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Antiangiogenic cancer therapy: why do mouse and human patients respond in a different way to the same drug?

YIHAI CAO*

Department of Microbiology, Tumor and Cell Biology, Karolinska Institute, Stockholm, Sweden

ABSTRACT The tumor vasculature is an increasingly attractive target for development of anticancer drugs. The fundamental principle for antiangiogenic cancer therapy is based on the inhibitory effect of chemical compounds, proteins or nucleotides on tumor angiogenesis. Indeed, in almost all preclinical tumor models, antiangiogenic monotherapy with different agents shows potent effects on suppression of tumor growth. However, antiangiogenic monotherapy has barely produced any clinical benefits in cancer patients. Although in combination with chemotherapy some antiangiogenic drugs demonstrate survival improvement in patients with certain types of cancers, the overall benefits by addition of antiangiogenic drugs (ADs) to chemotherapy remain modest. The disparity of AD responses between preclinical models and clinical cancer patients has raised important issues, which include: 1) Are current animal tumor models appropriate for assessing the therapeutic efficacy of ADs for clinical development? 2) What are the key differences between mouse tumor models and human cancer patients? 3) Are anti-VEGF drugs off target in cancer patients? 4) What are alternative options for improvement of the clinical benefits of ADs? In this short review, I discuss these critical issues in relation to the clinical practice of ADs.

KEY WORDS: *angiogenesis, cancer, targeted therapy, ocular disease, cardiovascular disease*

Introduction

More than 40 years ago when Dr. Judah Folkman operated animal and human tumors, he noticed that tumor tissues are enriched in blood, which is supplied by tumor vessels (Folkman *et al.*, 1971). Folkman, in a theoretical paper, proposed that all solid tumor growth is dependent on angiogenesis and suppression of tumor angiogenesis might offer a new option of cancer therapy (Folkman, 1971). To approve his hypothesis, Folkman´s laboratory isolated blood vessel endothelial cells, purified the first angiogenic factor from tumors, developed ex-vivo and *in vivo* angiogenesis assays, isolated the first specific endogenous angiogenesis inhibitor and approved the antiangiogenic principle as a valid approach for cancer therapy in mice (Folkman *et al.*, 1979; Langer *et al.*, 1976; Shing *et al.*, 1984). All these landmark studies have paved today´s avenue for development of ADs by pharmaceutical companies and practice of antiangiogenic therapy in human cancer patients.

Among all known angiogenic pathways in tumors, the vascular endothelial growth factor (VEGF) signaling axis has become a central target for anticancer drug development (Cao, 2010, Hurwitz *et al.*, 2004; Kerbel, 2008; Torino *et al.*, 2009). Virtually all currently available ADs in the clinic contains anti-VEGF components, which include a VEGF neutralizing antibody, bevacizumab, and tyrosine kinase inhibitors targeting VEGF receptors such as sunitinib and sorafenib (Cao *et al.*, 2007; Cao *et al.*, 2009). Both genetic and epigenetic factors in the malignant tissue contribute to high expression levels of VEGF, which can be further elevated by hypoxia during tumor growth. Thus, VEGF is involved in the initial phase of tumor growth and later development and progression of the malignant disease. Constant high levels of VEGF in most tumors suggest anti-VEGF drugs should be persistently delivered to cancer patients (Cao *et al.*, 2010).

In preclinical tumor models, most antiangiogenic agents delivered as monotherapy have demonstrated potent anticancer effects in a variety of tumor types (Kim *et al.*, 1993; Millauer *et al.*, 1994). Consistent with tumor suppression, tumor neovascularization is usually markedly reduced after treatment with these agents. Thus, the antiangiogenic principle for cancer therapy in mouse tumor models has validated Folkman´s original hypothesis. However,

Abbreviations used in this paper: AD, antiangiogenic drug; Ang, angiopoietin; FGF-2, fibroblast growth factor-2; HGF, hepatocyte growth factor; IGF, insulin-like growth factor; TKRs, tyrosine kinase receptors; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor

***Address correspondence to:** Yihai Cao. Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, 171 77 Stockholm, Sweden. Fax: (+46)-8-33-1399. e-mail: yihai.cao@ki.se

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antiangiogenic monotherapy with most available drugs has not demonstrated clinical benefits in cancer patients (Cao, 2009; Hurwitz *et al*., 2004). Despite significant improvement of clinical outcome in combination with chemotherapy, survival benefits of ADs in combination settings remain modest in most cancer types (Kerbel, 2008). Although the common experience for general drug development shows a large gap between mouse and human responses, the antiangiogenic property of ADs should be the same for mouse and human tumor. Why do human cancer patients respond in a different way to the same drug? This review article attempts to discuss this important issue in relation to clinical practice of ADs.

Antiangiogenic principles

There are currently two classes of antiangiogenic agents that inhibit tumor angiogenesis: 1) Angiogenic pathway blockades and 2) Endogenous angiogenesis inhibitors (Folkman, 2007). Tumors produce a variety of angiogenic factors or cytokines to stimulate angiogenesis, which is essential for tumor growth and metastasis (Cao *et al*., 2007) (Fig. 1). These tumor-derived angiogenic factors include VEGF, fibroblast growth factor-2 (FGF-2), platelet-derived growth factor (PDGFs), angiopoietins (Angs), hepatocyte growth factor (HGF), and insulin-like growth factors (IGFs). The angiogenic signals triggered by these angiogenic factors are mediated by their specific tyrosine kinase receptors (TKRs) expressed in endothelial cells (Bjorndahl *et al*., 2005; Eriksson *et al*., 2002; Nissen *et al*., 2007; Xue *et al*., 2008). Giving the known information about signaling mechanisms, development of specific antagonists such as neutralizing antibodies, soluble receptors and intracellular tyrosine kinase inhibitors for therapeutic implications is a relatively straightforward approach (Cao, 2008; Dowlati, 2010; Force *et al*., 2007; Hurwitz *et al*., 2004; Kim *et al*., 1993; Millauer *et al*., 1994). Indeed, in preclinical models angiogenic factor antagonists such as bevacizumab show potent anticancer effects by neutralizing angiogenic signals in tumors (Kim *et al*., 1993). Because the molecular mechanisms of blocking angiogenic signaling pathways are obvious and well characterized, drug development based on these principles remains attractive for pharmaceutical companies.

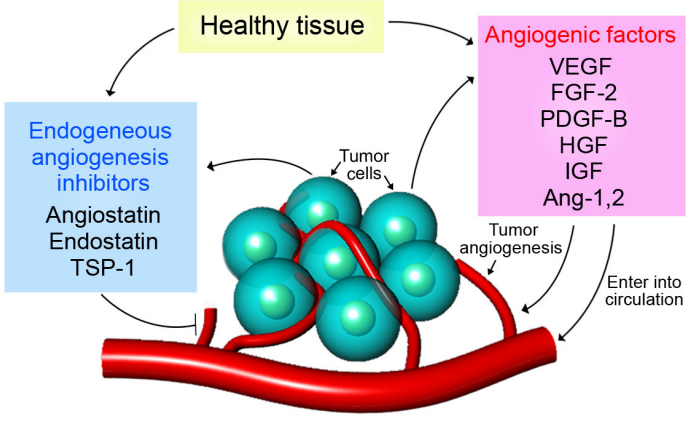

Fig. 1. Production of multiple angiogenic factors and inhibtors by both tumor cells and healthy tissues. *These angiogenic factors in the tumor environment stimulate local angiogenesis and they could also enter into the circulation to induce destructive effects on healthy tissues and organs of the host.*

Indeed, almost all currently available antiangiogenic drugs in the clinic are developed by antagonizing angiogenic pathways in tumors.

In contrast to angiogenic blockades, the molecular mechanisms of endogenous angiogenesis inhibitors such as angiostatin, endostatin and thrombospondin-1 remain enigmatic (Cao, 1998, 2001). Endogenous angiogenesis inhibitors have been reported to display broad-spectrum of inhibitory activities, which directly act on endothelial cells by blocking common pathways of various angiogenic factor-triggered angiogenesis (Fig. 1). However, they generally lack defined signaling pathways for endothelial inhibition. For example, specific endothelial cell receptors for angiostatin and endostatin and their signaling events remain unclear despite the early discovery of these inhibitors (O'Reilly *et al*., 1997; O'Reilly *et al*., 1994). This is probably one of the main reasons why pharmaceutical companies remain reluctant in further developing these inhibitors toward clinical use. Endogenous angiogenesis inhibitors displaying a broad spectrum of inhibitory activity would in principle more effective for therapy. The only clinically approved drug based on endogenous angiogenesis inhibitor is endostatin, which is only available in China for the treatment of lung cancer (Yang *et al*., 2006; Han *et al*., 2011).

Differences between preclinical tumor models and human patients in relation to antiangiogenic therapy

The most commonly used preclinical models for assessment of antiangiogenic and antitumor activities are xenograft tumor models in mice that carry implanted mouse or human tumors (Table 1). Typically, a large number of tumor cells ($1 \times 10^{5\text{-}6}$) are subcutaneously implanted into each of mice and tumors grow to a size of 1.0 cm^3 within a few weeks. Although this is a commonly used animal tumor model for studying antiangiogenic and antitumor effects of different molecules, the relevance of this xenograft model to clinical setting is far from reality. First, the subcutaneous implantation site does not usually represent physiologically orthotropic sites where human tumors arise. How many types of cancers do humans develop subcutaneously? In fact, the subcutaneous region is a rare site for development of human cancers. The tissue site is probably one of the important issues related to drug responses because angiogenic vessels in various tissues may express different receptors that are activated by specific ligands. Further, selective expression of different subsets of the same ligand receptors exists in different tissues. VEGFR1 and VEGFR2, for example, are differentially expressed in the retinal vasculature and blood vessels in other tissues (Cao *et al*., 2010; Saint-Geniez *et al*., 2008). Differential expression of angiogenic factor receptors in various tissues and organs may lead to distinctive AD responses.

Another key difference between human cancers and mouse tumor models is the speed of cancer development (Table 1). In human patients, spontaneous development of a clinical detectable cancer may take years whereas development of a similar size of a mouse tumor may only take weeks (O'Reilly *et al*., 1994). The differential growth rates between human and mouse tumors may create completely different environments, leading to dissimilar angiogenic profiles and drug responses. Typically, in a fast-growing mouse tumor, the growth of angiogenic vessels occurs behind the malignant tissue expansion, leading to a hypoxia environment. Tissue hypoxia is known to switch on hypoxia-inducible gene transcription of certain angiogenic factors such as VEGF and

thus alter angiogenic profiles (Makino *et al*., 2001). In relatively slow-growing human tumors however, tissue hypoxia-induced angigiogenic profiles are less enunciated as mouse tumors. The difference of angiogenic profiles between human and mouse tumors in relation to the tumor growth rate may determine variation of drug responses.

In humans, cancer is usually considered as a common disease in the older population whereas in mouse tumor models relatively young animals are used for experimentation (Table 1). It is known that angiogenesis occurs at different rates in various aged populations (Rivard *et al*., 1999). For example, impaired angiogenesis in respond to tissue ischemia has been reported in aged rabbits (Rivard *et al*., 1999). Young human or animal subjects are susceptible to angiogenic stimuli by triggering relatively robust angiogenic responses under physiological and pathological settings. In contrast, old human or animal subjects often show delayed or impaired angiogenic responses under the same conditions. In general, it is speculated that high angiogenic profiles in tumors would be more prone to angiogenesis inhibition because the newly formed vasculature is dependent angiogenic stimuli.

In addition to the variation between human and mouse tumors, the genetic backgrounds in human patients and experimental mice are completely different (Table 1). In experimental mouse models, genetically identical inbred syngeneic strains are often used for tumor studies. Conversely, human tumors arise from genetically divergent populations that may show diverse responds to the same drug. Recent clinical experiences with ADs demonstrate that the polymorphism of certain angiogenesis-genes in cancer patients significantly confers the difference of AD responses in the patients with the same type of cancer. For example, patients with different VEGF or VEGFR polymorphisms show polarized survival responses to anti-VEGF drugs (Cao, 2010; Schneider *et al*., 2008). Similarly, phase III clinical trials demonstrate that differential responses to the same drug such as gefitinib exists among different races of human patients (Branca, 2005; Duster, 2007; Takeda *et al*., 2010). The genetic variation of individual human cancer patients cannot be appropriately recapitulated using experimental mouse models.

In mouse xenograft tumor models, treatments with antiangiogenic agents usually begin shortly after implantation of tumor cells (Table 1). However, antiangiogenic therapy for human cancer patients is only initiated at the advanced stage of an established malignant disease. Strictly speaking, current regimens for mouse cancer therapy by ADs is aimed for prevention of tumor growth but not specifically designed for the treatment of established tumors. One argument in opposition to the treatment of established mouse tumors is that antiangiogenic agents are supposed to inhibit tumor growth rather than shrinkage of the tumor mass. Perhaps the critical and fundamental criteria for assessment of drug effects between preclinical models and clinical patients are different (see below). In mouse tumor models, we are usually studying the drug effect on tumor size, whereas in human patients survival improvements by ADs are often the endpoints of clinical outcomes.

TABLE 1

DIFFERENCES BETWEEN HUMAN CANCER AND MOUSE TUMOR MODELS

Features	Human patients	Experimental mouse patients
Assessment	Survivals	Tumor size
Tumor site	Intrinsic	Often artificial
Tumor growth rate	Slow (often years)	Fast (often weeks)
Age	Usually old population	Young age
Genetic background	Heterogeneous	Homogeneous
Treatment	Treatment begins usually at the advanced stage of cancer	Usually begins at the early stage of tumor development

Therapeutic efficacy of antiangiogenic agents is often assessed as monotherapy in mice whereas the same agents are delivered to cancer patients as combinatorial therapy with chemotoxic drugs (Table 1). The fact that antiangiogenic monotherapy often lacks clinical benefits undermines the relevance of mouse tumor models to human cancer patients. Despite this known fact, therapeutic assessments of antiangiogenic agents by monotherapy continue in mouse tumor models. Would combination therapy be more appropriate for preclinical evaluation of antiangiogenic agents? In mouse tumor models, delivery of chemotherapeutic drugs alone at the conventional dose levels often produces overwhelming antitumor effects and addition of antiangiogenic agents as an extra component would be difficult to enhance the chemotherapeutic effect.

To recapitulate clinical situation, spontaneous mouse tumor models have been used as an alternative option for preclinical assessment of antiangiogenic agents. In contrast to xenograft tumor models, spontaneous mouse tumor models provide an opportunity to study kinetics of tumor angiogenesis during the tumor development (Kandel *et al*., 1991). Furthermore, antiangiogenic agents can be delivered to animals at different stages of tumor development, allowing assessment of therapeutic efficacy of drugs under non-invasive conditions (Parangi *et al*., 1996). Despite these advantages, spontaneous mouse tumor models also suffer severe drawbacks, which include: 1) introduction of an activated endogenous oncoprotein into a specific cell type; 2) deletion or inactivation of tumor suppressor genes; 3) overexpression of viral oncoproteins in mice; and 4) exposure of animals to carcinogenic chemicals. Although in these spontaneous tumor models the angiogenic switch could occur at the predicted time point, genetic manipulation of mice by germ-line overexpressing oncogenes often lead to alteration of angiogenic profiles, which are far from clinic relevance. For example, in the Rip-Tap pancreatic tumor transgenic model the simian virus 40 T antigen (SV40 large T) potently induces VEGF expression (Catalano *et al*., 2002). If anti-VEGF agents are evaluated in this mouse tumor model, drug response would expectedly to be rigorous. However, tumors in human patients do not often express SV40 large T antigen. Recently, it has been reported that treatment of k-ras oncogene-driven genetically modified mouse tumors with antiangiogenic agents recapitulates clinical responses seen in patients (Francia *et al*., 2010; Singh *et al*., 2010). It remains to be seen if such animal tumor models would predict clinical benefits.

Off-tumor targets of antiangiogenic drugs

Current antiangiogenic drugs are delivered to cancer patients by systemic administration, which may lead to a global impact on healthy vasculatures distributed in multiple tissues and organs (Cao, 2010). In fact, current clinically available ADs significantly regress tumor-free microvasculatures in several tissues and organs in mouse models (Kamba *et al*., 2006). In the conventional view of anticancer drugs, off-tumor targets would be associated with

unwanted adverse effects of drugs. Interestingly, clinical benefits of ADs have been positively associated with systemic syndromes such as skin rashes and hypertension, which are resulted from the systemic effects of the drugs (Cao, 2010; Ravaud *et al.*, 2009). Why would these systemic effects be positively correlated with beneficial outcomes of these drugs? In preclinical tumor models, it has been demonstrated that antiangiogenic agents at a low dosage without affecting the tumor vasculature normalize vasculatures in healthy tissues including those fenestrated vasculatures in endocrine organs such as bone marrow, liver and adrenal gland (Xue *et al.*, 2008). Normalization of tumor VEGF-induced vascular tortuosity in non-tumor tissues significantly prolongs survivals of tumor-bearing mice by improving the cancer associated systemic syndrome. These findings suggest that off-tumor targets of antiangiogenic agents offer alternative mechanisms of clinical benefits of ADs. Unfortunately, clinical trials based on improvement of paraneoplastic syndrome and cancer cachexia by ADs have neither been designed nor reported. Improvement of the cancer-associated systemic syndrome by ADs in correlation with prolongation of patient survivals warrants future clinical validation.

Options for optimization of clinical benefits of antiangiogenic drugs

Current available ADs in combination with chemotherapy for the treatment of cancer patients have produced only modest beneficial effects (Cao *et al.*, 2009). Optimization of antiangiogenic therapy is urgently needed in order to maximize therapeutic efficacy of these drugs. Obviously, defining novel therapeutic targets other than VEGF would be an important approach to increase clinical responses because a majority of cancer patients remain intrinsic resistance to anti-VEGF therapy. Given the fact that most tumors produce a broad spectrum of angiogenic factors to stimulate angiogenesis and to sustain the established vasculature, it is unsurprising that blockade of a single angiogenic pathway would be insufficient to suppress tumor growth and multitargeted “dirty drugs” would be more effective. In support of this view, antiangiogenic monotherapy with tyrosine kinase inhibitors such as sunitinib and sorafenib targeting multiple signaling pathways demonstrates survival benefits in the treatment of metastatic renal cell carcinoma (Escudier *et al.*, 2007; Motzer *et al.*, 2006). Inversely, delivery of mono-specific ADs such as bevacizumab has not been demonstrated to be beneficial in clinical settings (Hurwitz *et al.*, 2004). Thus, development of new generation of drugs targeting diverse angiogenic pathways is expected to improve clinical benefits of ADs. In preclinical tumor models, it has been shown that a combination of antiangiogenic agents with different mechanistic principles yields a synergistic effect on tumor suppression (Dorrell *et al.*, 2007; Lode *et al.*, 1999). Translation of this preclinical finding to patient therapy would suggest an organizing principle of antiangiogenic cancer therapy should be considered in the future clinical practice.

Another important aspect of optimizing antiangiogenic therapy is to develop best possible drug delivery systems that enable kinetic personalized therapy (Cao *et al.*, 2010). Unlike preclinical animal tumor models, the genetic background of clinical patients is diversified. In the same origin of cancer, divergent but completely different patterns of angiogenic profiles may exist among patients with the same cancer. If so, a generalized therapeutic regimen with the same AD as the key composition would be irrational for all patients. Thus, a personalized or individualized therapeutic approach should be considered. Even though an individual patient originally is sensitive to a specific AD based on his/her angiogenic profiles, the same patient may develop evasive resistance to the same drug owing to alteration of angiogenic profiles in tumors (Cao *et al.*, 2009). In such a scenario, kinetically monitoring of changes of angiogenic profiles that predict clinical responses would be desirable. It has been recently suggested that smart microchips embedded with different antiangiogenic or chemostatic drugs may release any available drugs at any given time (Cao *et al.*, 2010). If such smart drug-microchips are available in the future, they would expected to substantially improve the therapeutic efficacy of antiangiogenic therapy.

Other approaches of optimization of antiangiogenic therapy include: 1) Define reliable biomarkers for patient selection and monitoring therapeutic benefits as exhaustively discussed elsewhere; 2) Development of appropriate animal tumor models that recapitulate drugs responses in cancer patients. Recently it has been reported that genetically modified mouse tumor models produce similar drug responses seen in patients (Singh *et al.*, 2010); 3) Understanding molecular mechanisms of intrinsic and evasive refractoriness to antiangiogenic drugs; 4) Design both preclinical and clinical studies by sequential delivery of ADs and chemotherapeutic drugs. Sequential delivery of ADs followed by chemotherapeutics has recently been shown in mice to produce superior beneficial effects (Zhang e*t al.*, 2011); 5) Explore metronomic therapy in combination antiangiogenic therapy; 6) Optimizing dosages for ADs in combination of chemotherapeutic drugs and 7) Considering long-term non-stop antiangiogenic therapy by reducing the high cost of these current expensive drugs.

Outlook

Cancer is a complex disease that encompasses both genetic and epigenetic alternation of malignant cells and the tumor environment (Hanahan *et al.*, 2000). In cancer patients, genetic diversity of human populations may generate completely different angiogenic profiles in tumors despite presence of the same type of cancer in different patients. The diversity of genetic backgrounds and angiogenic profiles implies an individualized therapeutic approach should be given to patients and generalized therapy with the same regimen may represent clinical failures. In fact, with an exception of bevacizumab almost every one of more than a dozen phase 3 trials designed for targeted drugs in combination with standard chemotherapy fails to show survival advantages in the front-line therapy for the treatment of lung cancer (Francia *et al.*, 2010). Taken considerations of the vast diversity of human cancer patients, such disappointing clinical outcomes may not be totally unexpected. Although clinical experiences show that antiangiogenic therapy remains ineffective and produces only modest survival benefits in a minority of patients, this statistically significant and clinically positive outcome is somehow surprisingly encouraging and validates Folkman´s original hypothesis that targeting blood vessels is probably a more universal approach for cancer therapy.

Unlike humans, genetically inbred experimental homogenous mice represent the same background and tumors are artificially manipulated to grow at the same or at least a similar pace. Unsurprisingly, these genetically identical animals would produce a similar response to the same drug. Indeed, antiangiogenic mono-

therapy in mice regardless of xenograft or genetic tumor models shows the predicted power of tumor suppression. Thus, this type of animal model would not be appropriate for assesment therapeutic efficacy of ADs in cancer patients. Do we have better options to do preclinical evaluation? The simple answer is probably not yet.

As new mechanistic information on tumor angiogenesis and new targeted ADs are available, it is expected that an organizing principle of antiangiogenic therapy would be more effective by employing different classes of drugs to overcome resistance. Development of smart drug-embedded microchip systems is probably an attractive approach for making personalized antiangiogenic therapy into a reality.

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