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REVIEW ARTICLE

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Current status and perspective of angiogenesis and antivascular therapeutic strategy: non-small cell lung cancer

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Abstract Lung cancer is the leading cause of cancer death worldwide, and most patients die of metastatic disease. Angiogenesis, namely, neovascularization from preexisting vasculature, is necessary for tumor growth in both primary and distant organs to supply oxygen and nutrition. Angiogenesis consists of sprouting and nonsprouting (the enlargement, splitting, and fusion of preexisting vessels) processes, and both can occur concurrently. The growth of non-small cell lung cancer (NSCLC), which accounts for more than 80% of all lung cancers, is usually dependent on angiogenesis, which is regulated by complex mechanisms in the presence of various angiogenesis-related molecules. Vascular endothelial growth factor (VEGF), also known as vascular permeability factor (VPF), is one of the most potent angiogenic molecules, while also regulating both angiogenesis and vascular permeability and hence promoting tumor progression and the development of malignant pleural effusions in NSCLC. Recent clinical trials showed that the anti-VEGF antibody bevacizumab, combined with standard first-line chemotherapy, provided a statistically and clinically significant survival advantage with tolerable toxicity. In addition, the combined use of the anti-VEGF antibody with an inhibitor of epidermal growth factor receptor (EGFR) has also shown favorable antitumor efficiency. These successes proved the validity of an antivasculature strategy for NSCLC. Furthermore, a large number of antivasculature agents have been shown to be effective against multiple targets. The efficiency of these compounds is currently being investigated in clinical trials for NSCLC.

Key words Lung cancer · Metastasis · VEGF · VPF · EGF receptor · Molecularly targeted therapeutics

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Introduction

Lung cancer has been the leading cause of malignancy-related death in Japan since 1998, and more than 50000 persons die of this disease every year.[1] Lung cancer is divided into two subgroups, including small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC), and more than 80% of all cases are NSCLC. A surgical resection is undoubtedly the first choice of treatment for NSCLC patients in the early stages (stage I and II). More than two-thirds of all patients, however, present at an advanced stage.[2] Radiation therapy is generally preferred for patients with locoregionally advanced NSCLC (stage III), whereas chemotherapy is administered for patients with disseminated disease (stage IV). Despite improvements in these therapeutic modalities, the prolongation of survival in patients presenting at advanced stages (especially stage IV) is still not satisfactory. Therefore, novel and more effective therapeutic modalities are necessary for NSCLC presenting at an advanced stage. One candidate is molecularly targeted therapy. An inhibitor of epidermal growth factor receptor (EGFR), gefitinib, has been approved in several countries, including Japan, and it has been shown to be effective for the treatment of chemotherapy-refractory NSCLC.[3] However, an Iressa Survival Evaluation in Lung Cancer (ISEL) study revealed that gefitinib treatment has shown no overall survival advantage in an unselected population.[4]

Angiogenesis, neovascularization to supply oxygen and nutrition,[5] has been shown to play a critical role in the progression of tumors (growth of both primary and metastatic tumors). Therefore, blockade of angiogenesis has been expected to prevent the growth of tumor cells at both primary and metastatic sites and to prevent the emergence of tumor progression, thereby improving the prognosis of patients with various types of malignant tumors, including NSCLC. From this point of view, dozens of compounds that target tumor vasculature have been developed, and many experimental studies have shown that antitumor vasculature agents can effectively control tumor cell growth and metastases.[6] In this article, the molecular mechanisms of

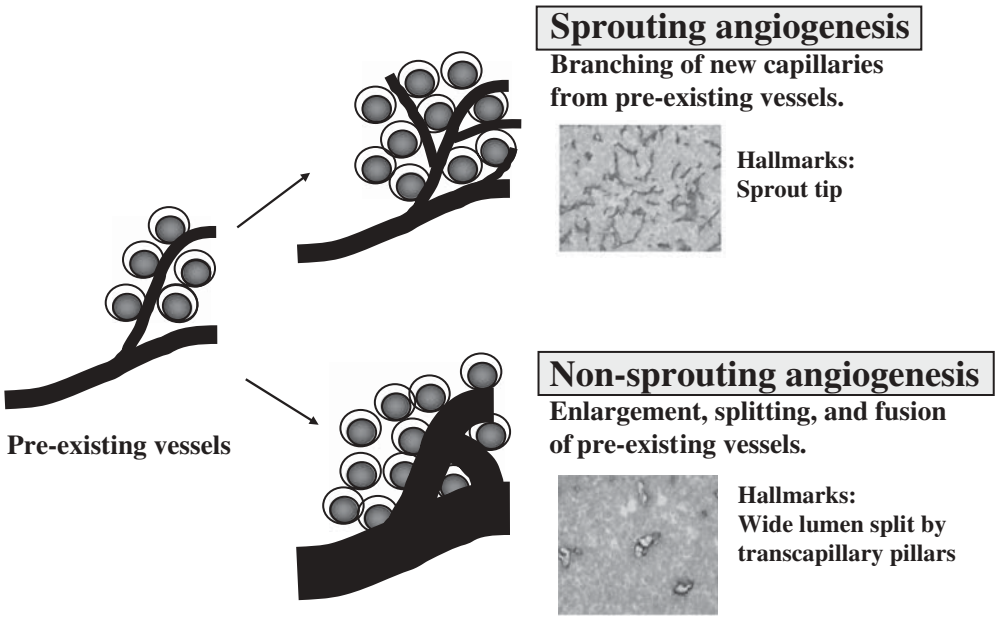

Fig. 1. Pattern of the angiogenesis processes. Sprouting angiogenesis involves the branching (true sprouting) of new capillaries from preexisting vessels. The hallmark of this type is sprout tips. Nonsprouting angiogenesis results from the enlargement, splitting, and fusion of preexisting vessels produced by the proliferation of endothelial cells at the wall of the vessel. Transvascular bridges are sometimes observed in enlarged vessels produced by nonsprouting angiogenesis. Sprouting angiogenesis and nonsprouting angiogenesis can thus occur concurrently

angiogenesis in NSCLC are briefly reviewed, and recent progress in the clinical development of antivascular therapeutics against NSCLC is discussed.

Molecular mechanism of tumor angiogenesis

Types of angiogenesis

Angiogenesis can occur by either sprouting or nonsprouting processes[7] (Fig. 1). Sprouting angiogenesis involves the branching (true sprouting) of new capillaries from preexisting vessels. This type of angiogenesis begins with an angiogenic stimulus, followed by the 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, thereby forming new capillary tubes. All these actions therefore require endothelial cell survival, which mandates the interplay of numerous factors (Table 1) that can act in a positive or negative fashion.

Nonsprouting angiogenesis results from the enlargement, splitting, and fusion of preexisting vessels. Transvascular bridges are sometimes observed in enlarged vessels produced by nonsprouting angiogenesis. Nonsprouting 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.[7] We previously reported this type of angiogenesis to be observed in brain metastasis with progressive growth.[8,9]

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 nonangiogenic NSCLC tumors characterized by a lack of parenchymal destruction and the absence of both tumor-associated stroma and new vessels; however, this type of tumor is found in less than 20% of all NSCLC.[10] Several lines of evidence suggest that the growth of solid tumors, including NSCLC, is usually dependent on angiogenesis.[11,12] In fact, intratumoral microvessel density (IMD) was inversely correlated with the survival of surgically resected NSCLC patients.[13] Glomeruloid microvascular proliferation (GMP) is a focal proliferative budding of endothelial cells resembling a renal glomerulus, and it has recently been suggested to have an aggressive angiogenic phenotype associated with a poor prognosis in NSCLC.[14] Vascularization is commonly evaluated by immunohistochemistry for several endothelial markers, including CD31, CD34, CD105, and factor VIII. Of these factors, CD105, a proliferative endothelial marker, has been reported to be a better predictive factor of a poor prognosis of NSCLC patients.[13] As already described, angiogenesis is regulated by the balance of angiogenic factors and antiangiogenic factors (see Table 1).

Important angiogenic factors in NSCLC

VEGF

VEGF, the prototype of the VEGF family, which consists of VEGF, VEGF-B, VEGF-C, VEGF-D, and VEGF-E, has been suggested to be a highly potent and specific growth factor for endothelial cells while also playing a very impor-

Table 1. Endogenous factors involved in the control of angiogenesis

Proangiogenic factors	Antiangiogenic factors
Growth factors	Matricellular glycoproteins
VEGF, FGF-2, EGF, TGF-α,	Thrombospondin-1 and -2
PDGF-AA, PDGF-BB,	
Proteases	Collagen fragments
Cathepsin, MMP-2, -7, -9	Angiostatin, endostatin, tumstatin
uPA	Canstatin, malignostatin
Cytokines	Cytokines
IL-1, IL-6, IL-8, MCP-1, TNF-α	IFN-α, IFN-β
Others	Others
Ang-1, Ang-2, integrins, hypoglycemia inhibitor	Vasohibin, vascular endothelial growth
Low levels of PO_2 and pH, NOS, COX-2	Pigment epithelium-derived factor

VEGF, vascular endothelial growth factor; FGF-2, fibroblast growth factor-2; EGF, epidermal growth factor; TGF-α, transforming growth factor-α; PDGF, platelet-derived growth factor; MMP, matrix metalloproteinase; uPA, urokinase type plasminogen activator; IL, interleukin; Ang, angiopoietin; NOS, nitric oxide synthese; COX, cyclooxygenase; IFN, interferon

tant role in angiogenesis.[15] VEGF consists of at least six isoforms (VEGF121, VEGF145, VEGF165, VEGF183, VEGF189, VEGF206), which are regulated by splicing at the mRNA level, and VEGF165 is the most abundant and biologically potent 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, autophosphorylation, and signal transduction. Several studies have demonstrated VEGF expression to be directly correlated with IMD while inversely correlated with survival in patients with NSCLC.[11] High tumoral expression of VEGF189, a membrane-associated form, was reported to be associated with high IMD, shorter survival time, and early postoperative relapse.[16] Recently, a new endogenous splice variant, VEGF165b, was identified. It binds to VEGFR-2 with the same affinity as VEGF165, thus preventing VEGFR-2 signaling by VEGF165 and hence acting as an inhibitor of angiogenesis.[17] Because VEGF can be produced by both cancer cells and host cells, host-derived VEGF also plays an important role in tumor angiogenesis. Recently, overexpression of *Wnt5a*, a member of the Wnt gene family that encodes the multifunctional signaling glycoproteins in NSCLC, was shown to be associated with tumor proliferation and stromal VEGF expression,[18] thereby suggesting *Wnt5a* to play a regulatory role in host-derived VEGF secretion.

VEGF as an angiogenic factor

VEGF has been reported to induce angiogenesis and facilitate both the growth of the primary tumors and the production of metastasis. 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 multiorgan metastasis of lung cancer.[8,19–21] In multiple organ metastases, brain metastasis is one of the most serious problems, because it restricts the quality of life of the patient and is also refractory against various anticancer modalities.

Brain metastasis was induced by the injection of tumor cells into the internal carotid artery of nude mice.[8] As described earlier, we found nonsprouting angiogenesis to be predominantly observed in brain metastasis and that the levels of VEGF production by NSCLC cells directly correlated with the potential to produce brain metastasis in this model.[8] Brain metastases developed by VEGF high-producing cell lines progressed rapidly and had many enlarged vessels split by transcapillary pillars (a hallmark of nonsprouting angiogenesis). In addition, the transfection of VEGF high-producing cells with the *antisense-VEGF165* gene resulted in a decrease in brain metastasis formation. However, the metastatic potential of VEGF low-producing cells was not augmented by transfection with the *sense-VEGF165* or *sense-VEGF121* genes. These results suggest that VEGF is essential for promoting brain metastasis via angiogenesis and that its inhibition could thus represent an important therapeutic target.

VEGF as a permeability factor

VEGF with multifunctional activities was recently found to be identical to vascular permeability factor (VPF).[15] Malignant pleural effusion (PE) is frequently associated with advanced lung cancer. We have shown that malignant PE fluid contained a high level of VEGF in lung cancer patients.[22] We also developed a valuable model for PE formation, which occurred after i.v. injection of human lung adenocarcinoma cell lines (PC14)[20] and its metastatic variant PC14PE6,[23] thus demonstrating that VEGF was responsible for the development of PE induced by these lung adenocarcinoma cells.[23] In addition, VEGF was reported to induce brain edema by its activity as VPF.[24] Collectively, VEGF/VPF has been shown to be essential not only for angiogenesis but also for the formation of pleural effusion and brain edema produced by lung cancer via the induction of vascular hyperpermeability (Fig. 2). Because VEGF/VPF and its receptors can be therapeutically targeted, VEGF receptor inhibitor could be useful for the control of malignant PE and brain edema in lung cancer patients.

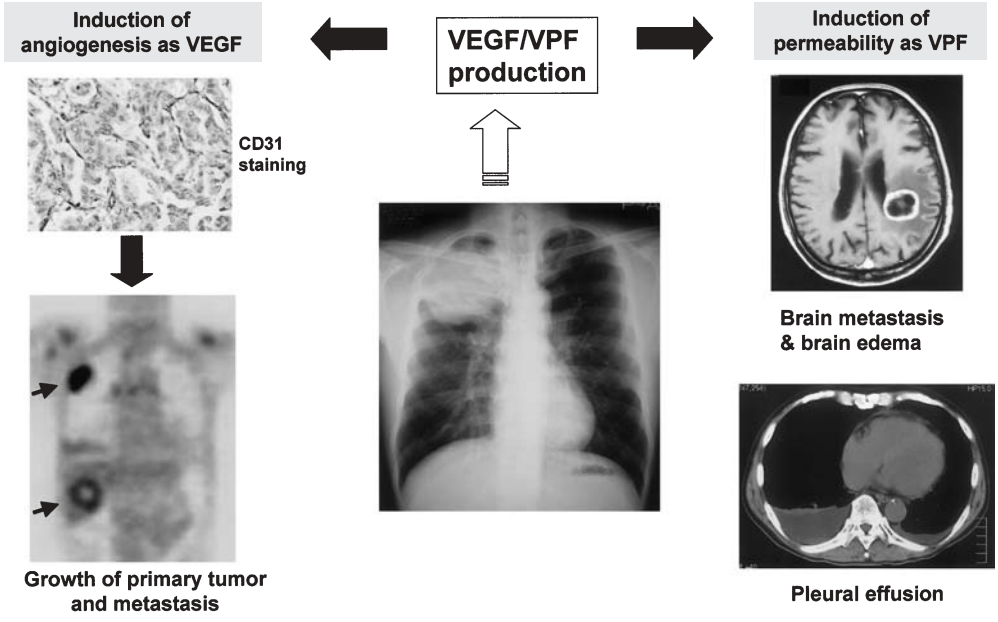

Fig. 2. The involvement of vascular endothelial growth factor (VEGF)/vascular permeability factor (VPF) in lung cancer progression. VEGF/VPF produced by tumors facilitates the growth of the primary tumors and metastasis via angiogenesis. VEGF/VPF also facilitates the formation of pleural effusion and brain edema by the induction of hyperpermeability. *Arrows* indicate the primary lung tumor and liver metastasis observed in positron emission tomography

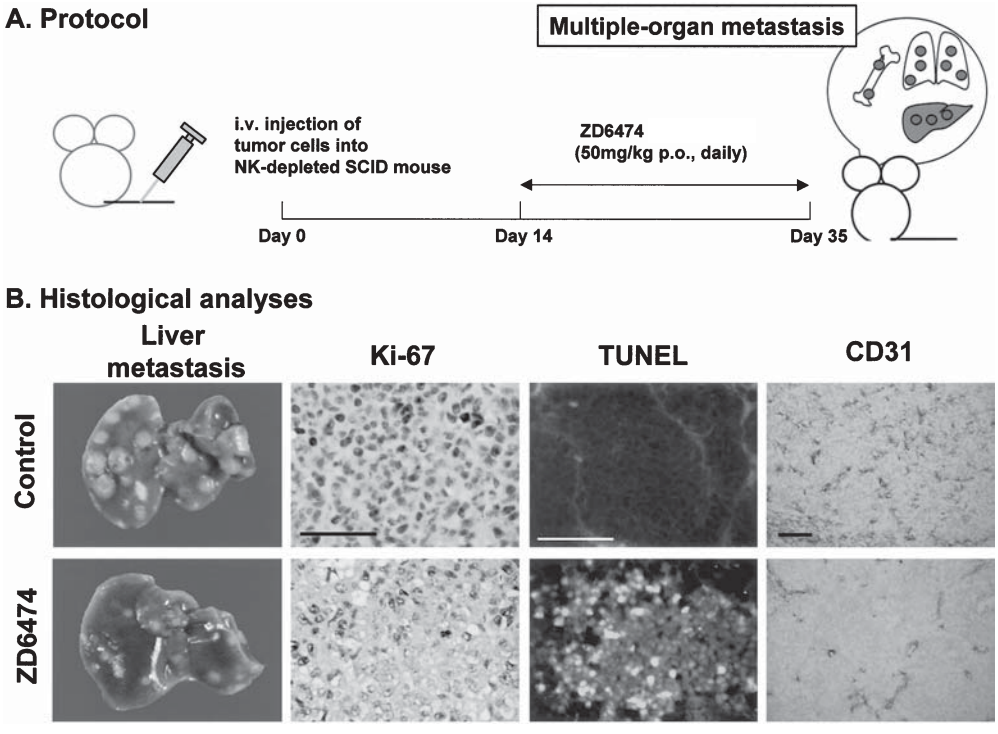

Fig. 3A,B. Antivasculature effect of ZD6474 in an experimental metastasis model. **A** Protocol. Human small cell lung cancer (SCLC) cells (SBC-5) were injected intravenously into NK cell-depleted SCID mice. From 14 days after the inoculation, mice were treated with ZD6474 (50 mg/kg/day orally). Five weeks after tumor cell inoculation, metastatic burden was determined. **B** Histological analyses. SBC-5 cells produced large metastases in the liver, some of which were larger than 3 mm in diameter. Treatment with ZD6474 inhibited production of large metastases in the liver. The liver metastases were analyzed by immunohistochemistry and immunofluorescence. Note that treatment with ZD6474 inhibited tumor vascularization (CD31) and tumor cell proliferation (Ki-67), while also enhancing total cell apoptosis (TUNEL)

VEGF family

Much attention is currently being paid to the VEGF family because of its implications not only for angiogenesis but also for lymphoangiogenesis.[15] VEGF-C and VEGF-D have been shown to bind to VEGFR-2 and VEGFR-3 and to play pivotal roles in developmental and tumoral lymphoangiogenesis.[25] In NSCLC, VEGF-C expression has been reported to correlate with lymph node metastasis, lymphatic invasion, and a poor prognosis.[26,27]

Collectively, these findings suggest that the VEGF family and its receptors may be ideal targets of both angiogenesis and lymphoangiogenesis in NSCLC.

Angiopoietins

Angiopoietin (Ang)-1 and -2 have been identified as ligands for Tie-2, which is a receptor tyrosine kinase specifically expressed on endothelial cells, and Angs play a critical role in angiogenesis in concert with VEGF.[28] Ang-1 binds to Tie-2 and maintains and stabilizes mature vessels by promoting the interaction between endothelial cells and surrounding extracellular matrix. Ang-2 competitively binds to Tie-2 and antagonizes the stabilizing action of Ang-1, thus resulting in the destabilization of vessels. These destabilized vessels may undergo regression in the absence of angiogenic factors such as VEGF; in the presence of VEGF, however,

these destabilized vessels may undergo angiogenic changes. A recent report indicated a positive Ang-2 expression to be significantly correlated with a poor prognosis, as well as with aggressive angiogenesis in resected NSCLC, which was even further enhanced in the presence of VEGF expression.[29] Therefore, Ang-2 may be one of the targets of novel antiangiogenic therapy for NSCLC.

Vasohibin

Vasohibin is a newly identified negative angiogenesis regulator.[30] It is selectively induced in endothelial cells by proangiogenic stimulatory growth factors such as VEGF via VEGFR-2-mediated signaling. It appears to operate as an intrinsic and highly specific feedback inhibitor of activated endothelial cells engaged in the process of angiogenesis. In addition, it was reported that transfection of vasohibin in Lewis lung carcinoma resulted in suppression of tumor growth via inhibition of angiogenesis.[30] A large amount of vasohibin was detected in tumor-associated endothelial cells in NSCLC patients, thus suggesting that vasohibin plays a regulatory role in the angiogenesis of NSCLC.

EGFR

EGFR, a 170-kDa transmembrane glycoprotein, is activated by the binding of specific ligands, such as EGF and transforming growth factor-α (TGF-α).[31] EGFR signaling (via ras – raf-1 – MAPK, STAT, and PI3K – Akt) facilitates the tumor cell cycle, proliferation, invasion, and decreased apoptosis. In addition, because (1) EGFR is detected in the dividing tumor vasculature endothelial cells,[32] (2) EGF induces neovascularization of mice cornea,[33] and (3) blockade of EGFR signaling downregulates the production of proangiogenic cytokines, including VEGF and IL-8, by several tumor cell lines expressing EGFR,[33] EGFR was suggested to be involved, at least indirectly, in the regulation of angiogenic processes.

EGFR is commonly overexpressed in various types of malignancies, including NSCLC.[31] Recently, somatic mutations in the tyrosine kinase domain of the EGFR gene have been discovered and reported to be one of the best prediction markers for the response to EGFR tyrosine kinase inhibitors in NSCLC patients.[34] However, a recent meta-analysis study showed that expression of wild-type EGFR or mutant EGFR itself was rarely related to patient outlook.[35,36] On the other hand, the expression of phosphorylated EGFR, but not an overexpression of EGFR protein, was inversely correlated with survival and time to tumor progression,[37] thus suggesting that activation of EGFR, but not its overexpression, may be one of the important prognostic factors for NSCLC patients. Further analyses to explore the correlation between vascularization and EGFR-activation are thus warranted.

Antitumor vasculature therapy

The concept of targeting tumor vasculature was first advocated 30 years ago,[1] and this concept has recently re-emerged because of the development of new antitumor vasculature agents. These agents have potential advantages, such as the physical accessibility and genetic stability of target cells, over conventional types of cytotoxic chemotherapy.[38]

There are several strategies for targeting tumor vasculature. The first is the modality selectively targeting angiogenesis: neovascularization during the growth of small tumors (antiangiogenic agents). Angiogenesis-related molecules, including VEGF and VEGFR-2, have been suggested to be ideal targets for this modality, and a large number of inhibitors have been developed.[6] The second strategy is the therapy selectively targeting established tumor-associated vessels in large tumors (vascular-targeting agents), and tubulin in the dividing endothelial cells is one of the targets for this type of modality. The third is long-term use of compounds that secondarily disrupt the tumor vasculature, including anticancer drugs (taxanes) and EGFR inhibitors. The fourth is the combined use of these compounds.

Antiangiogenic agents

VEGF inhibitors

A large number of compounds, including anti-VEGF antibody, anti-VEGFR-2 antibody, and phosphorylation inhibitors of VEGFR-2 tyrosine kinase, have been developed as VEGF inhibitors.[6] These compounds have been reported to inhibit the growth of a wide variety of tumor cell lines in various animal models, and their efficiency is now being evaluated in clinical trials.

The most thoroughly investigated compound is humanized anti-VEGF monoclonal antibody (bevacizumab: Avastin). As early clinical trials showed tumor regression was rarely observed when patients with solid tumors in advanced stages were treated with VEGF inhibitors alone, the therapeutic effect of bevacizumab in combination with cytotoxic chemotherapy was evaluated. The Eastern Cooperative Oncology Group (ECOG) 4599 phase III trial[39] was conducted to confirm the encouraging activity and survival observed in a small randomized phase II trial with the addition of bevacizumab to carboplatin plus paclitaxel chemotherapy in untreated advanced NSCLC.[40] In that trial, a higher incidence of severe tumor-related bleeding episodes was observed in patients with squamous histology and centrally located tumors treated with bevacizumab. Accordingly, in the ECOG trial 4599, any patients with squamous cell NSCLC, brain metastasis, or gross hemoptysis were excluded. From July 2001 to April 2004, 878 patients were randomized to receive either paclitaxel (200 mg/m^2) plus carboplatin [to an area under the concentration–time curve (AUC) of 6], or the same chemotherapy plus bevacizumab (15 mg/kg) on day 1 every 3 weeks. Chemotherapy was

continued up to six cycles; patients in the experimental arm received single-agent bevacizumab after the six cycles of chemotherapy until progressive disease or intolerable toxicity. The patients in the chemotherapy-alone arm were not allowed to cross over to bevacizumab. The results showed that there was a significant advantage for patients in the bevacizumab arm in terms of median survival (12.5 months versus 10.2 months; $P = 0.0075$). In addition, the patients treated with bevacizumab also had a significantly greater response rate (27% versus 10%; $P < 0.0001$) and a significantly longer progression-free survival time (6.4 months versus 4.5 months; $P < 0.0001$). Both regimens were well tolerated; however, a higher incidence of bleeding was associated with bevacizumab administration (4.5% versus 0.7%). There were 10 treatment-related deaths: 5 were the result of hemoptysis, and all were in the experimental arm.

These results showed that the addition of bevacizumab to carboplatin plus paclitaxel in patients with nonsquamous NSCLC provides a statistically and clinically significant survival advantage with tolerable toxicity. However, careful patient selection is mandatory to avoid fatal bleeding following bevacizumab administration.

Vascular targeting agents

Vascular targeting agents that damage established tumor vasculature are also of potential clinical use. We have shown that ZD6126 (ANG453), a tubulin inhibitor, selectively induced apoptosis of preexisting tumor-associated endothelial cells, shut down blood flow, and induced tumor necrosis within 24 h.[41] Even in vitro, ZD6126 inhibited the growth of endothelial cells more selectively than tumor cells. Although other tubulin-binding inhibitors, such as docetaxel and paclitaxel, have been reported to also have antiangiogenic activity,[41] their effect was not selective on endothelial cells. The mechanism of the selectivity of ZD6126 against tumor endothelial cells thus remains unclear. The monotherapy of daily injection with ZD6126 inhibited lung metastasis of human NSCLC cell lines,[41] and this effect could be further augmented by the combined use of cisplatin.[42]

Combretastatin A4 phosphate is another tubulin inhibitor. One case of anaplastic thyroid tumor that completely disappeared after treatment with combretastatin has been reported.[43] Phase II clinical trials are presently ongoing.

EGFR inhibitors

As EGFR inhibitors, monoclonal antibodies directed against the extracellular ligand-binding domain (e.g., cetuximab and ABX-EGF) and synthetic small compounds (e.g., gefitinib and erlotinib) that inhibit ATP binding to the tyrosine kinase domain have been developed. The furthest along in clinical development of EGFR inhibitors is a small compound, gefitinib. It has been approved for the treatment of NSCLC in several countries. Although gefitinib treatment has shown no overall survival advantage in unselected populations in an ISEL study,[4] it has demonstrated a favorable antitumor activity in a subset of NSCLC patients, especially in females, in Asian people, those with adenocarcinoma histology, those who have never smoked, and patients with a good performance status.[44] Several factors, including active mutations in EGFR gene,[34,45] copy numbers of the EGFR gene,[46] phosphorylation of Akt,[47] and k-*ras* mutations,[36] either directly or indirectly correlated with the responsiveness of NSCLC patients to gefitinib. We recently identified 12 "gefitinib resistance-related genes (GRRGs)" by cDNA microarray analyses.[48] GRRGs were predominantly expressed in progressive disease (PD) cases, and the responsiveness of NSCLC patients to gefitinib could thus be predicted by the evaluation of GRRGs.[48]

In preclinical studies, gefitinib was shown to induce apoptosis of EGFR-overexpressing tumor cells and to inhibit angiogenesis induced by EGF.[49] Further studies are warranted to clarify the antiangiogenic effect of EGFR inhibitors in clinical studies.

VEGFR tyrosine kinase inhibitors (TKI) as dual inhibitors

Small molecule tyrosine kinase inhibitors prevent activation of VEGFRs, thus inhibiting downstream signaling pathways rather than binding to VEGF directly. Many of these compounds have additional activity against other tyrosine kinase-type receptors, and they can be, therefore, classified into two groups, such as VEGFR inhibitors with PDGFR activity and VEGF inhibitors with EGFR activity (Table 2).

VEGFR TKI with PDGFR activity

PTK787 (vatalanib) is an oral inhibitor of VEGFR-1, -2, and -3 tyrosine kinases and related kinases such as PDGFR-β and c-*kit*. Several preclinical studies have shown significant growth inhibition in a range of tumor types and the potential to inhibit metastasis. In addition, we demonstrated that PTK787 suppressed the development of malignant pleural effusions by VEGF high-producing PC14PE6 cells via inhibition of vascular hyperpermeability,[50] thus suggesting that this compound may be useful for the control of tumor growth and pleural effusions. The role of PTK787 in patients with lung cancer is now being evaluated in a phase II study (the GOAL Study) in France and Germany.

BAY 43-9006 (sorafenib) is an inhibitor of VEGFR-2, VEGFR-3, and PDGFR-β. This compound also has strong activity against Raf-1, and it inhibits tumor cell proliferation and angiogenesis.[51] The clinical evaluation of BAY 43-9006 has been promising, with a prolongation of progression-free survival in renal cell carcinoma. BAY 43-9006 (400 mg twice daily) is currently in clinical trials for the treatment of NSCLC, both as monotherapy and in combination with chemotherapy.

Table 2. Inhibitors of VEGF-VEGFR signaling

Drug	Target	Phase of development	Company
Monoclonal antibody			
Bevacizumab/Avastin	VEGF	Approved	Genentech
IMC-1C11	VEGFR-2	Phase I	ImClone
Inhibitors of VEGFR tyrosine kinase			
GW-786034	VEGFR-2	Phase II	GSK
CEP-7055	VEGFR-1/2/3	Phase I	Cephalon
AZD2171	VEGFR-2,	Phase I	AstraZeneca
With PDGFR activity			
BAY 43-9006	Raf-1, VEGFR-2/3, PDGFR-β	Phase III	Bayer
PTK787	VEGFR-1/2/3, PDGFR-β, c-kit	Phase III	Novartis
SU11248	VEGFR-1/2/3, CSF-1 PDGFR-β, c-kit	Phase III	Pfizer
AG013736	VEGFR, PDGFR-β, c-kit	Phase II	Pfizer
AMG706	VEGFR, PDGFR-β, c-kit Ret	Phase II	Amgen
With EGFR activity			
ZD6474	VEGFR-2, EGFR	Phase II	AstraZeneca
AEE788	VEGFR-2, EGFR, erbB2	Phase I	Novartis
Others			
AVE-005/VEGF-Trap	VEGF	Phase I	Regeneron Sanofi-aventis
Neovastat (AE-941)	MMP, VEGF	Phase III	Æterna
NM-3	VEGF, FGF-2	Phase I	Genzyme

VEGFR, vascular endothelial growth factor receptor; PDGFR, platelet-derived growth factor receptor; EGFR, epidermal growth factor receptor; FGF-2, fibroblast growth factor-2

VEGFR TKI with EGFR activity

ZD6474 is a novel, orally active agent that inhibits VEGFR-2 and EGFR tyrosine kinases.[52,53] Consistent with the antiangiogenic mechanism of its action, ZD6474 inhibits VEGF signaling and angiogenesis in vivo and demonstrates a broad-spectrum antitumor activity in a range of histologically diverse tumor xenograft models.[52] We recently reported that ZD6474 successfully controlled the growth of established metastatic colonies produced by both NSCLC[54] and SCLC cell lines.[55] Other preclinical studies have demonstrated that combining ZD6474 with paclitaxel or radiation therapy produces a greater inhibition of tumor growth in comparison to any of these agents alone. In phase I clinical trials, the safety and tolerability of ZD6474 has been evaluated (Japanese[56] and Western[57] populations). Once-daily oral administration of ZD6474 at doses <300 mg/day was generally well tolerated, with adverse events including diarrhea, rash, and asymptomatic QTc (corrected Q–T interval) prolongation, all of which were manageable by dose interruption or reduction. In a Japanese study, four of nine patients with refractory NSCLC had partial responses. More than 40% of patients in the Western study had disease stabilization (>8 weeks). ZD6474 is currently being investigated in phase II trials in NSCLC, in which the efficacy of ZD6474 is being compared with that of the EGFR TKI gefitinib, in combination with the cytotoxic agent docetaxel versus docetaxel alone, or in combination with the carboplatin/paclitaxel regimen.

Combining treatment strategy

Because tumor angiogenesis is regulated by complex mechanisms, monotherapy with the majority of antivascular agents would be insufficient to obtain long-term disease control. Therefore, multiagents or multimodality therapy combined with other anticancer modalities may be necessary to obtain additional therapeutic benefit, as already described. There are at least three strategies as combining treatments for NSCLC. The first is the combined use of antiangiogenic agents and vascular targeting agents, focusing on tumor vasculature as a target. Because even vascular targeting agents induce extensive central necrosis in the tumor, and the narrow peripheral rim of the tumor would regrow in the presence of angiogenesis from the surrounding normal vessels, the addition of antiangiogenic agents appears to be logical. The second is the use of antivascular agents as an adjuvant of surgical resection, because antitumor vasculature agents, especially antiangiogenic agents, are expected to be more effective against residual tumors than bulky tumors.

The third strategy targets both tumor cells and tumor-associated endothelial cells. For this, antivasculature agents with cytotoxic agents or molecularly targeted drugs directed to tumor cells may be used in combination. As EGFR tyrosine kinase inhibitors demonstrated favorable clinical response to a particular population of NSCLC, VEGF inhibition with bevacizumab has been investigated in combina-

tion with the EGFR TKI erlotinib in 40 patients with a stage IIIB/IV or recurrent NSCLC of nonsquamous cell histology.[58] Treatment with bevacizumab (15 mg/kg intravenously every 21 days) plus erlotinib (150 mg/day orally) resulted in 8 partial responses (20%) and 26 patients with stable disease (65%). The median survival of 34 patients treated in the phase II part of the study was 12.6 months, with 52% of the patients alive at 1 year. No pharmacokinetic interactions were observed between the two agents and the most common adverse events were mild to moderate rash, diarrhea, and proteinuria. The combined use of VEGFR inhibitor (AZD2171) with gefitinib is also being evaluated, and the results of early clinical studies have demonstrated a favorable feasibility. These encouraging data support further evaluation of VEGF/EGF inhibition for the treatment of advanced NSCLC, and a randomized study is currently ongoing.

Biomarkers

One impediment to the successful and rapid development of antitumor vasculature therapy is the lack of validated assays capable of measuring an antivascular effect directly in patients.[59] Several techniques and assays, including dynamic magnetic resonance imaging (MRI) and $[^{15}O]H_2O$ positron emission tomography (PET) scanning to evaluate blood flow, [^{17}F]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.[60] The establishment of appropriate surrogate makers is required in the context of antitumor 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. Antitumor vascular therapy was thus suggested as a potentially effective candidate, and a large number of compounds targeting tumor vasculature have been developed. The success of bevacizumab combined with conventional cytotoxic agents or an EGFR tyrosine kinase inhibitor has raised hopes that antivascular therapies will provide benefits for the treatment of NSCLC, and it is expected that we can determine the optimal conditions, including optimal doses, optimal schedule, and optimal clinical setting with the establishment of appropriate biomarkers. An optimal combined modality with antivascular therapy is also necessary to obtain additional therapeutic benefits.

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