell growth factor; TSP-2, thrombospondin-2.

as vessel co-option, modification of the existing sinusoidal network, and, eventually, traditional angiogenesis (Fig. 4). Understanding the molecular phenotype of these vascular networks allows appropriate targeting with agents that inhibit the function of ligands or receptors on endothelial cells that mediate the angiogenic process.

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REFERENCES

1. Folkman J. What is the evidence that tumors are angiogenesis dependent? *J Natl Cancer Inst* 1990;82:4–6.
2. Fidler IJ, Ellis LM. The implications of angiogenesis for the biology and therapy of cancer metastasis. *Cell* 1994;79:185–8.
3. Fidler IJ. Critical factors in the biology of human cancer metastasis: twenty-eighth G.H.A. Clowes memorial award lecture. *Cancer Res* 1990;50:6130–8.
4. Bouck N. Tumor angiogenesis: the role of oncogenes and tumor suppressor genes. *Cancer Cells* 1990;2:179–85.
5. Kerbel RS. Growth dominance of the metastatic cancer cell: cellular and molecular aspects. *Adv Cancer Res* 1990;55:87–132.
6. Holash J, Maisonpierre PC, Compton D, et al. Vessel cooption, regression, and growth in tumors mediated by angiopoietins and VEGF. *Science* 1999;284:1994–8.
7. Fidler IJ. Modulation of the organ microenvironment for treatment of cancer metastasis. *J Interferon Cytokine Res* 1995;15:585–92.
8. Jung YD, Ahmad SA, Akagi Y, et al. Role of the tumor microenvironment in mediating response to anti-angiogenic therapy. *Cancer Metastasis Rev* 2000;19:147–57.
9. Radinsky R, Ellis LM. Molecular determinants in the biology of liver metastasis. *Surg Oncol Clin N Am* 1996;5:215–29.
10. Terayama N, Terada T, Nakanuma Y. An immunohistochemical study of tumour vessels in metastatic liver cancers and the surrounding liver tissue. *Histopathology* 1996;29:37–43.
11. Terayama N, Terada T, Nakanuma Y. Histologic growth patterns of metastatic carcinomas of the liver. *Jpn J Clin Oncol* 1996;26: 24–9.
12. Paku S, Lapis K. Morphological aspects of angiogenesis in experimental liver metastases. *Am J Pathol* 1993;143:926–36.
13. Gervaz P, Scholl B, Mainguene C, Poitry S, Gillet M, Wexner S. Angiogenesis of liver metastases: role of sinusoidal endothelial cells. *Dis Colon Rectum* 2000;43:980–6.
14. Warren RS, Yuan H, Matli MR, Gillett NA, Ferrara N. Regulation by vascular endothelial growth factor of human colon cancer tumorigenesis in a mouse model of experimental liver metastasis. *J Clin Invest* 1995;95:1789–97.
15. Tischer E, Mitchell R, Hartman T, et al. The human gene for vascular endothelial growth factor. Multiple protein forms are encoded through alternative exon splicing. *J Biol Chem* 1991;266: 11947–54.
16. Tokunaga T, Oshika Y, Abe Y, et al. Vascular endothelial growth factor (VEGF) mRNA isoform expression pattern is correlated with liver metastasis and poor prognosis in colon cancer. *Br J Cancer* 1998;77:998–1002.
17. Takahashi Y, Kitadai Y, Bucana CD, Cleary KR, Ellis LM. Expression of vascular endothelial growth factor and its receptor, KDR, correlates with vascularity, metastasis, and proliferation of human colon cancer. *Cancer Res* 1995;55:3964–8.
18. Etoh T, Shibuta K, Barnard GF, Kitano S, Mori M. Angiogenin expression in human colorectal cancer: the role of focal macrophage infiltration. *Clin Cancer Res* 2000;6:3545–51.
19. Adachi Y, Itoh F, Yamamoto H, et al. Expression of angiomodulin (tumor-derived adhesion factor/mac 25) in invading tumor cells correlates with poor prognosis in human colorectal cancer. *Int J Cancer* 2001;95:216–22.
20. Kawakami T, Tokunaga T, Hatanaka H, et al. Interleukin 10 expression is correlated with thrombospondin expression and decreased vascular involvement in colon cancer. *Int J Oncol* 2001; 18:487–91.
21. Takahashi Y, Bucana CD, Liu W, Yoneda J, Cleary KR, Ellis LM. Platelet-derived endothelial cell growth factor in human colon cancer angiogenesis: role of infiltrating cells. *J Natl Cancer Inst* 1996;88:1146–51.
22. Maeda K, Nishiguchi Y, Kang SM, et al. Expression of thrombospondin-1 inversely correlated with tumor vascularity and hematogenous metastasis in colon cancer. *Oncol Rep* 2001;8:763–6.
23. Nakata S, Ito K, Fujimori M, et al. Involvement of vascular endothelial growth factor and urokinase-type plasminogen activator receptor in microvessel invasion in human colorectal cancers. *Int J Cancer* 1998;79:179–86.
24. Werther K, Christensen IJ, Brunner N, Nielsen HJ. Soluble vascular endothelial growth factor levels in patients with primary colorectal carcinoma. The Danish RANX05 Colorectal Cancer Study Group. *Eur J Surg Oncol* 2000;26:657–62.
25. Cascinu S, Staccioli MP, Gasparini G, et al. Expression of vascular endothelial growth factor can predict event-free survival in stage II colon cancer. *Clin Cancer Res* 2000;6:2803–7.
26. Lee JC, Chow NH, Wang ST, Huang SM. Prognostic value of vascular endothelial growth factor expression in colorectal cancer patients. *Eur J Cancer* 2000;36:748–53.
27. Takahashi Y, Tucker SL, Kitadai Y, et al. Vessel counts and expression of vascular endothelial growth factor as prognostic factors in node-negative colon cancer. *Arch Surg* 1997;132:541–6.
28. Matsushima K, Baldwin ET, Mukaida N. Interleukin-8 and MCAF: novel leukocyte recruitment and activating cytokines. *Chem Immunol* 1992;51:236–65.
29. Shi Q, Abbruzzese JL, Huang S, Fidler IJ, Xiong Q, Xie K. Constitutive and inducible interleukin 8 expression by hypoxia and

acidosis renders human pancreatic cancer cells more tumorigenic and metastatic. *Clin Cancer Res* 1999;5:3711–21.
30. Li A, Varney ML, Singh RK. Expression of interleukin 8 and its receptors in human colon carcinoma cells with different metastatic potentials. *Clin Cancer Res* 2001;7:3298–304.
31. Ueda T, Shimada E, Urakawa T. Serum levels of cytokines in patients with colorectal cancer: possible involvement of interleukin-6 and interleukin-8 in hematogenous metastasis. *J Gastroenterol* 1994;29:423–9.
32. Kitadai Y, Ellis LM, Takahashi Y, et al. Multiparametric in situ messenger RNA hybridization analysis to detect metastasis-related genes in surgical specimens of human colon carcinomas. *Clin Cancer Res* 1995;1:1095–102.
33. Ura H, Denno R, Hirata K, Yamaguchi K, Yasoshima T. Separate functions of alpha2beta1 and alpha3beta1 integrins in the metastatic process of human gastric carcinoma. *Surg Today* 1998;28: 1001–6.
34. Yun Z, Menter DG, Nicolson GL. Involvement of integrin alphavbeta3 in cell adhesion, motility, and liver metastasis of murine RAW117 large cell lymphoma. *Cancer Res* 1996;56:3103–11.
35. Stoeltzing O, Liu W, Reinmuth N, et al. Reduction of colon cancer growth by a novel antiangiogenic agent that targets the integrin 51 (abstract). *Clin Cancer Res* 2001;7(Suppl):A17:4.
36. Reinmuth N, Liu W, Ahmad SA, et al. The (v)-(3) inhibitor S247 decreases colon cancer metastasis, angiogenesis and improves survival in a murine model of liver metastasis (abstract). *Clin Cancer Res* 2001;7(Suppl):A24:5.
37. Ishikawa F, Miyazono K, Hellman U, et al. Identification of angiogenic activity and the cloning and expression of platelet-derived endothelial cell growth factor. *Nature* 1989;338:557–62.
38. Moghaddam A, Zhang HT, Fan TP, et al. Thymidine phosphorylase is angiogenic and promotes tumor growth. *Proc Natl Acad Sci U S A* 1995;92:998–1002.
39. Maeda K, Chung YS, Ogawa Y, et al. Thymidine phosphorylase/ platelet-derived endothelial cell growth factor expression associated with hepatic metastasis in gastric carcinoma. *Br J Cancer* 1996;73:884–8.
40. Takahashi Y, Bucana CD, Akagi Y, et al. Significance of platelet-derived endothelial cell growth factor in the angiogenesis of human gastric cancer. *Clinl Cancer Res* 1998;4:429–34.
41. Takao S, Akiyama SI, Nakajo A, et al. Suppression of metastasis by thymidine phosphorylase inhibitor. *Cancer Res* 2000;60: 5345–8.
42. Baenziger NL, Brodie GN, Majerus PW. Isolation and properties of a thrombin-sensitive protein of human platelets. *J Biol Chem* 1972;247:2723–31.
43. Zabrenetzky V, Harris CC, Steeg PS, Roberts DD. Expression of the extracellular matrix molecule thrombospondin inversely correlates with malignant progression in melanoma, lung and breast carcinoma cell lines. *Int J Cancer* 1994;59:191–5.
44. Tokunaga T, Nakamura M, Oshika Y, et al. Thrombospondin 2 expression is correlated with inhibition of angiogenesis and metastasis of colon cancer. *Br J Cancer* 1999;79:354–9.
45. Shaheen RM, Davis DW, Liu W, et al. Antiangiogenic therapy targeting the tyrosine kinase receptor for vascular endothelial growth factor receptor inhibits the growth of colon cancer liver metastasis and induces tumor and endothelial cell apoptosis. *Cancer Res* 1999;59:5412–6.
46. Shaheen RM, Tseng WW, Vellagas R, et al. Effects of an antibody to vascular endothelial growth factor receptor-2 on survival, tumor vascularity, and apoptosis in a murine model of colon carcinomatosis. *Int J Oncol* 2001;18:221–6.
47. Shaheen RM, Tseng WW, Davis DW, et al. Tyrosine kinase inhibition of multiple angiogenic growth factor receptors improves survival in mice bearing colon cancer liver metastases by inhibition of endothelial cell survival mechanisms. *Cancer Res* 2001;61: 1464–8.
48. Bruns CJ, Liu W, Davis DW, et al. Vascular endothelial growth factor is an in vivo survival factor for tumor endothelium in a murine model of colorectal carcinoma liver metastases. *Cancer* 2000;89:488–99.