Molecular mechanisms of angiogenesis in non-small cell lung cancer, and therapeutics targeting related molecules

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Angiogenesis, neovascularization from pre-existing vasculature, is necessary to supply oxygen and nutrition for tumor growth in both primary and distant organs. It consists of sprouting and non-sprouting (the enlargement, splitting, and fusion of pre-existing vessels) processes, and both can occur concurrently. Growth of solid tumors, including non-small cell lung cancer (NSCLC), is usually dependent on angiogenesis, which is regulated by complex mechanisms involving various angiogenesis-related molecules. Vascular endothelial growth factor (VEGF), also known as vascular permeability factor (VPF), one of the most potent angiogenic molecules, regulates both angiogenesis and vascular permeability, and hence promotes tumor progression and development of malignant pleural effusions in NSCLC. Signals via epidermal growth factor receptor (EGFR), promote not only the tumor cell cycle, but also the process of angiogenesis. Therefore, these molecules are potential targets for anti-tumor vasculature therapy. Many agents targeting tumor vasculature have been developed, and several compounds have shown anti-tumor potential in preclinical studies. Their efficacy against NSCLC is currently being evaluated in clinical trials. (Cancer Sci 2003; 94: 479–485)

Lung cancer has been the leading cause of malignancy-related death in Japan since 1998, and more than 80% of cases are non-small cell lung cancer (NSCLC). Surgical resection is undoubtedly the first choice treatment for NSCLC patients in early stages (stage I and II). Over two-thirds of patients are, however, in advanced stages. Radiation therapy is generally preferred for patients with locoregionally advanced NSCLC (stage III), and chemotherapy for patients with disseminated disease (stage IV). Despite improvement of these therapeutic modalities, prolongation of survival of patients in advanced stages (especially stage IV) is not satisfactory. Therefore, novel and more effective therapeutic modalities are necessary for NSCLC in advanced stages. It has been well established that the progression of tumors (growth of both primary and metastatic tumors) is critically dependent on angiogenesis (neovascularization) to supply oxygen and nutrition. Therefore, blockade of angiogenesis has been expected to prevent the growth of tumor cells at both primary and metastatic sites, thereby preventing tumor progression, and thus improving the prognosis of patients with various types of malignant tumors, including NSCLC. From this point of view, dozens of compounds which target tumor vasculature have been developed, and many experimental studies have shown that anti-tumor vasculature agents are effective to control tumor-cell growth and metastases.

In this article, the molecular mechanisms of angiogenesis in NSCLC are briefly reviewed and the strategy for control of NSCLC by anti-vasculature agents is discussed.

Molecular mechanisms of tumor angiogenesis

Types of angiogenesis. Angiogenesis can occur through either sprouting or non-sprouting processes (Fig. 1). Sprouting angiogenesis involves the branching (true sprouting) of new capillaries from pre-existing vessels. This type of angiogenesis begins with an angiogenic stimulus, followed by local degradation of the basement membrane surrounding the capillaries. Endothelial cell migration is then accompanied by the proliferation of cells at the leading edge of the migrating column. As they move, the endothelial cells begin to organize into three-dimensional structures to form new capillary tubes. All of these, then, require endothelial cell survival, mandating the interplay of numerous factors, which can act in a positive or negative fashion. These events are tightly regulated by various angiogenic factors, including vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), angiotropins, interleukin-8 (IL-8), and thymidine phosphorylase (TP).

These factors can be produced not only by tumor cells, but also by host stromal cells.

Non-sprouting angiogenesis results from the enlargement, splitting, and fusion of pre-existing vessels produced by the proliferation of endothelial cells within the wall of a vessel. Transvascular bridges are sometimes observed in enlarged vessels produced by non-sprouting angiogenesis. Non-sprouting angiogenesis can occur concurrently with sprouting angiogenesis not only in the vascularization of organs or tissues (lung, heart, yolk sac) during development, but also in tumor angiogenesis.

We recently observed this type of angiogenesis in brain metastasis with progressive growth.

Angiogenesis as a poor prognosis factor of NSCLC. Tumors may be able to grow without neovascularization if a suitable vascular bed is available. Pezzella et al. reported non-angiogenic NSCLC tumors characterized by lack of parenchymal destruction and absence of both tumor-associated stroma and new vessels, though this type of tumor accounted for less than 20% of NSCLC. Several lines of evidence suggest that growth of solid tumors, including NSCLC, is usually dependent on angiogenesis. In fact, intratumoral microvessel density (IMD) was inversely correlated with survival of patients with surgically resected NSCLC. In addition, expression of several angiogenesis-related molecules, such as VEGF, matrix metalloproteinases (MMPs), epidermal growth factor receptor (EGFR), angiotropein-2, TP, cyclooxygenase 2 (COX-2), inversely correlated with prognosis of the patients with NSCLC. Among these molecules, we focused on VEGF, MMP.
and EGFR, and explored the involvement of these molecules in the progression of NSCLC.

**Involvement of angiogenesis-related molecules in NSCLC.**

VEGF: VEGF is the prototype of the VEGF family, which consists of VEGF, VEGF-B, VEGF-C, VEGF-D, and VEGF-E, is suggested to be the most potent and specific growth factor for endothelial cells and to play a very important role in angiogenesis. VEGF consists of at least 4 isoforms (VEGF121, VEGF165, VEGF189, VEGF206) which are regulated by splicing at the mRNA level, and VEGF165 is the most abundant isoform. VEGF binds with high affinity to two tyrosine kinase receptors, VEGFR-1 (Flt-1) and VEGFR-2 (Flk-1/KDR). Ligand binding causes receptor dimerization, auto-phosphorylation and signal transduction. Several studies have demonstrated that VEGF expression is directly correlated with IMD, and inversely correlated with the survival of patients with NSCLC. Yuan et al. further examined VEGF isoform expression in 57 surgically resected primary tumors from NSCLC patients using RT-PCR, and demonstrated that a high tumoral expression of VEGF189 was associated with a high IMD, shorter survival, and early postoperative relapse. VEGF189 is a membrane-associated form, unlike VEGF121 and VEGF165 (secreted form), VEGF189 might contribute more to local angiogenesis in NSCLC, while the amount of VEGF189 is less than 10% of the total VEGF expression.

Lung cancer frequently metastasizes to multiple organs, including the bone, lung, brain, and liver. We developed several metastasis models and determined the molecular mechanisms of multi-organ metastasis of lung cancer. Among multiple organ metastases, brain metastasis is one of the most serious problems, because it restricts the quality of life of the patient and is refractory to various anticancer modalities. We obtained a brain metastasis model by the injection of suitable cells into brains of nude mice (Fig. 2A). As described above, we found that non-sprouting angiogenesis was predominantly observed, and that levels of VEGF production by NSCLC cells directly correlated with the potential to produce brain metastasis in this model. Brain metastases developed by VEGF high-producing cell lines progressed rapidly and had many enlarged vessels split by transcapillary pillars (a hallmark of non-sprouting angiogenesis). We further showed that the metastatic potential of VEGF low-producing cells was not augmented by transfection with sense-VEGF165 or antisense-VEGF165 gene. However, the transfection of VEGF high-producing cells with antisense-VEGF165 gene resulted in a decrease in brain metastasis formation (Fig. 2B). These results suggest that VEGF is essential but not sufficient for promoting brain metastasis via angiogenesis, and that its inhibition could represent an important therapeutic target.

On the other hand, VEGF with multifunctional activities was recently found to be identical to vascular permeability factor (VPF). Malignant pleural effusion (PE) is frequently associated with advanced lung cancer. One study showed that malignant PE fluid contained a high level of VEGF in lung cancer patients. We recently developed a valuable model for PE formation after i.v. injection of human lung adenocarcinoma cell lines (PC14 and its metastatic variant PC14PE6). Interestingly, VEGF was found to be responsible for the development of PE induced by these lung adenocarcinoma cells. Thus, VEGF/VPF has been shown to be essential not only for angiogenesis, but also for PE formation in lung cancer, via the induction of vascular hyperpermeability. Since VEGF/VPF and its receptors can be therapeutically targeted, VEGF receptor inhibitors could be useful for the control of malignant PE in lung cancer patients.

There is currently much interest in the VEGF family because of its implication not only in angiogenesis, but also in lymphangiogenesis. Many MMPs are necessary for blood vessel penetration, MMPs were classified into subgroups depending on their substrates (i.e., collagenases, gelatinases, stromelysins, membrane-type, and nonclassified). Since MMPs are necessary for blood vessel penetration, MMPs were suggested to play a crucial role not only in the process of tu-

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**Fig. 1.** Patterns of angiogenesis. Sprouting angiogenesis involves the branching (true sprouting) of new capillaries from pre-existing vessels. The hallmark of this type is sprout tips. Non-sprouting angiogenesis results from the enlargement, splitting, and fusion of pre-existing vessels produced by the proliferation of endothelial cells at the wall of a vessel. Transvascular bridges are sometimes observed in enlarged vessels produced by non-sprouting angiogenesis. Sprouting angiogenesis and non-sprouting angiogenesis can occur concurrently.
mor-cell invasion, but also in the process of angiogenesis. Several studies have demonstrated that MMPs, especially MMP-2 and MMP-9 (gelatinase A and B), membrane type 1 MMP (MT1-MMP), the activator of MMP-2, and stromelysin-3 are overexpressed in various types of tumors, and that their level of expression is correlated with tumor aggressiveness, as implied by increased histological grade, advanced clinical stage, and poor patient outcome. On the other hand, we assessed the expression of MMP-2 and MMP-9 at the mRNA level by means of in situ hybridization in 60 formalin-fixed paraffin-embedded samples of stage I NSCLC, and found that the levels of MMP-2 and MMP-9, as single factors, did not correlate with disease-free or overall survival. Thus, the prognostic significance of MMPs in NSCLC is still controversial. MMPs are known to be expressed by both cancer cells and stromal cells. We reported previously that MMP activity produced by human lung cancer cells varied among cell lines in vitro and in vivo. In addition, MMP activity (gelatinolytic activity assessed by film in situ zymography) derived from host-stroma cells also varies among organs (high in the liver and low in the lung) (Fig. 3A). We further demonstrated that MMP derived from the liver parenchyma facilitated the formation of liver metastasis by MMP-low-expressing lung cancer cell lines, suggesting that MMP could promote metastasis, presumably via invasion and angiogenesis, irrespective of its origin.

**EGFR:** EGFR is a 170-kDa transmembrane glycoprotein. Binding of specific ligands, such as EGF and transforming growth factor α (TGF-α), to EGFR results in the dimerization of EGFR and leads to autophosphorylation of the intracellular receptor tyrosine kinase. EGFR signaling (via ras–raf-1– MAPK, STAT, and PI3K–Akt) facilitates tumor-cell cycling, proliferation, invasion, and decreased apoptosis. In addition, since 1) EGF is detected in the dividing tumor vasculature endothelial cells, 2) EGF induces neovascularization of mouse cornea, 3) blockade of EGFR signaling down-regulates the production of proangiogenic cytokines, including VEGF and IL-8, by several tumor cell lines expressing EGFR, EGFR is likely to be involved, at least indirectly, in the regulation of angiogenic processes.

EGFR is commonly overexpressed in various types of malignancy, including NSCLC. Several studies showed an association between overexpression of EGFR and prognosis or histological grade of differentiation in NSCLC. However, a recent meta-analysis study showed that EGFR expression was rarely related to patient outcome. Since activation (phosphorylation) of EGFR is essential for the signal transduction and hence progression of tumors, we hypothesized that activation of EGFR may more directly reflect the significance of this molecule in NSCLC, and thus evaluated the expression of phosphorylated EGFR, in addition to EGFR protein, in surgically resected tumors from NSCLC patients. We found that the expression of phosphorylated EGFR, but not overexpression of EGFR protein, inversely correlated with survival and time to tumor progression, suggesting that activation of EGFR, but not overexpression, may be an important prognostic factor for NSCLC patients. Further analysis to explore the correlation between vascularization and EGFR activation is ongoing.

### Anti-tumor vasculature therapy

The concept of targeting tumor vasculature was first advocated 30 years ago, and this concept has recently re-emerged because of the development of new anti-tumor vasculature agents. These agents have potential advantages, such as physical accessibility and genetic stability of target cells, over con-

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**Fig. 2.** Brain metastasis model and anti-metastatic effect by VEGF inhibition. A. Brain metastasis model based on internal carotid artery injection. B. Brain metastasis produced by lung cancer (PC14PE6) cells transfected with antisense VEGF gene. Note that PC14PE6 cells transfected with antisense VEGF gene (PC14PE6/AS) produced fewer brain metastases. VEGF expression and vascularization (determined by immunohistochemistry for CD31) were also decreased in brain tumors produced by PC14PE6/AS cells.
ventional cytotoxic chemotherapy.3, 38)

There are several strategies targeting tumor vasculature. The first is the modality selectively targeting angiogenesis (neovascularization) during the growth of small tumors (anti-angiogenic agents). Angiogenesis-related molecules, including VEGF, VEGF receptor 2, and MMP, were suggested as targets for this modality, and a large number of inhibitors have been developed. The second strategy is selective targeting of established tumor-associated vessels in large tumors (vascular-targeting agents), and tubulin in the dividing endothelial cells is one target of this type of modality. The third is long-term use of compounds which secondarily disrupt tumor vasculature, including anticancer drugs (taxans) and EGFR inhibitors.

**Anti-angiogenic agents**

**VEGF inhibitors:** Many compounds, including anti-VEGF antibody, anti-VEGF receptor 2 antibody, and phosphorylation inhibitors of VEGF receptor 2 tyrosine kinase, have been developed as VEGF inhibitors.3, 38) These compounds were reported to inhibit the growth of a wide variety of tumor cell lines in various animal models.3, 38) In addition, we demonstrated that a VEGF receptor tyrosine kinase inhibitor (PTK787) suppressed expression of malignant PEs by VEGF high-producing PC14PE6 cells via inhibition of vascular hyperpermeability,39) suggesting that VEGF inhibitors may be useful for control of tumor growth and PEs. Recently, neutralizing antibody for VEGF and inhibitors of VEGF receptor tyrosine kinase phosphorylation were evaluated for therapeutic potential in clinical trials. The early clinical trials showed that tumor regression was hardly observed when patients with solid tumors in advanced stages were treated with VEGF inhibitors alone. Based on these results, the therapeutic effect of anti-VEGF antibody in combination with cytotoxic chemotherapy was evaluated in recent trials. The combined use of anti-VEGF antibody (rhuMab VEGF) with chemotherapy (carboplatin + paclitaxel) improved the response rate and time to tumor progression in chemotherapy-naive advanced NSCLC patients.40) In addition, Langmuir reported the outcome of 28 patients with solid tumors who received anti-VEGF antibody (bevacizumab) for more than 1 year.41) In this particular population, the response rate was 50% (14/28 cases) and disease stabilization for more than 1 year was observed in an additional 13 cases, indicating the possibility of long-term disease stabilization by anti-VEGF antibody. Furthermore, in 16 patients with recurrent disease during the observation period, restarted bevacizumab treatment caused tumor regression in 2 cases and disease re-stabilization in 8 cases, suggesting that some patients who progress after long-term (6–12 months) bevacizumab +chemotherapy may have benefits from retreatment. Long-term treatment with VEGF inhibitors was reported to be well tolerated in the majority of the patients. Nevertheless, since adverse events, including deep venous thromboses and sudden and life-threatening hemoptysis, were reported in these studies, the potential toxicities of VEGF inhibitors must be considered in future trials.

**MMP inhibitors:** First-generation MMPIs, such as batimastat, have broad inhibitory activity against almost all MMPs and are not appropriate for oral administration. The second-generation inhibitors, such as marimastat, MMZ70 (CGS27023A), and prinomastat, also have a broad spectrum, but can be orally administered. The third-generation inhibitors, such as BAY12-9566 and ONO-4817, were designed to have a selective spectrum of MMP inhibition, and many members of this generation do not inhibit MMP-1.28) Many MMP inhibitors were reported to show promising anti-tumor efficiency against MMP-expressing tumor cells in preclinical studies.28) We recently reported that an MMP inhibitor (ONO-4817) could inhibit metastasis by MMP (gelatinases)-expressing lung cancer cell lines (PC14PE6, H226), and that the anti-metastatic effect of ONO-4817 could be augmented by the combined use of a cytotoxic antitumor drug (docetaxel) in our lung metastasis model.42) Interestingly, ONO-4817 also inhibited metastasis to the liver by MMP low-expressing cell lines (SBC-3/DOX, RERF-LC-AI) in our metastasis models. Film in situ zymography revealed that normal liver parenchyma expressed collagenase activity and facilitated metastasis by tumor cells without MMP expression in the liver.42) These results suggest that MMP inhibitors may inhibit metastasis if MMPs are expressed and involved in the growth of tumors, irrespective of their source (tumors or non-tumor parenchyma). However, the anti-metastatic effect of ONO-4817 was stronger when the drug was started by the time of establishment of micrometastasis.42) Fig. 3B, suggesting limited value of this compound in the clinical treatment of malignant diseases.

MMP inhibitors are being evaluated for in the treatment of various malignant diseases and are at various stages of clinical trials. The major dose-limiting toxicity of broad-spectrum MMPIs is time- and dose-dependent musculoskeletal adverse events, including joint pain and stiffness, that limit both the

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**Fig. 3.** Involvement of MMP in lung cancer progression. A. Heterogeneity of host-derived gelatinolytic activity determined by film in situ zymography. Note that high gelatinolytic activity was detected in the liver parenchyma, but gelatinolysis was not detected in the lung parenchyma. Gelatinolytic activity in the kidney was intermediate. B. Effect of treatment with MMP inhibitor for various periods on liver metastasis. SBC-3/DOX (1×10^6) cells were inoculated intravenously into NK-cell depleted SCID mice. The mice were given food with or without a MMP inhibitor (1% ONO-4817 content) for the indicated periods, and killed on day 28. Then, the formation of metastases was evaluated. Note that the anti-metastatic effect of MMP inhibitor was stronger when the drug was started by the time of establishment of micrometastasis (day 14).
dose level administered and the duration of therapy. These adverse events were suggested to be a toxicity associated with inhibition of MMP-1. A phase III clinical trial showed that marimastat, a second-generation MMPI, prolonged progression-free survival of patients with inoperable gastric cancer, especially for patients who had previously received chemotherapy and did not have clinically detectable metastasis at the time of enrollment. Some phase III trials with MMP inhibitors, including marimastat, prinomastat (AG3340), and BAY-12-9566, were also performed for lung cancer patients in advanced stages, but no clinical efficacy has been reported. The insufficient effect of MMP inhibitors in these trials might be due to the heterogenous expression of MMP in tumors in lung cancer patients. In view of the preclinical findings, MMP inhibitors might affect tumor growth only at early stages, but not when the disease has progressed to advanced stages. It is also possible that immune-mediated effects on tumor cells are inhibited by MMP inhibitors, because of the inability of immune cells to invade tumors and destroy them. Therefore, the design of further clinical trials with MMP inhibitors should be carefully considered (e.g., enroll only patients with early-stage cancer with high expression of MMP).

**Vascular targeting agents.** Vascular targeting agents that damage established tumor vascular structure are also of potential clinical use. Tubulin inhibitors (combretastatin A4 phosphate, ZD6126) disrupt tubulin dimerization, damaged endothelial cells, and shut off blood flow. One report claimed that an anaplastic thyroid tumor completely disappeared after treatment with combretastatin.

We have shown that another vascular targeting agent, ZD6126 (ANG453), can shut-down blood flow, inducing tumor necrosis within 24 h. Importantly, ZD6126 induced apoptosis of endothelial cells in tumors, but not in nontumor parenchyma, suggesting selectivity of this drug for tumor-associated endothelial cells (Fig. 4A). In addition, ZD6126 at higher concentrations (more than 125 ng/ml) inhibited proliferation of both endothelial cells and tumor cells, but it inhibited only endothelial proliferation at lower concentrations (less than 30 ng/ml) (Fig. 4B), suggesting that ZD6126 inhibits the growth of endothelial cells more selectively than tumor cells. Although other tubulin binding inhibitors, such as docetaxel and paclitaxel, were also reported to have anti-angiogenic activity, their effect was not selective on endothelial cells (Fig. 4). The mechanism of the selectivity of ZD6126 against tumor endothelial cells remains unclear. Further examinations are warranted to clarify this mechanism.

On the other hand, monotherapy involving daily injection with ZD6126 inhibited lung metastasis of human NSCLC cell lines, and this effect could be further augmented by combined use of cisplatin (submitted for publication). The therapeutic efficacy of ZD6126 is being evaluated in clinical trials.

**EGFR inhibitors.** There are two strategies to target EGFR. The first is monoclonal antibody directed against the extracellular ligand-binding domain (e.g., IMC-C225 and ABX-EGF), and the other is synthetic small compounds which inhibit ATP binding to the tyrosine kinase domain, thereby shutting down the signal. ZD1839 and OSI-774 are selective, reversible inhibitors of EGFR tyrosine kinases, and CI-1033 is an irreversible inhibitor of all four EGF receptors. The EGFR inhibitor that is furthest along in clinical development is a small compound, ZD1839. In preclinical studies, ZD1839 was shown to induce apoptosis of EGFR-overexpressing tumor cells and it inhibited angiogenesis induced by EGF.

Phase I clinical trials with ZD1839 have shown excellent tolerance (with toxicity limited to mild rash and diarrhea) and efficacy in some advanced NSCLC patients. In a phase II trial (IDEAL 1: a multi-center, international study), 208 evaluable patients were randomized to either 250 or 500 mg oral daily administration of ZD1839. The overall objective response rate at 250 or 500 mg was 18.4% and 19.0%, respectively. Disease control rate (complete response + partial response + stable disease) at 250 or 500 mg was 54.4% and 51.4%, respectively. Although multivariate analysis suggested that ethnicity was not a significant factor, Japanese patients showed better results in terms of response rate (27.5% for Japanese vs. 10.4% for non-Japanese) and disease control rate (65.7% for Japanese vs. 40.6% for non-Japanese). In addition, female sex and adenocarcinoma were found to be associated with a favorable response to ZD1839. Progression-free survival measured for 12 weeks was comparable with prior findings of second-line therapy in NSCLC: docetaxel gave a response rate of 7.1% and time to progression of 10.6 weeks. A provocative aspect of this trial was that symptom improvement was reported by a majority of patients after only 8 days of ZD1839 treatment. Based on these results, ZD1839 (250 mg/day) was approved in Japan in July, 2002 and has been widely used for NSCLC patients.

Bronchioalveolar carcinoma (BAC), one of the subtypes of lung papillary adenocarcinoma, presents unique clinical features, such as endobronchial spread and bronchorrhea in advanced stages. The prognosis for BAC patients in advanced stages is extremely poor, similar to patients with other NSCLC...
types, because of the very low susceptibility to conventional chemotherapy and radiotherapy.62 Although alternative modalities, such as indomethacin, interferon α2, and streptococcal preparation by inhalation, have been investigated in patients with extended BAC and bronchorrhea, the effect was quite unsatisfactory. Recently, we experienced two patients with chemotherapy-refractory BAC who were successfully treated with ZD1839.54 In both cases (females), serous sputum production (greater than 200 ml/day before treatment) was markedly reduced within 3 days of starting the treatment with ZD1839, and hypoxia and radiographic signs of bilateral lung consolidation were visibly improved within 7 days. Following more than 10 months of treatment, no evidence of recurrence or severe adverse events were observed. Although large-scale studies are necessary to evaluate the efficiency of ZD1839 against BAC, these results suggest the presence of a population of BAC patients who show favorable response to ZD1839.

In Western countries, two phase III trials (INTACT 1 and 2) were recently performed to determine whether a combined use of cytotoxic agents and anti-tumor vascular effect could augment the efficacy of ZD1839 in chemotherapy-naïve patients with advanced NSCLC.55,56 More than 1000 patients were entered in each trial, and the patients underwent 6 cycles of chemotherapy (paclitaxel and carboplatin, or gemcitabine and cisplatin) in combination with daily ZD1839 (250 mg or 500 mg) or placebo. Although no major new adverse events were observed, ZD1839 did not improve survival, progression-free survival, or response rate. These results suggest no added benefit of concurrent use of ZD1839 with chemotherapy as the first line therapy.

While ZD1839 was reported to be well tolerated in clinical trials, pulmonary damage including life-threatening interstitial pneumonia was reported in more than 1% of the NSCLC patients treated with ZD1839.57 Therefore, the safety of this drug should be carefully re-evaluated. In addition, since the response to EGFR inhibitors was not suggested to correlate with EGFR expression in tumor cells, the mechanism of the anti-tumor action needs to be clarified. It is also necessary to determine whether tumor vascularization is affected by treatment with EGFR inhibitors.

Future directions
Since tumor angiogenesis is regulated by complex mechanisms, monotherapy with the majority of anti-vascular agents would be insufficient to obtain long-term disease control. Therefore, multi-agents or multi-modality therapy combined with other anticancer modalities may be necessary to obtain additional therapeutic benefit. There are at least three strategies. The first is the combined use of anti-angiogenic agents and vascular targeting agents focusing on tumor vasculature as a target. Since even vascular targeting agents induce extensive central necrosis in the tumor and the narrow peripheral rim of the tumor would re-grow in the presence of angiogenesis from the surrounding normal vessels, the addition of anti-angiogenic agents appears to be logical. The second is the use of anti-vascular agents as an adjuvant of surgical resection, because anti-tumor vasculature agents, especially anti-angiogenic agents, are expected to be more effective against residual tumors rather than bulky tumors. The third is the strategy which targets both tumor cells and tumor-associated endothelial cells. For this, anti-vasculature agents with cytotoxic agents or molecular-targeted drugs directed to tumor cells may be used in combination.

One impediment to the successful and rapid development of anti-tumor vasculature therapy is the lack of validated assays capable of measuring an anti-vascular effect directly in patients.59 Several techniques and assays (including dynamic magnetic resonance imaging (MRI) and [15O]H2O positron emission tomography (PET) scanning to evaluate blood flow, [18F]fluorodeoxyglucose PET scanning to evaluate metabolism, and detection of apoptotic endothelial cells in the tumors and peripheral blood) are currently being tested in ongoing clinical trials.56 Establishment of appropriate surrogate makers is required in the context of anti-tumor vasculature therapy.

Conclusions
Angiogenesis plays a critical role in the progression of the majority of NSCLC, and is regulated by complex mechanisms in the presence of various angiogenesis-related molecules. Anti-tumor vascular therapy is considered promising, and many compounds targeting tumor vasculature have been developed. Since several compounds have shown favorable anti-tumor potential in preclinical studies, their efficiency is currently being evaluated in clinical trials against various solid tumors, including NSCLC; these trials are expected to determine the optimal conditions, including optimal doses, optimal schedule, and optimal clinical setting, with establishment of appropriate surrogate markers. An optimal combined modality with anti-vascular therapy is probably necessary to obtain additional therapeutic benefits.