Clinical implications of angiogenesis in cancers

Roberta WC Pang[1]
Ronnie TP Poon[2]

Departments of [1]Medicine and [2]Surgery, The University of Hong Kong, Pokfulam, Hong Kong, China

Abstract: Angiogenesis plays an important role in the growth and progression of cancer. The regulation of tumor angiogenesis depends on a net balance of angiogenic factors and antiangiogenic factors, which are secreted by both tumor cells and host-infiltrating cells. Numerous studies have indicated that assessment of angiogenic activity by either microvessel density or expression of angiogenic factors in cancer can provide prognostic information independent of conventional clinicopathological factors such as tumor staging. Some studies also suggested that assessment of tumor angiogenesis may predict cancer response to chemotherapy or radiotherapy. However, the most important clinical implication of tumor angiogenesis is the development of a novel strategy of anticancer therapy targeting tumor vessels instead of cancer cells. Antiangiogenic therapy aims to inhibit the growth of tumor, and current evidence suggests that it works best in combination with conventional cytotoxic chemotherapy. Recently, a monoclonal antibody against vascular endothelial growth factor, which is one of the most potent angiogenic factors, has been approved for clinical use in colorectal cancer patients after a clinical trial confirmed that combining the antibody with standard chemotherapy regimen could prolong patient survival. The clinical implications of angiogenesis in cancer are reviewed in this article.

Keywords: angiogenesis, antiangiogenic therapy, cancer, prognosis

Angiogenesis

Angiogenesis refers to the sprouting of new blood vessels from pre-existing capillaries. It is a multi-step process involving proliferation of activated endothelial cells, migration of the endothelial cells to reach remote targets, assembly of the endothelial cells into new capillary tubes, followed by the synthesis of a new basement membrane and maturation of vessels with formation of a vascular lumen. Angiogenesis is different from vasculogenesis, which involves de novo differentiation of endothelial cells from in situ mesoderm-derived precursor cells. Although sprouting from pre-existing blood vessels is the principal process in angiogenesis, recent evidence has suggested that recruitment and in situ differentiation of bone marrow-derived endothelial progenitor cells are involved in angiogenesis in physiological and pathological conditions (Asahara et al 1999). Angiogenesis is a critical process in embryogenesis. In the adult, new blood vessel formation is required in some physiological conditions such as the female reproductive cycle, tissue repair, and wound healing. More importantly, angiogenesis is now known to play an essential role in pathological conditions such as cardiac and limb ischemia, diabetic retinopathy, rheumatoid arthritis, and neoplasms (Carmeliet and Jain 2000).

The concept that tumor growth and metastasis are dependent on the development of new blood vessels was first formulated by Folkman (1971), who suggested that a solid tumor starts as a dormant avascular nodule which could only grow and develop if it becomes vascularized. Neovascularization must occur to provide oxygen and nutrients to the tumor cells. Furthermore, the immature neovessels enhance tumor

Correspondence: Ronnie TP Poon
Department of Surgery, The University of Hong Kong, Queen Mary Hospital, 102 Pokfulam Road, Hong Kong, China
Tel +852 2855 3641
Fax +852 2817 5475
Email poontp@hkucc.hku.hk

cell entry into the circulation and hence distant metastasis (Liotta and Stracke 1988). It is now also recognized that neovascularization dependence goes beyond solid tumors; it also plays an important role in the development of hematological malignancies (Perez-Atayde et al 1997). The understanding of the fundamental role of angiogenesis in cancer growth and metastasis has led to tremendous interest in research in its regulatory mechanisms and clinical implications in the management of cancer patients in the past three decades.

Regulatory mechanisms of cancer angiogenesis

The control of tumor angiogenesis depends on a net balance of several activators (angiogenic factors) and inhibitors (antiangiogenic factors), which are secreted by both tumor cells and host infiltrating cells such as macrophages and fibroblasts. During tumor progression, environmental and genetic changes induce an "angiogenic switch", with either upregulation of angiogenic factors or downregulation of angiogenesis inhibitors (Hanahan and Folkman 1996). Environmental signals that can trigger angiogenesis include hypoxia, change in pH, metabolic stress, and cytokines from inflammatory response (Shweiki et al 1992, 1995; Akagi et al 1999). Angiogenesis is also potentiated by certain oncogenes such as Src and Ras (Kerbel et al 1998; Rak et al 2000), and downregulated by certain tumor suppressor genes such as p53 and von Hippel-Lindau genes (Pal et al 1997; Bouvet et al 1998). There is also evidence that angiogenesis may be stimulated by hormones such as androgen (Jain et al 1998), progesterone (Wu et al 2004), and estrogen (Dabrosin et al 2003), which may contribute to carcinogenesis and tumor progression in hormone-dependent cancers such as prostate and breast cancer.

The development of new blood vessels in a tumor starts with the release of angiogenic factors, which bind to specific receptors of endothelial cells of pre-existing blood vessels to trigger the process of angiogenesis. In addition to angiogenic factors, proteinases such as matrix metalloproteinases (MMPs) and plasminogen activators are required to dissolve the extracellular matrix in front of the sprouting vessels (Bergers et al 2000). During the process of angiogenesis, endothelial cell adhesion molecules such as integrin $\alpha_v\beta_3$ and vascular adhesion molecule-1 help to connect new vessels with the pre-existing ones to produce the intratumoral vascular network (Koch et al 1995; Eliceri and Cherish 1999).

In the initial hypothesis of Folkman (1971), the development of new blood vessels during angiogenesis was presumed to originate from endothelial cells in pre-existing vessels. However, there is now strong evidence from studies in animal tumor models that tumor endothelial cells may also be derived from circulating endothelial precursor cells originating from the bone marrow (Asahara et al 1999; Ruzinova et al 2003). Furthermore, it has been proposed that other types of bone marrow progenitor cells from different lineages, such as hematopoietic progenitor cells, can be co-recruited to tumor angiogenesis foci (Lyden et al 2001). A recent study has demonstrated the presence of bone marrow-derived circulating endothelial precursor cells in human cancers (Peters et al 2005). However, the exact contribution of bone marrow-derived circulating endothelial precursor cells to the formation of neovessels in tumors remains uncertain, and its regulatory mechanisms are also far from clear. Besides sprouting from pre-existing host vessels and development of new vessels from bone marrow-derived endothelial progenitor cells, it has also been proposed that by co-option, cancer cells can grow around an existing host vessel and form vessel-like networks, a process termed vascular mimicry, although the significance of this tumor vascularization model is controversial (Holash et al 1999).

There are more than 40 known endogenous inducers and inhibitors of angiogenesis to date. Table 1 shows the relatively well-characterized endogenous angiogenic and antiangiogenic factors. The best characterized angiogenic factor is vascular endothelial growth factor (VEGF), which is secreted by almost all solid cancers (Leung et al 1989). VEGF is a heparin-binding peptide with a specific mitogenic effect on endothelial cells, and it also increases vascular permeability. VEGF is the central mediator of tumor angiogenesis stimulated by hypoxia and certain oncogenes. The effects of VEGF on endothelial cells are mediated via its receptors, Flt-1 and KDR (Veikkola et al 2000). Basic fibroblast growth factor (bFGF) is another potent angiogenic factor secreted by most solid tumors. It acts synergistically with VEGF in inducing angiogenesis (Asahara et al 1995). Platelet-derived endothelial cell growth factor (PD-ECGF), also known as thymidine phosphorylase, stimulates endothelial cell migration rather than proliferation, and its angiogenic effect is mediated by the release of 2-deoxy-D-ribose as a result of breakdown of thymidine by PD-ECGF (Griffiths and Stratford 1997). Angiogenin, a peptide that belongs to the family of pancreatic ribonucleases, is a potent inducer of angiogenesis in vivo (Badet 1999). Angiopoietins

Table 1 Endogenous angiogenic and antiangiogenic factors

Angiogenic factors	Antiangiogenic factors
Vascular endothelial growth factor	Thrombospondin-1, 2
Acidic and basic fibroblast growth factors	Endostatin
Transforming growth factor-α/β	Angiostatin
Platelet-derived endothelial cell growth factor	Interferon-α/β
Hepatocyte growth factor	Interleukin-12
Tumor necrosis factor-α	Platelet factor 4 fragment
Epidermal growth factor	Angiopoietin-2
Placental growth factor	Human macrophage metalloelastase
Tissue factor	Tissue inhibitor of metalloproteinase-1/2
Interleukin-6/8	Vascular endothelial growth inhibitor
Angiogenin	Vasostatin
Angiopoietin-1	Anti-thrombin III fragment
Cyclooxygenase-2	Osteopontin fragment
Nitric oxide	

are more recently identified mediators of angiogenesis. Angiopoietin-1 binds to Tie-2, an endothelial cell specific tyrosine kinase receptor, leading to endothelial cell stabilization (Papapetropoulos et al 1999). In contrast, angiopoietin-2 binds to Tie-2 and leads to endothelial cell destabilization and vascular regression (Maisonpierre et al 1997). Cyclooxygenase-2 (Cox-2), an enzyme known to regulate cellular processes such as apoptosis, also has an angiogenic effect via thromboxane-A2 (Daniel et al 1999). Tissue factor is a primary physiological initiator of blood coagulation that has been shown to enhance tumor angiogenesis (Daniel et al 1999). Among the antiangiogenic factors, thrombospondin-1 is considered an important inhibitor of tumor angiogenesis (Gupta et al 1999). Two other potent antiangiogenic factors are angiostatin and endostatin, which are produced by tumor cells themselves and are generated by proteolysis of inactive circulating precursors plasminogen and collagen XVIII, respectively (O'Reilly et al 1996, 1997). It has been postulated that endogenous inhibitors of angiogenesis produced by a tumor, such as angiostatin and endostatin, may play an important role in tumor dormancy.

Angiogenic factors secreted by tumor cells act in a paracrine fashion on tumor endothelium during the different phases of the tumor angiogenic process. There is recent evidence that some of the angiogenic factors may act in an autocrine fashion on the cancer cells. VEGF was initially thought to be a specific angiogenic factor, and VEGF receptors were thought to be expressed on the cell surface of endothelial cells only. However, recent studies have demonstrated that VEGF receptors are expressed on cancer cells of several types of human cancers and may mediate proliferation and invasion of cancer cells (Herold-Mende et al 1999; Dias et al 2000). Our group has demonstrated that VEGF receptors are expressed on several hepatocellular carcinoma cell lines, and VEGF stimulates proliferation of hepatocellular carcinoma cells (Liu et al 2005). The autocrine effect of VEGF may explain in part the antitumor effect observed with anti-VEGF antibody in combination with chemotherapy in addition to its antiangiogenic effect. Tyrosine kinase receptors of other angiogenic factors such as bFGF and PD-ECGF have also been identified in cancer cells of some human cancers (Dickson et al 2000; George 2003). The functional role of these receptors in cancer cells has not been fully clarified yet. These tyrosine kinase receptors are expressed in endothelial cells and they mediate the angiogenic effects of the angiogenic factors by modulating the signaling of these factors, hence they are important targets for antiangiogenic therapy. Small molecule inhibitors of these tyrosine kinase receptors that compete with the adenosine 5'-triphosphate (ATP)-binding site of the catalytic domain of the tyrosine kinases have been developed and are now under clinical trials in cancer patients.

Prognostic implications of cancer angiogenesis

Because cancer angiogenesis is related to tumor growth and metastasis, assessment of tumor angiogenesis activity may be useful in prognostic prediction. Weidner et al (1991) first reported the prognostic significance of tumor angiogenesis in breast cancer patients. Tumor neovascularization was quantified by immunohistochemistry using endothelial markers to stain microvessels, which are not seen in a conventional histological examination. After immunostaining, the entire tumor section was scanned at low power (x 40) field to identify "hot spots", which are the areas of the highest neovascularization. Individual microvessels were then counted under a high power (x 200) field to obtain a vessel count in a defined area, and the average vessel count in five hot spots was taken as the microvessel density (MVD). Commonly used endothelial markers for assessing MVD include CD31, CD34, and von Willebrand factor (vWF). Subsequent to the study by Weidner et al, numerous studies have shown the prognostic

significance of tumor MVD on survival and/or disease recurrence after surgical resection of different cancers, some showing that tumor MVD was a prognostic factor independent of conventional pathological prognostic factors (Maeda et al 1995; Takebayashi, Akiyama, et al 1996; Ellis et al 1998; Igarashi et al 1998). Assessment of tumor angiogenesis may be particularly useful in prognostic classification of patients with apparently early cancer by conventional tumor staging, some of whom may still develop early recurrence or metastasis despite being staged as having early cancers by conventional parameters such as tumor size. In a study conducted by our group, tumor MVD was found to be an independent prognostic factor in patients with small hepatocellular carcinomas <5 cm (Poon et al 2002). Tumor angiogenesis is not only of prognostic value in solid cancers, but it may also predict prognosis in patients with hematological malignancies. Studies have demonstrated adverse prognosis in leukemia patients with high bone marrow angiogenesis (Molica et al 2002; Rabitsch et al 2004). Overall, most studies showed that the degree of neovascularization in various human cancers is a prognostic indicator. However, there remains some controversy regarding the prognostic value of tumor MVD because negative results have been demonstrated in a few studies (Ellis et al 1998; Torres et al 1999; Pietra et al 2000). The inconsistent results may be related to the lack of a standardized and objective method of assessing tumor MVD. The counting of microvessels in selected hot spots under microscopy is likely to be associated with subjective bias and inter-observer variation, which is further aggravated by the lack of a standardized site of tumor sampling in retrospective studies that employed archived tumor specimens. The use of different endothelial markers in different studies is also a potential source of variation. Automated computerized image analysis for quantifying the MVD may reduce subjective bias during the counting process (Poon et al 2002). In a study of breast cancer, tumor MVD obtained by automated computerized image analysis, but not the MVD obtained by manual counting, was an independent prognostic factor (Acenero et al 1998). The use of more specific antibodies to stain activated endothelial cells has also been proposed to improve tumor MVD assessment. Immunostaining for integrin $\alpha_v\beta_3$, which is a vascular endothelial adhesion molecule upregulated in angiogenesis, may allow selective staining of activated endothelial cells (Gasparini et al 1998).

The evaluation of expression of angiogenic factors in tumor specimens provides an alternative to MVD in assessing tumor angiogenic activity. This method may potentially reduce the bias associated with the selection of hot spots for MVD evaluation, and may provide more functional information on the tumor angiogenic activity than MVD. VEGF is the most widely studied angiogenic factor for its clinical significance. Numerous studies have demonstrated that tumor overexpression of VEGF correlates with high tumor MVD and is associated with advanced tumor stage or tumor invasiveness in various common human cancers (Maeda et al 1996; Kitadai et al 1998; Seo et al 2000; Verstovsek et al 2002). In some studies, VEGF expression in the tumor has been shown to be a prognostic factor independent of conventional prognostic factors (Maeda et al 1996; Seo et al 2000). VEGF expression in cancer cells has also been shown to be a prognostic factor in hematological malignancies (Verstovsek et al 2002). The effect of VEGF on angiogenesis depends on not only tumor cell expression of VEGF, but also on the VEGF receptors in the endothelial cells. A study from our group showed that the VEGF receptor Flt-1 was upregulated in the endothelial cells of hepatocellular carcinoma and correlated significantly with the VEGF level in the tumor and intrahepatic metastasis (Tokunaga et al 1998). The overexpression of other angiogenic factors such as PD-ECGF (Takebayashi, Aklyama, et al 1996; Matsumura et al 1998; Konno et al 2001), bFGF (Yamanaka et al 1993; El-Assal et al 2001), transforming growth factor (TGF)-β (Friess et al 1993; Maehara et al 1999; Saito et al 1999), angiogenin (Shimoyama et al 1996; Etoh et al 2000), tissue factor (Seto et al 2000; Poon, Lau, Ho, et al 2003), and COX-2 (Cianchi et al 2001; Tang et al 2005) in various cancers has been shown to correlate with advanced tumor stage and decreased patient survival.

The majority of angiogenic factors are soluble and diffusible peptides. Hence, the circulating level of angiogenic factors has been investigated as a surrogate marker of the angiogenic activity of the tumors. This may have advantages in clinical application because the measurement of circulating angiogenic factors is a convenient and noninvasive approach that does not require tumor specimens, and it can be repeated before and after operation or other treatments. Dynamic contrast enhanced magnetic resonance and computed tomography are widely used in monitoring tumor angiogenesis before and after antiangiogenic therapy. Compared with these complex imaging assays of tumor vascularity, measurement of circulating angiogenic factors as biomarkers is easier and less expensive. The concentration of circulating angiogenic

factors can be measured easily by enzyme-linked immunosorbent assay (ELISA). Folkman's group first reported a significant association between high serum or urine levels of bFGF and progressive disease in patients with different types of cancers (Nguyen et al 1994). Subsequently, it was also shown that serum VEGF levels of cancer patients were significantly higher than those of healthy controls (Yamamoto et al 1996). The clinical significance of circulating angiogenic factors in various types of cancers has been studied extensively, and a detailed review of the topic by our group has been previously published (Poon, Fan, Wong, et al 2001). Most studies evaluated the prognostic value of preoperative levels of circulating angiogenic factors and suggested a prognostic value of circulating level of angiogenic factors in cancer patients. The prognostic significance of circulating VEGF has been most widely studied (Jacobson et al 2000; Yoshikawa et al 2000; Aguayo et al 2002; Werther et al 2002; Poon et al 2004). There are data that suggest the prognostic significance of the circulating levels of other angiogenic factors such as bFGF (Poon et al 2001a) and PD-ECGF (Shimada et al 2002). Because of its pivotal role in angiogenesis, VEGF appears to be the most promising angiogenic biomarker. The prognostic value of serum VEGF may have significant clinical implications because it can be used before an operation to select patients with more invasive tumors who may benefit from neoadjuvant therapy or alternative treatments (Poon et al 2001b). However, there is still some controversy regarding the clinical value of measurement of serum VEGF level because the VEGF in the circulation is found largely in the platelet, and there is a debate whether the serum VEGF level truly reflects tumor expression of VEGF or whether there are other possible sources of circulating VEGF, such as the blood cells (Poon, Fan, Wong, et al 2001). More recent studies since our last review in 2001 have provided better insight into the significance of circulating angiogenic factors. In a study by our group, we demonstrated that the serum VEGF per platelet, which reflects the platelet load of VEGF in the circulation, correlated positively with tumor expression of VEGF in patients with hepatocellular carcinoma (Poon, Lau, Cheung, et al 2003). In recent years, there have been several reports on the use of circulating VEGF in predicting or monitoring response to antiangiogenic therapy in clinical trials. There is some evidence that post-treatment decline in circulating VEGF levels is associated with response or stabilization of the disease (Drake et al 2003; Hernberg et al 2003; Zangari et al 2004), but pre-treatment serum VEGF level does not appear to predict treatment response (Stadler et al 2004). Post-treatment change of circulating VEGF level may be most valuable in monitoring response to antiangiogenic therapy targeting VEGF (Drevs et al 2005). Theoretically, measurement of circulating angiogenic factors may also be of value in selecting the optimum antiangiogenic therapy, but so far there have been little data on this aspect. Recently, there is evidence that measurement of soluble VEGF receptors, in addition to serum VEGF level, may also provide prognostic information in cancer patients (Hu et al 2004). The ratio of VEGF and soluble VEGFR1 has been shown to provide better prognostic value than serum VEGF, VEGFR1 or VEGFR2 alone (Hoar et al 2004; Aref et al 2005).

A few studies have evaluated the relationship between tumor angiogenesis and tumor response to chemotherapy and/or radiotherapy in gastrointestinal cancers. As tumor growth depends on angiogenesis, the rate of tumor cell proliferation is related to angiogenic activity. The microvascularization of the tumor may also affect tissue distribution of anticancer drugs. Furthermore, the degree of intratumoral hypoxia, which is an important determinant of tumor response to radiotherapy, is also influenced by angiogenesis. Hence, it is reasonable to speculate that there may be a relationship between the angiogenic activity of a tumor and its responsiveness to cytotoxic drugs or radiotherapy. Dirix et al (1997) first showed that serum VEGF and bFGF levels were higher in progressive disease compared with responsive disease in patients treated with chemotherapy for metastatic cancers from various origins. Subsequently, Hyodo et al (1998) studied 34 patients with metastatic gastric or colorectal cancers treated with systemic chemotherapy and found that a low pre-treatment plasma VEGF level was associated with a significantly higher response rate and better prognosis; such a predictive power was not observed with carcinoembryonic antigen (CA)-19.9 levels. Another study showed that high pre-treatment serum VEGF levels were predictive of poor response and survival in patients undergoing chemoirradiation for esophageal squamous cell carcinoma (Shimada et al 2001).

While most studies have suggested an adverse prognostic value of high serum VEGF level and possible value in predicting or monitoring tumor response to antiangiogenic or other anticancer therapies, thus far it has not been successfully translated to clinical use in bedside. In addition to the fact that results on its prognostic and predictive value of various studies in the literature are not entirely consistent, the range of serum VEGF level varies widely among studies

of different cancers, and there is substantial overlap of the serum levels of cancer patients and healthy subjects. This makes it difficult to define a cutoff value for use in individual cancer patients and hence limits its potential to become a clinically useful biomarker.

Therapeutic implications of tumor angiogenesis

The potential therapeutic implications of tumor angiogenesis were envisaged by Folkman (1971) when he first introduced the concept of tumor angiogenesis. Tumor cells were the target of conventional cytotoxic chemotherapy. The proliferating endothelial cells that are present in all cancers provide a common target in the cancers for a novel anticancer therapy, which may have the following theoretical advantages over cytotoxic chemotherapy: (1) The microvascular endothelial cells are genetically stable cells with an extremely low mutation rate, and hence drugs targeted at the endothelial cells are less likely than cytotoxic drugs to induce drug resistance; (2) Since antiangiogenic therapy targets the specific immature characteristics of tumor vasculature, which differs from normal quiescent vasculature, little or no toxicity has been demonstrated in pre-clinical studies (Burke and DeNardo 2001); (3) Endothelial cells are directly exposed to bloodborne agents, circumventing the problem of drug delivery to tumor cells, which is a major obstacle to conventional anticancer therapy; (4) Tumor blood flow is measurable in the clinic, allowing monitoring of the therapeutic efficacy of antiangiogenic therapy.

Antiangiogenic therapy can be classified according to their mechanisms of action (Table 2). The first approach is to block the angiogenic factors or receptors, of which VEGF and VEGF receptors have been the most commonly targeted ones because of their central role in tumor angiogenesis. In nude mice models, antibody against VEGF or blockage of VEGF receptors could inhibit the growth of human xenotransplants of different cancers such as gastric carcinoma (Kamiya et al 1999), colonic carcinoma (Shaheen, Ahmad, et al 2001), pancreatic carcinoma (Solorzano et al 2001), and hepatocellular carcinoma (Liu et al 2005). Inhibition of multiple angiogenic factors may be more effective than inhibition of a single angiogenic factor because a tumor may be able to overcome the effect of inhibition of one angiogenic factor by upregulated expression of other angiogenic factors. A recent study showed that the use of a tyrosine kinase inhibitor for multiple angiogenic factor receptors including VEGF, bFGF, and PD-ECGF receptors was effective in improving survival in mice bearing colon cancer liver metastasis (Shaheen, Tseng, et al 2001). Several drugs targeting multiple tyrosine kinase receptors of angiogenic factors are being developed and tested in pre-clinical studies or phase I/II clinical trials. A second approach of antiangiogenic therapy is to use drugs that directly inhibit the proliferation or survival of endothelial cells. Some of the naturally occurring antiangiogenic factors known to be expressed by tumors

Table 2 Classes of antiangiogenic agents

Antiangiogenic mechanisms	Examples
Inhibitors of angiogenic factors or receptors	
Monoclonal antibodies against angiogenic factors	Bevacizumab (monoclonal anti-VEGF antibody)
Antibodies that act as soluble receptors of angiogenic factors	VEGF Trap (Inhibitor of VEGFR1 and VEGFR-2)
Inhibitors of tyrosine kinase receptors for angiogenic factors	PTK787/ZK225 (blocks VEGF receptors)
	SU11248 (blocks VEGFR-1 and -2, FLT3, KIT, PDGFR-α and -β)
	BAY43-9006 (blocks VEGFR-2 and 3, PDGFR-β)
Direct inhibitors of endothelial proliferation and survival	
Endogenous inhibitors	Endostatin
	Angiostatin
Exogenous inhibitors	Thalidomide
Inhibitors of extracellular matrix breakdown	
Inhibitors of matrix metalloproteinases	Marimastat
	Neovastat
Inhibitors of vascular adhesion molecules	
Inhibits integrin $\alpha_v\beta_3$ receptor	Vitaxin

Abbreviations: PDGFR, platelet-derived growth factor receptor; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor.

have been shown to inhibit tumor growth in animal models of cancers (Schmitz et al 2004; te Velde et al 2005). Thalidomide is a drug that inhibits endothelial proliferation, but the exact mechanism is unclear. It has been found to inhibit the growth of human cancer implanted in nude mice (Kotoh et al 1999). A third approach is to use drugs that prevent the degradation of extracellular matrix and basal membrane, a step essential for angiogenesis. For example, an inhibitor of MMP-2 and MMP-9 has been shown to reduce tumor vascularity and liver metastasis in human colon cancer xenograft implanted in mice (Oba et al 2002). A fourth approach is to inhibit vascular cellular adhesion molecules such as integrin $\alpha_v\beta_3$ (Lode et al 1999). Based on currently available evidence, blockage of angiogenic factors such as VEGF offers the greatest promise, while drugs directly inhibiting the proliferation of endothelial cells, such as endostatin and thalidomide, have not been found to have significant antitumor efficacy in clinical trials.

Some clinically available drugs previously known for other effects are now recognized to have an antiangiogenic effect as well. For example, interferon-alpha is an immunomodulatory agent that has been used in the treatment of unresectable hepatocellular carcinoma. It has been recently reported that interferon-alpha inhibits the growth of human hepatocellular carcinoma (HCC) implanted in nude mice by an antiangiogenic effect probably mediated by inhibition of bFGF and VEGF production (Wang et al 2000). However, interferon-alpha monotherapy has not been found to be an effective therapy for advanced HCC and is associated with significant side effects (Llovet et al 2000). Celecoxib, a COX-2 inhibitor, is an antiinflammatory drug that can induce apoptosis, and it is used to inhibit the growth of adenomatous colorectal polyps in patients with familial adenomatous polyposis. A recent study showed that celecoxib can suppress tumor growth in nude mice by antiangiogenic effect (Leahy et al 2002).

A combination of antiangiogenic agents may be more effective than monotherapy. Although antiangiogenic therapy is thought to have a low risk of drug resistance, there is some preclinical and clinical evidence that suggests the possibility of acquired drug resistance in antiangiogenic therapy (Kerbel 2001). In particular, indirect antiangiogenic therapy that depends on the blockade of tumor-derived angiogenic factors has a high risk of drug resistance, because tumor cells may eventually release a different angiogenic factor. The expression of multiple angiogenic factors in a cancer implies possible antiangiogenic drug evasion by alternate pathways of angiogenesis in tumor cells, likely induced by antiangiogenic drug-mediated increases in tumor hypoxia. Definitive preclinical evidence of antiangiogenic drug resistance has been recently reported in a study, which showed that phenotypic resistance to VEGFR2 blockade emerged as tumors regrew during treatment with function-blocking antibody to VEGFR2 after an initial period of growth suppression, and that this resistance to VEGF blockade involved reactivation of tumor angiogenesis independent of VEGF and associated with hypoxia-mediated induction of other proangiogenic factors (Casanovas et al 2005). The combination of two antiangiogenic agents may delay or avoid the problem of drug resistance. This may involve combination of two drugs targeting different angiogenic factors (Ciardiello et al 2000), or a combination of antiangiogenic drugs that act through different mechanisms to obtain a synergistic effect and to reduce the chance development of drug resistance. For example, it has been shown that combination of soluble vascular endothelial growth factor [VEGF] receptor 1 as an indirect inhibitor of angiogenesis and endostatin as a direct inhibitor of endothelial proliferation synergistically enhanced their inhibitory effect on the growth of hepatocellular carcinoma in a rat model (Graepler et al 2005). Combination of an antiangiogenic agent with a vascular disrupting agent is another approach that has been shown to achieve significant enhancement of antitumor efficacy in models of human cancers, and such combination therapy may have significant therapeutic benefit even in tumors insensitive to either treatment alone (Shi and Siemann 2005).

Early clinical trials of antiangiogenic therapy in human cancers involved the use of antiangiogenic drugs as monotherapy for patients with advanced cancers refractory to conventional chemotherapy. Thalidomide is one of the earliest antiangiogenic drugs tested in clinical trials. Although there is some evidence of significant efficacy of thalidomide for multiple myeloma (Singhal et al 1999), results of thalidomide monotherapy for advanced cancers of other organs were generally unsatisfactory, with low tumor response rates (Reiriz et al 2004; Lin et al 2005). Trials of monotherapy using another antiangiogenic agent, endostatin, for advanced solid cancers were also disappointing (Thomas et al 2003). Antiangiogenesis is mainly a cytostatic therapy that it is likely to have the greatest effect when combined with cytotoxic chemotherapy or radiotherapy. It has been shown in animal studies that a combination of VEGF neutralizing antibody or VEGF receptor antibody and cytotoxic chemotherapy drug was more effective than either agent alone as anticancer therapy

(Klement et al 2000; Matsumoto et al 2000). Lee et al (2000) showed that anti-VEGF monoclonal antibody could augment the tumor response to radiation in human colon adenocarcinoma xenograft in mice, possibly because the anti-VEGF monoclonal antibody treatment can compensate for the resistance to radiation induced by hypoxia. However, the benefit of combination of antiangiogenic therapy with radiation therapy remains uncertain. As demonstrated by a recent study (Casanovas et al 2005), tumor response to antiangiogenic therapy can lead to hypoxia and upregulation of hypoxia-related growth factors, which might ultimately make these cells more aggressive.

The combination of a recombinant humanized anti-VEGF monoclonal antibody, bevacizumab (Avastin, Genentech, South San Francisco, CA, USA), with a chemotherapy regimen consisting of irinotecan, 5-flourouracil, and leucovorin, has been recently demonstrated to prolong survival of patients with metastatic colorectal cancer compared with patients who received the chemotherapy regimen alone in a large randomized trial (Hurwitz et al 2004). This has led to the approval of bevacizumab by the US Food and Drug Administration for the treatment of patients with metastatic colorectal cancer. Promising data are emerging showing that bevacizumab in combination with chemotherapy improves tumor response or survival of patients with other cancers such as lung cancer, breast cancer, ovarian carcinoma, renal cell carcinoma, pancreatic cancer, and other tumor types (Johnson et al 2004, Miller, Chap, et al 2005, de Gramont and Van Cutsem 2005). An alternative approach of targeting VEGF pathway is the use of small molecule inhibitors that inhibit VEGF receptors. Several of these tyrosine kinase inhibitors, including PTK787/ZK 222584 (Vatalanib), SU5416, ZD6474, and BAY 43-9006 (Sorafenib) are now in clinical trials in patients with advanced cancers (Heymach et al 2004; Miller, Trigo, et al 2005; Strumberg et al 2005; Thomas et al 2005). Most of these tyrosine kinase receptor inhibitors have inhibitory effect on other tyrosine kinase receptors, and this may theoretically be an advantage over monoclonal antibody against VEGF, which has a single action of inhibition of VEGF. For example, PTK787/ZK 222584 inhibits PD-ECGF and c-kit protein receptor kinases in addition to VEGF receptors. These small molecule inhibitors are orally active, have a favorable safety profile and can be easily combined with other forms of chemotherapy. However, preliminary data from some of the early trials did not reveal a dramatic antitumor effect as demonstrated with anti-VEGF antibody therapy. In a phase II trial testing SU5416 (an inhibitor of VEGF and kit receptor tyrosine kinases) in patients with advanced soft tissue sarcoma, it was observed that VEGF receptor or kit inhibition was incomplete in at least some cases, providing a possible explanation for the observed lack of antitumor activity (Heymach et al 2004). The development of resistance of cancer cells to tyrosine kinase inhibitors is another problem that needs to be addressed to enhance the clinical efficacy of tyrosine kinase inhibitors (Ozvegy-Laczka et al 2005). Currently, more than 40 angiogenesis inhibitors are undergoing clinical trials in patients with various cancers, and in addition to bevacizumab, several other agents have reached phase III trials. The role of antiangiogenic therapy in cancer treatment is going to expand with the availability of more effective agents in the future.

Currently most studies on measurement of angiogenic factors focus its role in monitoring response and predicting outcome of treatment. A clinical study that evaluates the role of measurement of angiogenic factors in selecting patients for specific therapies has not yet been published. Clinical use of antiangiogenic therapy in cancer treatment is still in its early phase of development. Measurement of angiogenic factors may be useful in selecting patients for specific therapies targeting at individual angiogenic factors. This is worthy of evaluation in future clinical trials on antiangiogenic therapy.

Conclusions

In recent years, tumor angiogenesis research has been translated from the laboratory to the bedside at a rapid pace. Hence, it is important for clinicians involved in care of cancer patients to be aware of the potential clinical implications of tumor angiogenesis. The vast number of studies on the prognostic impact of tumor angiogenesis has shown a direct correlation between tumor vascularity and prognosis. However, routine clinical assessment of tumor angiogenesis as a prognostic parameter cannot be justified with the existing data. With few exceptions, available data were derived from retrospective studies using archived surgical specimens. Large prospective studies using a standardized methodology and prospectively collected data are mandatory to validate the prognostic significance of tumor angiogenesis. Furthermore, the assessment of tumor MVD or expression of angiogenic factors requires tumor specimens, which may not be available in all cases of cancer patients. The prognostic role of circulating angiogenic factors remains controversial. Recent studies have suggested that color Doppler ultrasonography may provide a reliable

preoperative quantitation of tumor angiogenesis and prognostic information in cancer patients (Ogura et al 2001; Chen et al 2002). Other methods of noninvasive assessment of tumor angiogenesis, such as magnetic resonance imaging conjugated with biotinylated antibodies to integrin $\alpha_v\beta_3$, and positron emission tomography with specific radiolabelled glycopeptide to determine integrin $\alpha_v\beta_3$ status in the tumors, are under investigation (Sipkins et al 1998; Haubner et al 2001). Such noninvasive methods for assessing tumor angiogenesis may not only provide prognostic information, but may also represent a useful tool to monitor changes in tumor microvasculature in response to antiangiogenic therapy.

Antiangiogenic therapy is a novel strategy for treating cancers that has been shown to increase patient survival in combination with conventional chemotherapy. One major problem confronting clinical trials of antiangiogenic therapy is the lack of an established surrogate marker to measure antiangiogenic activity in vivo in cancer patients. Tumor response in terms of shrinkage alone may not be an appropriate index of treatment efficacy because of the cytostatic nature of the treatment. The ability of an antiangiogenic drug to induce prolonged stabilization of the disease and increase survival may be more meaningful end-points for clinical trials on antiangiogenic therapy. Noninvasive imaging of tumor vascularity may provide a better index of the response of the tumor to antiangiogenic therapy. Currently, antiangiogenic therapy is being tested in combination with chemotherapy mainly in end-stage inoperable disease. The role of antiangiogenic drugs in other clinical settings such as neoadjuvant or adjuvant therapy in combination with surgery remains to be studied. With the approval of the anti-VEGF monoclonal antibody bevacizumab for clinical use in cancer patients, it is foreseeable that active research will be directed to the study of the role of antiangiogenic therapy using various other agents, leading to novel angiogenesis-based treatments in the near future.

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