REVIEW ARTICLE

Clinical Implications of Circulating Angiogenic Factors in Cancer Patients

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Purpose: **Angiogenesis, a process fundamental to tumor growth, is regulated by angiogenic factors. This article reviews prognostic and other clinical implications of circulating angiogenic factors in cancer patients.**

Methods: **A MEDLINE search of literature was performed using the names of various angiogenic factors as the key words. Studies pertaining to circulating angiogenic factors in cancer patients were reviewed. Pertinent literature regarding tumor expression of common angiogenic factors and their prognostic relevance in human cancers were also examined.**

Results: **A substantial number of studies have demonstrated a strong association between elevated tumor expression of vascular endothelial growth factor (VEGF) and advanced disease or poor prognosis in various cancers. This supports the pivotal role of VEGF in regulating tumor angiogenesis. More recently, there is mounting evidence that the level of circulating VEGF in patients with different types of cancer may be predictive of tumor status and prognosis. Preliminary data also suggest that circulating VEGF may be useful in predicting and monitoring tumor response to anticancer therapies and in follow-up surveillance for tumor relapse. There are reports supporting the prognostic value of other circulating angiogenic factors such as basic fibroblast growth factor, platelet-derived endothelial cell growth factor, transforming growth factor-beta, and angiogenin, but their clinical significance is less conclusive because of limited data.**

Conclusion: **Circulating VEGF seems to be a reliable surrogate marker of angiogenic activity and tumor progression in cancer patients. Evaluation of circulating angiogenic factors is a promising novel approach of prognostication in cancer patients that has the advantages of being convenient and noninvasive, and it may provide new prognostic information that is not afforded by conventional clinicopathologic prognostic indicators.**

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ANGIOGENESIS REFERS to the development of new blood vessels from preexisting vasculature, a process that is fundamental to tumor growth. The role of angiogenesis in cancer biology was championed by Folkman[1], who first postulated in 1971 that solid tumors would remain dormant at a size of only 2 to 3 mm^3 in the absence of neovascularization, the size being limited by the diffusion of oxygen and nutrients. Subsequent research has provided definite evidence, both in experimental and human studies, that tumor growth is angiogenesis-dependent. The supporting data were summarized by Folkman in 1990.[2] It is now recognized that angiogenesis is not only essential for tumor growth but is also implicated in the initial progression from a premalignant tumor to a cancer,[3] invasion of the cancer cells into the circulation,[4] and growth of dormant micrometastases into frank metastatic lesions.[5] Hence, angiogenesis is involved from the very first stage of cancer formation to the final stage of distant metastasis (Fig 1).

Over the last decade, angiogenesis research has evolved from cell culture and animal models to applications in humans. In 1995, Folkman[6] reviewed the emerging clinical applications of research on angiogenesis, which have taken two main directions in cancer patients: the quantitation of angiogenesis for use in diagnosis and prognosis, and the inhibition of angiogenesis to halt tumor growth. In the past 5 years, major progress has been attained in both directions. Several antiangiogenic agents have entered clinical trials, and the potential role of antiangiogenic therapy has been reviewed in recent articles.[7,8] The real efficacy of antiangiogenic therapy will only become apparent when the results of current trials are released. There is more solid evidence that supports the evaluation of tumor angiogenesis for prognosis in cancer patients. In 1991, Weidner et al[9] reported the first study showing the prognostic influence of tumor neovascularization in breast carcinoma. Intratumoral microvessel density assessed by immunohistochemical staining with specific endothelial cell markers has subsequently been shown to be an independent prognostic factor in different solid tumors.[10] Quantitation of angiogenesis seems to be a more sensitive prognostic indicator than conventional pathologic parameters. For example, it has

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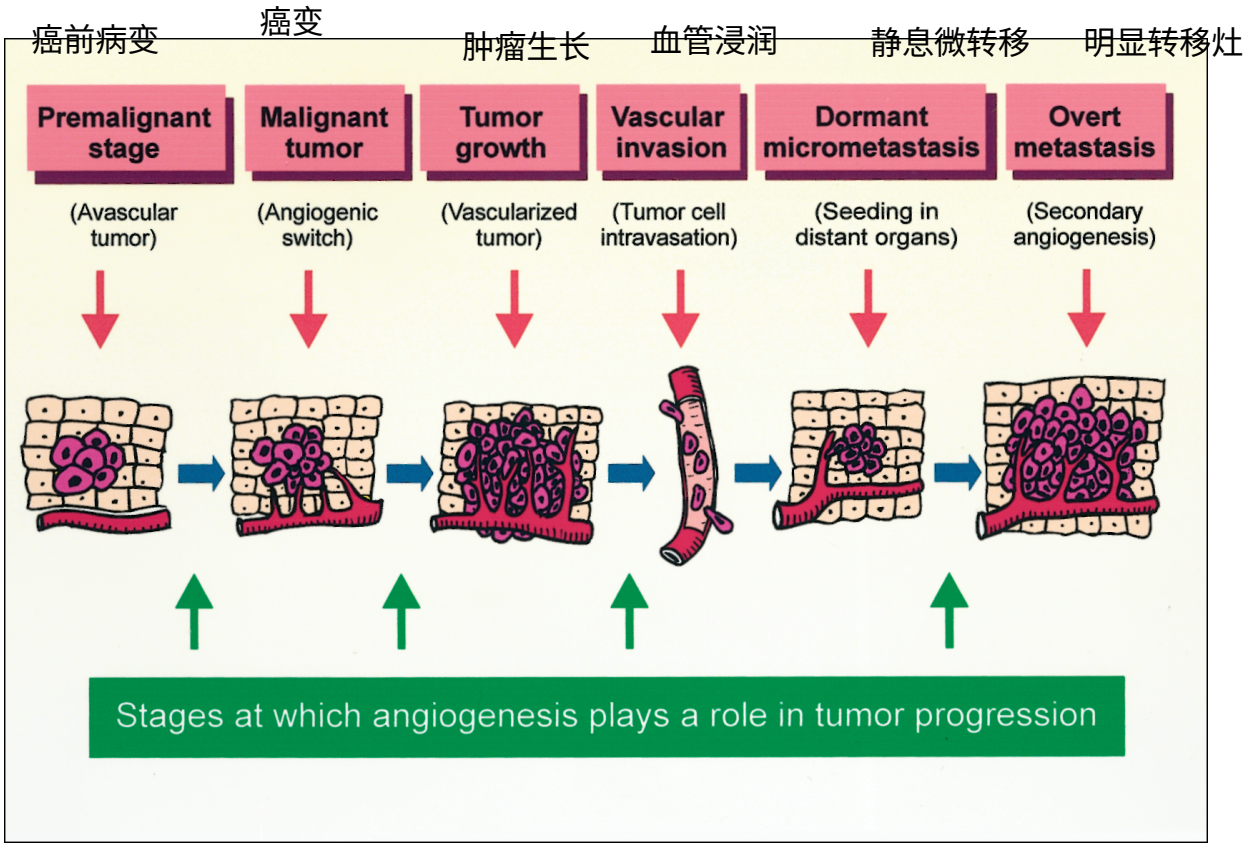

Fig 1. Angiogenesis in cancer development, growth, and metastasis. Angiogenic switch is a prerequisite for the development from premalignant stage to invasive cancer. Neovascularization provides nutrients for tumor growth and facilitates tumor cell intravasation. Secondary angiogenesis is required for the growth of avascular micrometastases to overt metastases.

been shown to be predictive of prognosis in lymph node–negative breast or colon cancer.[11,12] The latest studies have demonstrated an association between microvessel density and outcome even in lymphoma and leukemia.[13,14]

An indirect way to measure angiogenic activity in cancers is to evaluate the expression of angiogenic factors in tumor tissue or the quantity of angiogenic proteins secreted into body fluids. Measurement of circulating angiogenic factors is a convenient and noninvasive method that is potentially applicable to every cancer patient. The detection of serum angiogenic factors in cancer patients was first reported in early 1990s.[15,16] The last 5 years have witnessed an ever-growing interest in the clinical significance of circulating angiogenic factors that has resulted in the publication of more than 100 articles from researchers all over the world. There is growing evidence that the level of circulating angiogenic factors may have significant prognostic value and other clinical implications in various human cancers. Hence, it is time to examine the global picture of the available data in the perspective of its clinical use in cancer patients. In this article, data in the literature pertaining to circulating angiogenic factors in cancer patients are summarized, and suggestions for future directions of research are made. As a background, the biologic properties of the main angiogenic factors and recent data on their role in the progression of human cancers are briefly reviewed first. Relevant articles in the literature up to July 2000 were searched through MEDLINE database, and the bibliographies of the articles were reviewed for additional references.

ANGIOGENIC FACTORS

Considerable research has been directed toward dissecting the regulatory mechanism of angiogenesis since the recognition of its vital role in tumor growth, leading to the identification of several paracrine or autocrine positive regulators that stimulate angiogenesis. Angiogenic factors are derived from both tumor cells and infiltrating cells such as macrophages and fibroblasts.[17] In the development of a tumor, angiogenic switch is associated with the onset of expression and secretion of angiogenic factors by tumor cells. Tumors are also known to produce inhibitors of angiogenesis. The degree of angiogenesis in a tumor de-

Table 1. Endogenous Angiogenic and Antiangiogenic Factors That Regulate Angiogenesis

Angiogenic Factors	Antiangiogenic Factors
Vascular endothelial growth factor	Thrombospondin
Acidic and basic fibroblast growth factors	Angiostatin
Platelet-derived endothelial cell growth factor	Endostatin
Angiogenin	Interferon-α,β,γ
Transforming growth factors α, β	Interleukin-12
Epidermal growth factor	Platelet factor 4
Hepatocyte growth factor	Angiopoietin 2
Platelet-activating factor	Tissue inhibitors of metalloproteinase
Tumor necrosis factor-α	
Granulocyte colony-stimulating factor	
Interleukin-8	
Prostaglandins E1, E2	
Vascular integrin $\alpha_v\beta_3$	
Matrix metalloproteinases	

pends on a net balance of the effects of angiogenic and antiangiogenic factors. Over the past two decades, more than 20 growth factors, cytokines, and other substances have been found to possess proangiogenic activity, and a number of inhibitors of angiogenesis have also been identified. Table 1 lists the better defined endogenous angiogenic and antiangiogenic factors.

Vascular endothelial growth factor (VEGF) is one of the most potent angiogenic factors. It has a specific mitogenic activity on endothelial cells, and it is apparently devoid of mitogenic activity for other cell types.[18] It is a heparin-binding peptide with five molecular isoforms generated by alternative splicing of mRNA, composed of 206-, 189-, 165-, 145- and 121-amino acid residues.[19] The shorter isoforms, $VEGF_{165}$, $VEGF_{145}$, and $VEGF_{121}$, are secreted peptides that may act as diffusible agents, whereas the longer isoforms remain cell associated.[19] The various isoforms of VEGF bind to two tyrosine-kinase receptors, VEGFR-1 (flt-1) and VEGFR-2 (KDR/flk-1), which are expressed almost exclusively in endothelial cells.[19] VEGF not only induces proliferation of endothelial cells but also increases vascular permeability and promotes extravasation of proteins from tumor vessels, leading to the formation of a fibrin matrix that makes invasion of stromal cells into the developing tumor possible.[20] Most tumor cell types produce several VEGF isoforms simultaneously, but $VEGF_{121}$ and $VEGF_{165}$ are usually the predominant variants.[19] VEGF seems to play a central role in the regulation of tumor angiogenesis. The secretion of VEGF by tumor cells is stimulated by hypoxia.[21] As solid tumors grow in size, the cells within the expanding mass frequently become hypoxic because of increasing distance from the nearest blood vessels, and the VEGF-mediated angiogenesis in response to hypoxia seems to be a general mechanism involved in the growth of many cancers. The expression of VEGF by tumor cells is also potentiated by activation of oncogenes, such as ras,[22] or inactivation of tumor suppressor genes, such as *p53*,[23] and by other cytokines, such as transforming growth factor beta (TGF-β)[24] and nitric oxide.[25] Inhibition of VEGF has been shown to suppress tumor growth in vivo.[26]

Basic fibroblast growth factor (bFGF) is another angiogenic factor commonly encountered in tumors. It is a soluble heparin-binding polypeptide with several isoforms of different molecular masses ranging from 18 to 24 kd.[27] It has a mitogenic effect on endothelial cells, and like VEGF, it is a potent inducer of angiogenesis.[28] However, unlike VEGF, its mitogenic activity is nonspecific as it also enhances the proliferation of a wide variety of ectoderm- and mesoderm-derived cells, such as epithelial cells and fibroblasts.[29] Apart from tumor cell-derived bFGF that acts as a paracrine endothelial cell mitogen in tumor angiogenesis, endothelial cells themselves also produce and release bFGF, which acts in an autocrine fashion.[30] In addition to an independent effect on endothelial cells, bFGF acts synergistically with VEGF in the induction of angiogenesis.[31] The release of bFGF from tumor cells has been demonstrated to be associated with angiogenic switch in the multistep cancer development,[32] and monoclonal antibody against human bFGF has been shown to inhibit tumor growth.[33] Acidic fibroblast growth factor is another member of the fibroblast growth factors that is expressed by tumor cells and promotes endothelial cell proliferation.[34] However, it seems to play a less important role in tumor angiogenesis compared with bFGF.

Platelet-derived endothelial cell growth factor (PD-ECGF) is first isolated from platelets.[35] Compared with VEGF and bFGF, it is a less potent endothelial cell mitogen, but it also stimulates chemotactic migration of endothelial cells.[36] It is now known to be identical to thymidine phosphorylase, an enzyme that catalyzes the reversible breakdown of thymidine to thymine and deoxyribose-1-phosphate. The thymidine phosphorylase activity is indispensable for its angiogenic effect.[37] PD-ECGF is expressed by a wide variety of malignancies,[38] but some normal cells, such as macrophages, stromal cells, and glial cells, have also been found to produce PD-ECGF.[39] Its expression in tumor cells is known to be modulated by tumor microenvironment such as hypoxia.[40]

TGFs consist of two structurally distinct peptides, TGF-α and TGF-β, both of which stimulate endothelial cell proliferation.[34] TGF-β is a multifunctional cytokine that exists in three human isoforms (β1, β2, and β3), and it is involved in the regulation of cellular replication and synthesis of many components of the extracellular matrix.[41] TGF-β has been

shown to enhance angiogenesis in vivo,[42] and TGF-β1 seems to be the isoform most actively involved in this regard.

Angiogenin is a 14-kd peptide belonging to the family of pancreatic ribonucleases.[34] It is a potent inducer of angiogenesis in vivo, but unlike other angiogenic factors described above, it does not stimulate the proliferation of endothelial cells. The mechanism of action for its angiogenic activity seems to be indirect, probably mediated through interactions with other protein molecules.[43]

The aforementioned angiogenic factors have been most widely studied in relation to their expression and clinical significance in human cancers, and thus will be the main focuses of this review. A number of other growth factors or cytokines have been shown to stimulate angiogenesis, such as epidermal growth factor, hepatocyte growth factor, and platelet-activating factor (Table 1). These are multifunctional cytokines with biologic activities other than angiogenic activity, and their roles in tumor angiogenesis have not been as well defined as those described above. Angiogenesis is a multistep process involving endothelial cell proliferation, differentiation, migration, and organization to form tubules. It is likely that different angiogenic molecules may differentially regulate distinct steps of angiogenesis.[44]

Among the antiangiogenic factors, thrombospondin is one that has been relatively well characterized. It is a potent inhibitor of endothelial proliferation and migration, and it is downregulated during tumorigenesis.[45] Two other potent antiangiogenic factors recently isolated from tumors are angiostatin and endostatin.[46,47] Angiostatin is a 38-kd fragment of plasminogen,[46] and endostatin is a fragment of collagen XVIII.[47] On the basis of the clinical observation that removal of the primary tumor is sometimes followed by rapid development of distant metastases, it has been postulated that primary tumor may produce antiangiogenic factors such as angiostatin and endostatin that inhibit its metastatic growth. A detailed review of these antiangiogenic factors is beyond the scope of this article. The most important clinical implication regarding these angiogenesis inhibitors is their potential use for antiangiogenic therapy, which has been reviewed elsewhere.[7,8]

COMMON ANGIOGENIC FACTORS IN HUMAN CANCERS AND CLINICAL SIGNIFICANCE

Angiogenic factors regulate the angiogenic activity and growth of tumors, but the relative importance of various angiogenic factors in different human cancers has not yet been clarified. Tumor expression of angiogenic factors can be studied at protein level by techniques such as immunohistochemical staining and Western blot analysis, and at mRNA level by reverse transcriptase polymerase chain reaction or in situ hybridization. Quantitation of the expression of these angiogenic factors in the tumor provides an alternative to microvessel count in assessing tumor angiogenic activity. One of the main difficulties with the latter technique is to identify the most vascularized areas (hot spots) within the tumor. As a result, considerable interobserver variation is possible and may limit the reproducibility of microvessel density studies.

Table 2 summarizes the results of studies on the expression of common angiogenic factors and their correlation with tumor progression or survival in 10 prevalent human cancers that have been most widely examined in this aspect.[48-113] With very few exceptions,[76,88,96,110] studies have found a strong association between high tumor VEGF expression and advanced tumor stage or poor survival. Such an association has also been extensively confirmed in other malignancies, such as osteosarcoma,[114] melanoma,[115] and even leukemia.[116] In many cancers, VEGF expression in the tumor has been shown to be a significant prognostic factor of recurrence or survival independent of other conventional clinicopathologic prognosticators.[50,59,66,75,109,116] In some studies, it was identified to be the strongest predictor of survival by multivariate analysis.[50,109]

Studies on the prognostic impact of bFGF expression in tumors are less conclusive. Several studies have demonstrated a significant association between high tumor expression of bFGF and advanced tumor or poor prognosis,[60,69,77,82,89,97,103,111] but others did not find such a correlation.[61,68,112] It is interesting to note that in two studies, one in breast cancer[54] and the other in ovarian cancer,[104] a low bFGF level in the tumor was observed in patients with advanced tumor stage or poor prognosis. It seems difficult to explain these paradoxical findings, but it may be related to functions of bFGF other than its proangiogenic activity. Many stromal cell types in the tumor are known to express bFGF. In the study of breast cancer, low level of bFGF in tumors was found to be an indicator of poor prognosis.[54] The authors postulated that breast myoepithelial cells might be the main source of bFGF and that the reverse correlation between tumor bFGF content and disease stage might be ascribed to the loss of myoepithelial cells in more invasive tumors. In the other study of ovarian cancer, a high bFGF level in the tumor turned out to be an independent favorable prognostic factor for survival, and it was suggested that bFGF might induce a fibroblastic response via its stimulatory effect on fibroblasts, thereby resulting in less aggressive tumors.[104] Both of these studies measured bFGF in tumor cytosol extracts and thus could not differentiate bFGF produced by tumor cells and stromal cells. Further studies should evaluate specifically the correlation between tumor cell expression of bFGF and prognosis in these cancers.

Likewise, the prognostic significance of tumor PD-ECGF expression is not completely clear. The majority of studies showed a positive correlation between increased PD-ECGF expression and advanced tumor or adverse prognosis.[55,62,63,70,71,78,79,83,87,98,105,113] However, there were inconsistent results in studies of colorectal carcinoma. In two studies, active expression of PD-ECGF in tumor cells was correlated with high microvessel count, advanced tumor stage, and poor survival in colorectal cancer,[70,71] whereas another study failed to find a correlation between PD-ECGF expression and tumor vascularity.[72] In the latter study, a high expression of PD-ECGF in tumor stromal macrophages was predictive of good prognosis, a finding that was attributed to the role of PD-ECGF expressed by stromal cells in enhancing immune reaction against the tumor cells.

TGF-β and angiogenin have been examined for their prognostic relevance in only a few human cancers (Table 2). In general, these studies showed a positive association between TGF-β or angiogenin expression and the extent of tumor. Further research is required to elucidate the prognostic role of these angiogenic factors in various types of cancer.

EVALUATING ANGIOGENESIS BY CIRCULATING ANGIOGENIC FACTORS: POTENTIAL ADVANTAGES

Evaluation of tumor expression of angiogenic factors, especially VEGF, seems to be useful in providing prognostic information with regard to tumor recurrence and patient survival. However, like assessment of tumor microvessel count, it depends on the availability of resected surgical specimens or biopsy material, and there is considerable observer-related variability when using semiquantitative techniques such as immunohistochemical staining. Furthermore, heterogeneity of angiogenic activity in different parts of a tumor may also limit the accuracy of assessment of angiogenesis in tumor tissue. For example, VEGF expression is known to be the highest in hypoxic regions of the tumor near necrotic areas; hence, the site of tumor tissue examined may have a profound influence on the results when evaluating VEGF expression in a certain tumor. These factors may contribute to the variation in the results of studies on tumor expression of angiogenic factors.

As the majority of angiogenic factors are soluble and diffusible peptides secreted by tumors, the circulating level of angiogenic factors could theoretically reflect the overall angiogenic activity of the tumor, and it has certain potential advantages compared with the evaluation of angiogenic activity in tumor tissue. First, it can be performed in all cancer patients without the need of surgical specimens or biopsy material, a point that is particularly important for those patients in whom biopsy material is difficult to obtain. Second, it may allow preoperative evaluation of angiogenic

Table 2. Angiogenic Factors in Common Human Cancers and Correlation With Tumor Progression or Patient Survival

Cancer Type	Angiogenic Factor	Correlation With Tumor Progression or Patient Survival*	Ref
Breast carcinoma	VEGF	Positive	48-52
	bFGF	No correlation	51, 53
		Reverse correlation	54
	PD-ECGF	Positive	55
	Angiogenin	Positive	51, 56
Lung carcinoma	VEGF	Positive	57-59
	bFGF	Positive	60
		No correlation	61
	PD-ECGF	Positive	62, 63
Colorectal carcinoma	VEGF	Positive	12, 64-68
	bFGF	Positive	69
		No correlation	68
	PD-ECGF	Positive	70, 71
		Reverse correlation	72
	Angiogenin	Not available	73
Gastric carcinoma	VEGF	Positive	74, 75
		No correlation	76
	bFGF	Positive	77
	PD-ECGF	Positive	78, 79
	TGF-β	Positive	80
	Angiogenin	Not available	73
Pancreatic carcinoma	VEGF	Positive	81
	bFGF	Positive	82
	PD-ECGF	Positive	83
	TGF-β	Positive	84
	Angiogenin	Positive	85
Hepatocellular carcinoma	VEGF	Positive	86, 87
		No correlation	88
	bFGF	Positive	89
	PD-ECGF	Positive	87
		No correlation	90
	TGF-β	Not available	91
Prostate carcinoma	VEGF	Positive	92
	bFGF	Not available	93
	PD-ECGF	Not available	94
Bladder carcinoma	VEGF	Positive	95
		No correlation	96
	bFGF	Positive	97
	PD-ECGF	Positive	98
	Angiogenin	Positive	99
Ovarian carcinoma	VEGF	Positive	100-102
	bFGF	Positive	103
		Reverse correlation	104
	PD-ECGF	Positive	105
	TGF-β	Positive	106
Head and neck carcinoma	VEGF	Positive	107-109
		No correlation	110
	bFGF	Positive	111
		No correlation	112
	PD-ECGF	Positive	113

*Positive correlation indicates more advanced tumor or poorer survival with increased expression of the angiogenic factor; reverse correlation indicates earlier tumor stage or better survival with increased expression of the angiogenic factor.

Table 3. Studies of Circulating Vascular Endothelial Growth Factor in Cancer Patients

Cancer Type	Correlation With Tumor Progression or Patient Survival*	Ref
Breast cancer	Positive	118(S), 120(S)
Lung cancer	Positive	59(S), 121(S)
	No correlation	122(S), 123(S)
Gastrointestinal cancers		
Colorectal carcinoma	Positive	124(S), 125(S), 126(S), 127(P), 128(S), 129(P)
Gastric carcinoma	Positive	127(P), 130(S), 131(S), 132(P)
Hepatocellular carcinoma	Positive	133(P), 134(S), 135(S)
Urologic cancers		
Prostate carcinoma	Positive	136(S), 137(P)
Bladder carcinoma	Positive	138(S)
Renal cell carcinoma	Positive	139(P), 140(S), 141(S)
	No correlation	142(S)
Gynecologic cancers		
Ovarian carcinoma	Positive	143(S), 144(S), 145(S)
	No correlation	146(S)
Vulvar carcinoma	Positive	147(S)
Head and neck cancers		
Nasopharyngeal carcinoma	Positive	148(S)
Squamous cell carcinoma	No correlation	149(S)
Hematological malignancies		
Lymphoma	Positive	150(S), 151(S)
Leukemia	Positive	152(S)
Soft tissue sarcoma	Positive	153(S)

Abbreviations: S, serum samples used for the assay; P, plasma samples used for the assay.

*Positive correlation indicates more advanced tumor or poorer survival with higher circulating VEGF level.

activity before surgical removal of a tumor, which may be helpful in selecting patients for neoadjuvant therapy or other therapeutic considerations. Third, it is noninvasive and can be repeated serially. Fourth, measurement of circulating angiogenic factor levels by quantitative immunoassay is more precise compared with semiquantitative techniques such as immunostaining commonly used in the evaluation of tumor expression of angiogenic factors. Fifth, it is less expensive and time-consuming. Hence, in the past few years, there has been tremendous interest among researchers in studying the role of circulating level of various angiogenic factors as a reflection of the angiogenic activity and prognosis in cancer patients. Almost all the studies used an enzyme-linked immunosorbent assay to determine the concentration of angiogenic factors in the circulation; it seems to be a reliable and reproducible technique.

CIRCULATING VASCULAR ENDOTHELIAL GROWTH FACTOR IN CANCER PATIENTS

VEGF is a soluble peptide that has been found to be secreted by tumors into various body fluids. It is detected in very high levels in malignant pleural and ascitic effusions, and its enhancing effect on vascular permeability is thought to be an important mechanism mediating the formation of malignant effusions.[117] The finding of significantly higher serum VEGF levels in cancer patients than in normal subjects was first reported in 1994,[16] and was verified in a subsequent study in patients with various types of cancer.[118] In the latter study, a significant correlation of serum VEGF level with tumor stage, microvessel density, and tumor VEGF expression was noted. A further study also demonstrated a higher serum VEGF level in disseminated disease than in localized disease among patients with a variety of cancers, irrespective of the histologic type.[119] The findings of these pilot studies sparked off great interest in research to determine the clinical value of circulating VEGF as a biomarker and prognostic indicator in various types of human cancer.

Table 3 summarizes the results of studies correlating circulating VEGF level with tumor progression or patient survival in different types of cancer.[120-153] Some of these studies have been performed using archived serum samples in storage and retrospective data,[121-123,144,150] but others have been conducted with prospectively collected blood samples and clinical data.[120,125,133,135,139] In many instances, however, it was not clearly stated whether the studies were retrospective or prospective in nature.

With very few exceptions, these studies demonstrated a positive correlation of elevated serum or plasma VEGF level with advanced tumor stage or poor survival. This is in

line with studies of the prognostic significance of tumor VEGF expression and suggests that circulating VEGF level is a good reflection of tumor angiogenic activity in various cancers. However, a few studies have failed to find a significant correlation between circulating VEGF level and the tumor status or prognosis.[122,123,142,146,149] The reason for such discrepancies is not clear, but it may be related to difference in study population and methodology. In some of the latter studies, the number of patients was too small to demonstrate a statistically significant correlation, and thus the data need to be interpreted with caution.

Breast Cancer

Two studies have demonstrated a significant correlation between high serum VEGF level and advanced stage or metastatic disease in breast cancer.[118,120] However, it has not yet been established whether the circulating VEGF level has a prognostic influence on patient survival in breast cancer.

Lung Cancer

Salven et al[121] showed that among 68 patients with small-cell lung carcinoma undergoing combination chemotherapy, a high pretreatment serum VEGF level was an adverse prognostic factor of survival independent of tumor stage. In another study of 91 patients with resection of non–small-cell lung cancer, serum VEGF level was significantly higher in patients with stage T3/4 than those with T1/2 disease, but serum VEGF level was not prognostic of patients' survival after surgery.[59] A third study found significantly higher serum VEGF levels in 70 patients with small-cell or non–small-cell lung cancer of different histologic types compared with healthy controls, but no significant correlation between serum VEGF level and tumor stage or distant metastasis was observed.[122] Brattstrom et al[123] evaluated serum VEGF levels among 68 patients with non–small-cell lung carcinoma undergoing radiotherapy and found that elevated serum VEGF level was not prognostic of survival. These four studies investigated serum VEGF levels in patients with different histologic types of lung cancer undergoing different treatments. It is possible that the prognostic value of serum VEGF may be influenced by both the histologic type and treatment modality.

Gastrointestinal Cancers

Studies in gastrointestinal cancers have consistently indicated a prognostic value of circulating VEGF level. Colorectal carcinoma has been most widely studied in this respect. Dirix et al[124] examined the relationship between serum VEGF level and tumor growth kinetics in colorectal cancer by correlating serial serum VEGF levels with tumor volume changes in consecutive computed tomography scans in 44 untreated patients with advanced tumors. Patients with a short tumor volume doubling time were noted to have higher serum VEGF levels. Another group showed that preoperative serum VEGF level could predict nodal involvement and tumor stage with high sensitivity and specificity in colorectal carcinoma.[125] These findings were substantiated by two more studies that also demonstrated a significant association between serum VEGF level and tumor stage in colorectal carcinoma.[126,128] Studies of plasma instead of serum VEGF level revealed a similar correlation with tumor vascularity or prognosis.[127,129]

In gastric cancer, a significant association between serum VEGF level and depth of tumor invasion, venous infiltration, or tumor stage has been reported.[130,131] It has been further demonstrated that pretreatment plasma VEGF level was a prognostic factor of survival in patients with gastric cancer undergoing chemotherapy or surgical resection.[127,132]

Hepatocellular carcinoma is a highly invasive tumor characterized by neovascularization, and it is reasonable to speculate an important role of angiogenesis in relation to its invasiveness. A Japanese group first observed significantly elevated plasma VEGF levels in patients with metastatic disease compared with those with disease localized to the liver.[133] Recently, a Chinese group has reported an association between high serum VEGF level and portal vein emboli or nonencapsulated tumors, thereby suggesting a role of VEGF in the invasiveness of this cancer.[134] Their results concur with our own findings in a prospective study of serum VEGF level in patients undergoing resection of hepatocellular carcinoma.[135] In our study, a high preoperative serum VEGF level was significantly correlated with venous invasion, intrahepatic metastasis, and lack of tumor capsule, and a progressive elevation of serum VEGF level was observed with advancing tumor stage.

Urologic Cancers

Two studies have assessed the relationship between circulating VEGF level and the extent of disease in prostatic carcinoma.[136,137] One study found a significantly higher serum VEGF level with metastatic prostate cancer compared with localized disease,[136] and the other reported higher plasma VEGF levels in hormone-escaped prostate cancers compared with hormone-responsive cancers.[137] Whether circulating VEGF level is prognostic of survival in prostate cancer remains to be clarified.

In urothelial transitional cell carcinoma, it has been shown that elevated serum VEGF level was a significant prognostic factor of postoperative recurrence and survival in superficial bladder cancer.[138]

Dosquet et al[139] appraised the prognostic significance of several circulating angiogenic factors in patients with un-

treated renal cell carcinoma. In their study, plasma VEGF level was significantly higher in patients with lymph node or distant metastasis compared with those with undisseminated cancer. In agreement with this finding, two studies of patients with renal cell carcinoma treated by surgical resection also revealed a significant association between elevated serum VEGF level and tumor stage, histopathologic grade, and survival.[140,141] In contrast, another study did not find a significant relationship between serum VEGF and tumor grade, stage, or patient survival in renal cell carcinoma.[142] However, only 35 patients were involved in the latter study, and the number was probably too small to demonstrate any statistically significant correlation.

Gynecologic Cancers

Among various gynecologic malignancies, ovarian cancer has been the main focus of research on circulating VEGF. Yamamoto et al[101] first detected higher levels of serum VEGF in ovarian cancer patients compared with those with tumors of low malignant potential or benign tumors. Two subsequent studies demonstrated that preoperative serum VEGF level among patients undergoing surgical therapy was a significant prognostic factor of disease-free and overall survival independent of tumor stage, lymph node involvement, or histologic grade.[143,144] Another study, however, did not reveal a prognostic influence of preoperative serum VEGF on survival of patients undergoing surgical therapy, although this study did reveal an association between high serum VEGF level and advanced cancer.[145] Similarly, a more recent study did not find a significant correlation between serum VEGF level and survival in patients with recurrent ovarian cancer treated by chemotherapy.[146] Hence, the prognostic implication of serum VEGF level in ovarian cancer has not yet been fully clarified.

So far, only one study has investigated the clinical significance of serum VEGF in other gynecologic malignancies. It showed that a high serum VEGF level was associated with shorter disease-free and overall survival in patients with vulvar cancer.[147]

Head and Neck Cancers

Very few data are available regarding the prognostic influence of circulating VEGF in head and neck cancers. A study of 87 patients with nasopharyngeal carcinoma revealed that serum VEGF levels were significantly higher in metastatic disease compared with nonmetastatic disease.[148] This result was in accordance with the finding of a significant association between tumor tissue VEGF expression and metastatic disease in this cancer.[108] On the other hand, a study of serum VEGF in patients with head and neck squamous cell carcinoma did not identify a significant relationship between serum VEGF level and either tumor stage or lymph node metastasis.[149]

Hematologic Malignancies

It is well established that the growth of solid tumors is angiogenesis-dependent, but it was only recently discovered that angiogenesis also plays a role in the progression of hematologic malignancies. Two studies in patients with non-Hodgkin's lymphoma indicated a prognostic value of serum VEGF for patient survival.[150,151] Increased serum VEGF level has also been found to predict the risk of disease progression in B-cell chronic lymphocytic leukemia.[152] This finding is in line with another study showing that increased cellular level of VEGF in acute myeloid leukemia was an adverse prognostic factor of survival independent of other established prognostic factors.[116] Taken together with the observation of increased bone marrow microvessel count in leukemia patients,[14] these data suggest that leukemic cells can induce angiogenesis in the bone marrow, which seems to be important for disease progression.

Sarcoma

The prognostic relevance of angiogenesis in sarcoma has not been evaluated until recently. In a study of 85 patients with soft tissue sarcoma, serum VEGF level was positively related to tumor volume and histologic grade, hinting that serum VEGF might be a prognostic marker.[153] Studies of circulating VEGF in other types of sarcoma are not yet available.

Source and Biologic Significance of VEGF in the Circulation

Overall, the available data in the literature suggest that circulating VEGF level is a useful marker of tumor status in most types of human cancer. The majority of studies also indicate that it is a useful predictor of patient survival. In some studies, it has been demonstrated to be a strong prognostic factor independent of established prognostic parameters such as lymph node metastasis and tumor stage.[121,132,138,144] However, a few studies have failed to establish a correlation between serum VEGF and tumor stage or prognosis in cancers such as lung or ovarian carcinoma,[122,123,146] despite convincing evidence of the prognostic importance of tumor VEGF expression in these cancers. Such a disparity was often attributed to the possibility of other sources contributing to VEGF in the circulation.

Lymphocytes and macrophages infiltrating tumors, as well as various blood cells such as neutrophils and platelets, are known to express VEGF.[154-156] In healthy adults, a low level of VEGF in the circulation is maintained, probably because of secretion from these normal cells. Almost all studies reviewed above have reported an increased circulat-

ing VEGF level in cancer patients compared with healthy subjects, even in those that failed to demonstrate its prognostic value. The relative contribution of tumor cells and nontumorous cells to the VEGF in the circulation of cancer patients has been a subject of dispute. Several lines of evidence suggest that the tumor is a major source of circulating VEGF in cancer patients. First, a positive correlation between serum VEGF level and tumor VEGF expression has been demonstrated in some studies.[118,134,135] A second line of evidence is the observation of remarkable decrease in serum VEGF level after surgical resection of tumors,[100,117,118,126] which suggests that a considerable proportion of circulating VEGF is derived from the tumor. Third, it has been demonstrated that the VEGF level in mesenteric blood draining from the tumor was several-fold higher compared with peripheral blood in colorectal cancer patients,[68] and similarly, a significantly higher level of VEGF in tumor-bearing renal vein compared with contralateral renal vein in patients with renal cell carcinoma has also been observed.[140] Both findings vindicate tumor secretion of significant amount of VEGF into the circulation. Finally, extremely high VEGF level in malignant ascitic or pleural effusions also indicates secretion of a large amount of VEGF by tumors into the surrounding body fluid.[117] This indirectly supports the contention that tumors secrete a substantial amount of VEGF into the circulation.

In relation to the source of VEGF in the circulation, it has been argued whether serum or plasma should be used for measuring circulating VEGF. Recent studies have shown that most of the VEGF in serum is derived from platelets during coagulation, and the plasma level of VEGF is much lower than serum level.[157-159] A significant correlation between platelet number and serum VEGF level in cancer patients has been documented.[133,135,160] Hence, some authors recommended that plasma instead of serum should be used in studies of circulating VEGF level.[158] However, as summarized in Table 3, the majority of studies of circulating VEGF were performed using serum samples and they did show a positive correlation between serum VEGF and tumor stage or patient survival, thereby supporting the clinical relevance of measuring serum VEGF in cancer patients. In fact, both serum and plasma levels of VEGF have been shown to be of prognostic significance in most studies. It has been speculated that platelets may serve the role of storage of biologically active VEGF in the circulation by endocytosing and concentrating secreted VEGF from the tumor rather than actively producing VEGF themselves.[157,160] This hypothesis is supported by recent evidence from a study that demonstrated several-fold higher cellular content of VEGF in platelets of cancer patients when compared with healthy individuals, so that the level of whole blood VEGF in cancer patients was significantly higher than that of healthy individuals even when the platelet count was taken into account.[161] The authors of this study and others recommended that serum or whole blood but not plasma should be used in evaluating circulating VEGF in cancer patients.[160,161]

With regard to the VEGF storage function of platelets, it has been postulated that by concentrating VEGF in the circulation, platelets may prevent circulating VEGF from inducing angiogenesis except where coagulation takes place, such as at sites of wound healing or tumor.[160] There is some evidence that platelets may play a central role in hematogenous dissemination of cancer, which involves enhanced platelet aggregation and activation mediated by tumor cell-platelet adhesion.[162] A recent study has demonstrated that aggregation and activation of platelets is strongly associated with enhanced VEGF expression in tumor specimens from sarcoma patients.[163] Thus, an indirect link between tumor expression of VEGF and serum VEGF may actually exist via increased platelet activation, which may partly account for the high serum VEGF levels in cancer patients.

The biologic significance of increased VEGF in the circulation of cancer patients is largely unexplored. The release of VEGF from activated platelets at sites of interaction between circulating tumor cells and endothelial cells may have a role in tumor cell extravasation and metastasis in distant organs.[160,161] The biologic role of a low level of circulating VEGF in healthy subjects is also unknown. It is tempting to speculate a function of circulating VEGF in sustaining a constant low-grade regeneration of vascular endothelium to compensate for "wear and tear" in the vasculature of normal organs.

CIRCULATING bFGF IN CANCER PATIENTS

Elevation of serum and urine levels of bFGF has been documented in patients with a wide variety of cancers since early 1990s.[15,164] However, it was only within the last 5 years that there was intense research on the clinical implications of circulating bFGF in cancer patients. In 1997, a study reported elevated levels of bFGF in 132 patients with metastatic cancer from colorectal, breast, ovarian, or renal carcinoma and suggested a nonspecific correlation between serum bFGF level and the progression kinetics of metastatic carcinoma.[165] Subsequently, various groups of researchers have investigated specifically the clinical significance of circulating bFGF level in individual type of cancer, although data for each cancer are available only from one or two studies (Table 4).[166-179] The clinical significance of circulating bFGF in relation to tumor status and prognosis is

Table 4. Studies of Circulating Basic Fibroblast Growth Factor in Cancer Patients

Cancer Type	Correlation With Tumor Progression or Patient Survival*	Ref
Breast cancer	No correlation	166(S), 167(S)
Lung cancer	Reverse correlation	123(S)
Gastrointestinal cancers		
Colorectal carcinoma	Positive	124(S)
	No correlation	68(S), 129(P)
Gastric carcinoma	No correlation	132(P)
Hepatocellular carcinoma	Positive	168(S)
Urologic cancers		
Prostate carcinoma	Positive	169(S), 170(S)
	No correlation	171(S)
Renal cell carcinoma	Positive	15(S), 172(S)
Gynecologic cancers		
Ovarian carcinoma	Positive	173(S)
Endometrial carcinoma	Positive	174(S)
Cervical carcinoma	No correlation	175(S)
Head and neck cancers	Positive	176(S), 177(S)
Hematologic malignancies		
Lymphoma	Positive	151(S), 178(S)
Leukemia	Positive	179(S)
Soft tissue sarcoma	Positive	153(S)

*Positive correlation indicates more advanced tumor or poorer survival with higher circulating bFGF level, reverse correlation indicates earlier tumor stage or better survival with higher circulating bFGF level.

more controversial compared with what has been documented for VEGF.

Breast Cancer

So far, there has been no evidence in support of a prognostic role of circulating bFGF in breast cancer. Sliutz et al[166] evaluated the serum bFGF level in 20 breast cancer patients before and after surgery and found no correlation between preoperative serum bFGF and prognosis or lymph node status. Another study of 166 patients also failed to find a significant correlation between serum bFGF and tumor stage or prognosis in breast cancer.[167]

Lung Cancer

Only one study of serum bFGF in lung cancer has been reported at the time of this review. It showed that elevated serum bFGF was a favorable prognostic factor for survival among 68 patients with non–small-cell lung cancer treated with radiotherapy.[123] The authors have postulated some possible explanations for this unexpected finding, including a direct inhibitory effect of bFGF on cancer cell proliferation and a fibrosis-inducing effect of bFGF in response to radiotherapy. Studies in patients undergoing other forms of treatment, such as chemotherapy and surgery, may help clarify the prognostic significance of circulating bFGF in lung cancer.

Gastrointestinal Cancers

Unlike studies of circulating VEGF, studies on circulating bFGF in colorectal cancer have so far produced inconsistent data. Dirix et al[124] showed that high serum bFGF level was associated with shorter tumor volume doubling time in metastatic or recurrent disease of colorectal cancer, thus suggesting a positive correlation between serum bFGF level and tumor progression. In contrast, other authors reported that plasma or serum bFGF level did not correlate with microvessel count, tumor volume, or tumor stage in colorectal carcinoma.[68,129]

There was only one reported study each on the prognostic role of circulating bFGF in gastric and hepatocellular carcinoma.[132,168] The study on gastric cancer revealed no prognostic relevance of plasma bFGF level,[132] whereas the study on hepatocellular carcinoma found that a high serum bFGF level was positively correlated with advanced tumor stage.[168]

Urologic Cancers

Studies of circulating bFGF in prostate cancer were also not in agreement with each other. Two groups observed an increase in serum bFGF level with progression of tumor to advanced stage.[169,170] On the contrary, Meyer et al[171] found no significant correlation between serum bFGF level and tumor stage, Gleason score, or tumor volume.

Two studies in renal cell carcinoma have shown a positive correlation between serum bFGF level and tumor stage, tumor grade, or presence of pulmonary metastasis.[15,172] The relationship between circulating bFGF level and tumor status or prognosis is yet to be studied in urothelial carcinoma.

Gynecologic Cancers

Data on circulating bFGF in gynecologic cancers were scarce. Increased serum bFGF level has been shown to be associated with advance tumors in ovarian carcinoma[173] and endometrial carcinoma.[174] On the other hand, serum bFGF level has not been found to have an impact on prognosis in a study of cervical cancer patients.[175]

Head and Neck Cancers

Two groups of investigators have studied circulating bFGF in head and neck cancer patients. Leunig et al[176] reported elevated serum and urine levels of bFGF in head and neck cancer patients, and the levels of serum bFGF were positively related to the tumor volume. Dietz et al[177]

showed that bFGF was an independent prognostic factor for tumor control in advanced head and neck cancer after primary radiochemotherapy, with a shorter period of locoregional control in patients with a level of serum bFGF above the upper limit of normal controls. These preliminary data suggest a possible prognostic role of serum bFGF in head and neck cancer patients.

Hematologic Malignancies

In a study that measured bFGF in the sera of 160 non-Hodgkin's lymphoma patients taken before starting chemotherapy or radiotherapy, a high serum bFGF within the highest quartile was found to be an independent prognostic factor of survival.[178] In this study, serum bFGF had a stronger prognostic value than serum lactate dehydrogenase and the number of extranodal tumor sites, both of which are well established prognostic factors of non-Hodgkin's lymphoma. Another group has also provided data in support of a prognostic value of serum bFGF level in non-Hodgkin's lymphoma.[151]

Increase in intracellular and plasma level of bFGF has been reported in chronic lymphocytic leukemia.[179] A more recent study has shown that intracellular level of bFGF was correlated with disease stage in patients with chronic lymphocytic leukemia.[180]

Sarcoma

Little has been documented on the clinical significance of circulating bFGF in sarcoma patients. One study has demonstrated a significant correlation between serum bFGF level and tumor mass or histologic grade in patients with soft tissue sarcoma.[153]

Source and Biologic Significance of bFGF in the Circulation

Circulating bFGF level may have a prognostic value at least in certain cancers, although the supporting data are limited. The biologic significance of circulating bFGF is also unknown. Like VEGF, the exact source of bFGF in the circulation has not been established. Apart from tumor cells, peripheral-blood cells and tumor-infiltrating cells such as mononuclear cells and lymphocytes also have the capacity to produce bFGF.[181] In a tumor-bearing mouse model, the origin of elevated bFGF levels in the urine was found to be exclusively from tumor cells, thus indicating that the source of elevated bFGF in the urine of human cancer patients is, at least in part, the tumor itself.[182] In a study in colorectal cancer, the level of bFGF in blood taken from mesenteric vein draining the tumor was more than four-fold higher than peripheral blood in the same patients, implying that a large proportion of the circulating bFGF might be derived from abnormal release from the tumors.[68]

OTHER ANGIOGENIC FACTORS IN THE CIRCULATION

VEGF and bFGF are the two angiogenic factors most widely studied regarding their clinical relevance in the circulation. Other angiogenic factors such as PD-ECGF, TGF-β, and angiogenin have also been examined with respect to their clinical significance in the circulation, but the available data are confined to a few specific cancers only.

PD-ECGF

Circulating PD-ECGF level has been found to be elevated in cancer patients compared with normal controls.[183] In a study of hepatocellular carcinoma, plasma PD-ECGF levels in patients with the cancer were significantly higher compared with cirrhotic patients or normal controls, and there was significantly increased PD-ECGF expression in hepatocellular cancer cells compared with normal liver cells.[184] The same study also demonstrated a positive correlation between elevated plasma PD-ECGF and advanced tumor stage. As tumor expression of PD-ECGF has been shown to be of prognostic significance in several human cancers (Table 2), it is worthwhile to study the prognostic influence of circulating PD-ECGF level in these cancers.

TGF-β

Increased serum TGF-β level compared with healthy controls has been demonstrated in patients with renal cell carcinoma,[185] nasopharyngeal carcinoma,[186] and colorectal cancer.[187] Two of these studies also revealed a significant association between high serum TGF-β level and advanced tumor stage or presence of metastasis.[186,187] In contrast, a study in patients with prostatic disease did not find a significant difference in the serum TGF-β level in patients with prostatic carcinoma compared with those harboring benign prostate hypertrophy, and there was no correlation with tumor stage in prostatic carcinoma.[188]

Angiogenin

Elevated serum angiogenin level has been documented in patients with pancreatic cancer,[85] colorectal cancer,[189] and urothelial cancer.[99] A high serum angiogenin level was found to be related to disease progression or poor survival in all these studies. In one study, the serum angiogenin level decreased to normal levels after resection of colorectal cancer, thereby suggesting that at least part of the circulating angiogenin is derived from the tumor.[189]

CLINICAL IMPLICATIONS OF CIRCULATING ANGIOGENIC FACTORS IN CANCER PATIENTS

Prognostic Implications

Studies of circulating angiogenic factors in cancer patients have so far focused mainly on their prognostic implication in relation to tumor status and survival. Although the results of studies have not been completely homogeneous, there is compelling evidence that circulating angiogenic factors are of prognostic significance. The evidence is most overwhelming in the case of VEGF. Circulating VEGF level seems to be particularly useful as a prognostic tool in clinical subsets of patients, such as those with early-stage or lymph node–negative cancers, for which current clinicopathologic parameters are limited in their prognostic capacity. Apart from providing prognostic information, the level of circulating angiogenic factors may also have therapeutic implication in the selection of patients at high risk of recurrence for adjuvant therapy.

The use of preoperative circulating angiogenic factor level before surgical resection to predict disease invasiveness of a tumor, such as the presence of vascular invasion and lymph node metastasis, is particularly attractive in that it may help selecting patients with invasive tumors for neoadjuvant therapy before surgery. For example, we have shown that preoperative serum VEGF level is predictive of microscopic venous invasion in hepatocellular carcinoma patients,[135] and another group has demonstrated that preoperative serum VEGF level is predictive of tumor stage in colorectal carcinoma.[125] Previously, reliable information about tumor invasiveness and stage might not be available until after histologic examination of the resected specimens.

Diagnostic Implications

When early studies found that the levels of circulating angiogenic factors were significantly higher in cancer patients compared with healthy controls, it was hoped that the circulating angiogenic factors might also have a diagnostic utility in early detection of cancers. However, more recent studies have shown that the level of circulating VEGF is elevated mainly in patients with advanced stage tumor, whereas the level of VEGF in patients with early-stage cancers may not be significantly higher than healthy controls.[135,148] Hence, there seems to be little role of circulating VEGF level in early cancer detection.

Other authors have recently investigated the role of circulating angiogenic factors in discriminating patients with cancers from those with benign disease. Studies have found a significantly higher VEGF level in ovarian carcinoma compared with benign ovarian pathologies such as cystoadenoma, suggesting that serum VEGF might have a diagnostic value in differentiating benign and malignant ovarian neoplasms.[101,145,190] However, another group showed that CA-125 but not VEGF differentiated between benign ovarian cysts and cancers when both markers were analyzed in a multivariate model for their ability to predict malignancy in patients with ovarian masses.[191] Two studies in prostatic disease also indicated that neither serum bFGF nor TGF-β1 levels could differentiate between prostatic carcinoma and benign prostate hyperplasia.[171,188] Based on these data, the diagnostic value of circulating angiogenic factors in cancer patients seems to be limited.

Monitor for Tumor Recurrence

Another potential clinical application of circulating angiogenic factors is to monitor for recurrent disease after treatment of primary tumors, especially after surgical resection. Remarkable fall in serum VEGF level has been documented after surgical removal of breast cancer,[117,118] colorectal cancer,[126] and ovarian cancer.[101] In some of these studies, a persistent elevation or reelevation after initial fall of serum VEGF has been observed in patients with recurrence after resection of the primary tumor[101,117,118] In contrast, patients with no recurrence tended to have a persistent decrease in serum VEGF level after surgical removal of the cancers. In a study of serum bFGF in cervical cancer, a continuous increase in serum bFGF was observed months before clinical detection of disease relapse in patients after complete remission, thus indicating that serum bFGF might be useful in the surveillance of disease relapse.[175] However, these results should be considered preliminary, as the numbers of patients evaluated were small, and the level of circulating angiogenic factors has not been monitored serially in a prospective manner. The potential role of monitoring circulating angiogenic factors for early detection of tumor recurrence is an important subject for further studies.

Prediction and Monitor of Response to Cancer Therapy

Currently, there is no reliable and standardized indictor to predict response to cancer therapies. The angiogenic activity of a tumor is a major factor affecting the proliferation rate of tumor cells, and hence there may be a relationship between tumor angiogenesis and the responsiveness to cytotoxic anticancer therapy. Furthermore, the microvascularization within a tumor may affect tissue diffusion of anticancer drugs. Angiogenic activity also influences local oxygenation within the tumor and thus may affect the responsiveness of the tumor to radiotherapy.

Dirix et al[165] first showed that serum VEGF and bFGF levels were higher in progressive disease compared with responsive disease in patients with metastatic cancer from

various origins treated with chemotherapy. A subsequent study found that a high pretreatment plasma VEGF level was predictive of poor response to systemic chemotherapy in patients with advanced gastric or colorectal carcinoma; such a predictive power was not observed with carcinoembryonic antigen or CA-19.9 levels.[127] Another group showed that a high pretreatment serum VEGF was predictive of poor response to combination chemotherapy in patients with small-cell lung cancer.[121] These data indicated a possible predictive value of serum VEGF concerning the responsiveness of the tumor to chemotherapy.

A further potential application of circulating VEGF level is to monitor tumor response to chemotherapy. In a study comparing pre and postchemotherapy serum VEGF levels among patients with gastric cancer, a decrease in serum VEGF in those with a partial response to chemotherapy was observed, while patients who had progressive disease despite chemotherapy showed an increase of VEGF level.[130] Another group monitored serial serum VEGF levels in patients with various types of carcinoma and sarcoma treated with different combinations of chemotherapy, radiotherapy, and interferon, and the level of serum VEGF in nonresponders was found to have increased more than two-fold after treatment, whereas a decrease in serum VEGF level was noted in responders.[192] An interesting case report has also documented a close correlation between changes in serum VEGF levels and response to ex vivo gene therapy in a patient with metastatic renal cell carcioma.[193] These studies provide preliminary evidence for a potential role of serum VEGF in monitoring response of tumor to anticancer therapies, but further studies are required to testify the results before it can be considered for application in clinical practice.

Antiangiogenic therapy is a promising novel approach of anticancer treatment. Many of the antiangiogenic agents targeting at the inhibition of the expression or activity of specific angiogenic factors are now under clinical trials in cancer patients.[194] It is reasonable to envisage that the circulating level of a specific angiogenic factor may be useful in predicting and monitoring the response of a tumor to antiangiogenic therapy directed towards that particular angiogenic factor, although data are not yet available in this aspect.

FUTURE PERSPECTIVES

Over the past few years, rapid progress has been observed in the translation of angiogenesis research into clinical applications. There is now fairly convincing evidence in support of a prognostic value of tumor angiogenic activity in cancer patients, hence the assessment of angiogenesis may become a part of routine prognostic evaluation in cancer patients in the near future. In addition, evaluation of tumor angiogenic activity may have other far-reaching implications in cancer management. Up-to-date knowledge in this area should be of interest to all clinicians involved in the care of cancer patients. To this end, we have presented an overview of the biologic role and the clinical relevance of angiogenic factors in human cancers. To our knowledge, this review is the first attempt to summarize the substantial amount of data from recent studies on the prognostic value and other clinical implications of circulating angiogenic factors in cancer patients.

In clinical practice, measurement of angiogenic factors in the circulation is a more appealing approach than assessing the expression of angiogenic factors in tumor tissue, as the former is noninvasive, convenient and more practicable. The availability of commercial immunoassay kits for various angiogenic factors means that this technique can be widely adopted for clinical application once it is proven to be of clinical benefit. The global evidence from the available literature indicates that circulating VEGF is a useful tumor marker for evaluating the disease status in cancer patients, and it seems to have a prognostic value in a wide variety of human cancers. This corroborates the pivotal role of VEGF in tumor angiogenesis. An important advantage of circulating VEGF over the currently used serum tumor markers in cancer patients is its apparently universal prognostic value in almost all types of cancers. However, before embarking on routine use in cancer patients, prospective studies recruiting a large number of patients should be conducted to validate the results of existing reports. Large-scale studies are also needed to determine the cutoff level of circulating VEGF that best discriminates between patients with favorable and adverse prognosis. The cutoff level has been set according to different criteria in previous studies. Further research should be conducted to clarify the source of VEGF in the circulation and the intriguing role of platelets in relation to circulating VEGF to determine whether serum, plasma, or whole blood should be the best sample for measurement of circulating VEGF.

Existing data with respect to circulating bFGF in cancer patients are less conclusive, as there is considerable disparity among the limited number of studies. There is a paucity of clinical data on the other circulating angiogenic factors, although preliminary reports in a few cancers suggest that they may also be of some prognostic value. Further studies should be performed to elucidate the clinical significance of each of these angiogenic factors in human cancers.

Studies on circulating angiogenic factors have focused on the prognostic significance of single angiogenic factor in cancer patients. A recent study evaluating both serum VEGF and bFGF levels in patients with different types of

carcinoma and sarcoma revealed that VEGF and bFGF were generally not elevated in the same patient, and covariation of VEGF and bFGF levels was rarely observed during tumor progression, arguing for independent expression of the two angiogenic factors.[192] Another study in patients with ovarian cancer also found no correlation in the levels of serum VEGF, bFGF, and angiogenin, suggesting that these angiogenic factors might be independently regulated even in patients with the same type of cancer.[173] Appraisal of an angiogenic profile encompassing multiple angiogenic factors in the circulation may provide more accurate prognostic information than the level of a single angiogenic factor. It has also been suggested that measurement of both angiogenic and antiangiogenic factors in the circulation may reflect the net balance of tumor angiogenic activity, and hence may have a better prognostic value.[195] Little has been addressed to the clinical implication of circulating antiangiogenic factors in cancer patients, a research area that deserves attention in the future.

Although the clinical relevance of circulating angiogenic factors has been investigated extensively, very few studies have alluded to the biologic significance of angiogenic factors in the circulation of cancer patients. Molecular research to unveil interactions between circulating angiogenic factors, endothelial cells, normal cell components, and tumor cells in the circulation may help clarify the mechanism of cancer vascular invasion and metastasis. VEGF seems to be an angiogenic factor of particular interest in this aspect, as its enhancing effect on vascular permeability may contribute to the process of cancer cell intravasation and extravasation. Emerging data suggest that circulating VEGF may play an important role in tumor cell dissemination. Understanding the biologic role of circulating angiogenic factors in cancer patients may provide insight into potential strategies to prevent the development of metastasis. For example, a recent study has demonstrated an immediate increase of serum VEGF level in patients after resection of pulmonary metastasis, which might contribute to the development of further metastasis in the early postoperative period.[196] The authors showed that in an animal model, an increase in circulating VEGF level resulted in rapid growth of dormant micrometastases in the lung, which could be abolished by an angiogenesis inhibitor. This opens up a potential role of antiangiogenic therapy in suppressing the development of metastases after cancer resection.

In conclusion, there is mounting evidence that the level of circulating angiogenic factors, in particular VEGF, has a prognostic significance in various human cancers. Further studies should evaluate its use in clinical practice. Other potential clinical applications of circulating angiogenic factors such as monitoring for tumor recurrence and prediction of response to anticancer treatment should also be explored by more intense research. Antiangiogenic therapy is now being evaluated in clinical trials, and the potential relevance of circulating angiogenic factors in patients undergoing antiangiogenic treatment is a particularly interesting area for future research. Ultimately, the prognostic and therapeutic applications of tumor angiogenesis may merge with the clinical use of circulating angiogenic factors to predict and monitor the response of tumors to antiangiogenic therapy.

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