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REVIEW ARTICLE

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Molecular diagnosis of tumor angiogenesis and anti-angiogenic cancer therapy

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Abstract Angiogenesis is regulated by the balance of pro-angiogenic factors and angiogenesis inhibitors, and the imbalance of these regulators is the cause of pathological angiogenesis, including tumor angiogenesis. Angiogenesis is required for tumor growth and metastasis, and thus constitutes an important target for the control of tumor progression. While the benefit of anti-angiogenic therapy is potentially profound, limitations have also been recognized by the results obtained thus far by clinical trials. Precise understanding of the process of angiogenesis should lead us to new regimens for more efficient anti-angiogenic therapy. This review focuses on our current understanding of the molecular mechanism of tumor angiogenic and the status of the anti-angiogenesis approach for cancer treatment.

Key words Angiogenesis · VEGF · Angiopoietin · Anti-angiogenesis

Introduction

It has been revealed that carcinogenesis is a consequence of multiple steps of oncogene and tumor suppressor gene mutation. However, the growth of tumors beyond a size of 2 mm^3 in a body requires the additional step of angiogenesis. The original hypothesis for the dependence of solid tumors on angiogenesis was presented by Folkman in 1971.[1] Since then, numerous studies have been performed to prove this hypothesis. Animal studies clearly showed the dependence of solid tumors on angiogenesis. Moreover, multiple clinical studies for the assessment of tumor microvascular density in human cancers have supported the view that tumors are angiogenesis-dependent.

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Blood vessels are constructed by two processes: vasculogenesis and angiogenesis. Vasculogenesis is achieved by the in situ differentiation of endothelial progenitor cells to endothelial cells (ECs), whereas angiogenesis is achieved by capillary sprouting from pre-existing small vessels.[2] Although the involvement of vasculogenesis in tumors was suggested in an animal model,[3] its significance in human cancers has not been established. The capacity for vasculogenesis in the adult may not be large, and, thus, angiogenesis is thought to play a major role in neovascularization in the adult.[4]

Physiological angiogenesis in the adult is observed only in limited places, such as the endometrium and ovarian follicle, and this process is normally transient. However, persistent angiogenesis appears to play a crucial role in several pathological conditions including tumors. Indeed, the induction of angiogenesis, or the so-called "angiogenic switch," is recognized as a critical step for tumor progression.[5]

This review focuses on the molecules that regulate angiogenesis, the characteristic features of tumor vessels, and the current status of anti-angiogenic cancer therapy.

Angiogenesis and its regulators

Angiogenesis regulators

Angiogenesis is regulated by the balance between angiogenic factors and angiogenesis inhibitors. Many factors are reported to be involved in angiogenesis (Fig. 1). Among them, endothelium-specific factors and their receptors on ECs have been extensively studied. They include vascular endothelial growth factor (VEGF) family members, VEGF receptor-1 (VEGFR-1, also known as Flt-1), VEGF receptor-2 (VEGFR-2, also known as KDR/Flk-1), VEGF receptor-3 (VEGFR-3, also known as Flt-4), neuropilin-1, neuropilin-2, angiopoietins, and tyrosine kinase with Ig and EGF homology domain (TIE) receptors.

VEGFR-1 and VEGFR-2 are expressed on vascular endothelium, whereas VEGFR-3 is preferentially expressed

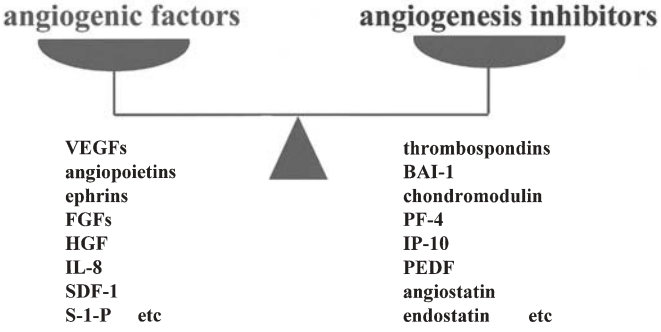

Fig. 1. Balance of angiogenesis regulators. Angiogenesis is regulated by the balance between angiogenic factors and angiogenesis inhibitors. Representative angiogenesis regulators are shown. *VEGF*, vascular endothelial growth factor; *FGF*, fibroblast growth factor; *HGF*, hepatocyte growth factor; *IL-8*, interlenkin-8; *SDF-1*, stromal cell-derived factor 1; *S-1-P*, sphingosine 1-phosphate; *BAI-1*, brain-specific angiogenesis inhititor-1; *PF-4*, platelet factor 4; *IP-10*, interferon-gamma inducible protein 10; *PEDF*, pigment epithelium-derived factor

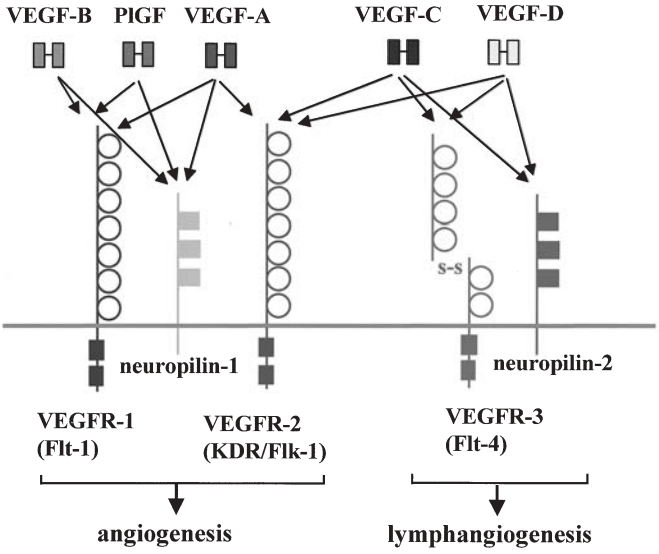

Fig. 2. VEGF family members and their cognate receptors. VEGF family members are homodimer proteins. VEGF receptor-1 (*VEGFR-1*; Flt-1), *VEGFR-2* (KDR/Flk-1), and *VEGFR-3* (Flt-4) are signal transducing receptors, and neuropilin-1 and neuropilin-2 are cell surface binding sites. VEGF (or VEGF-A) binds to VEGFR-1 and VEGFR-2; placenta growth factor (PlGF) and VEGF-B bind to VEGFR-1; and VEGF-C and VEGF-D bind to VEGFR-2 and VEGFR-3. VEGFR-1, VEGFR-2, and neuropilin-1 are important for angiogenesis, whereas VEGFR-3 and neuropilin-2 are important for lymphangiogenesis

on lymphatic endothelium in the adult. Neuropilins, originally identified as regulators of axon guidance, are expressed on ECs and act as cofactors of VEGF receptors. Targeted gene inactivation has revealed that neuropilin-1 is important for blood vessel formation,[6] whereas neuropilin-2 is important for lymphatic vessel formation in the embryo.[7] VEGF (or VEGF-A) binds to VEGFR-1 and VEGFR-2; placenta growth factor (PlGF) and VEGF-B bind to VEGFR-1; and VEGF-C and VEGF-D bind to VEGFR-2 and VEGFR-3 (Fig. 2). Among the VEGF family members, VEGF is the most important factor for angiogenesis, stimulating protease synthesis in and migration and proliferation of ECs; most of the VEGF-mediated signals are transduced via VEGFR-2.[8]

Angiopoietins are ligands of the TIE-2 receptor and are classified into two subgroups. Angiopoietin-1 (Ang-1) and angiopoietin-4 (Ang-4), comprising one subgroup, are agonistic ligands of the TIE-2 receptor, whereas angiopoietin-2 (Ang-2) and angiopoietin-3 (Ang-3), in the other subgroup, are very weak and act as antagonistic ligands of this receptor (Fig. 3).[9–11] No ligand of the TIE-1 receptor has yet been characterized.

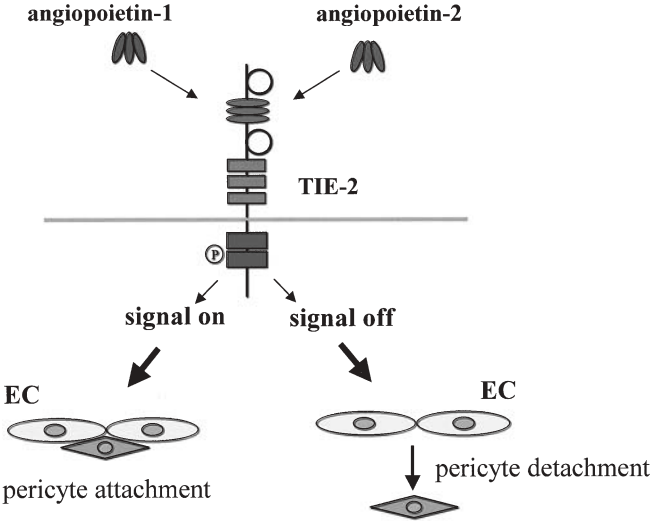

Fig. 3. Angiopoietins and tyrosine kinase with Ig and EGF homology domain (*TIE-2*) receptor. Angiopoietins are ligands of the TIE-2 receptor and are classified into two subgroups. Angiopoietin-1 is an agonistic ligand of TIE-2 and its action results in the attachment of pericytes to the vascular wall, whereas angiopoietin-2 is an antagonistic ligand of TIE-2 and results in the detachment of pericytes from the vascular wall. *EC*, endothelial cell

Process of angiogenesis

Angiogenesis normally includes six sequential steps: detachment of mural pericytes for vascular destabilization, extracellular matrix degradation by endothelial proteases, migration of ECs, proliferation of ECs, tube formation by ECs, and reattachment of pericytes for vascular stabilization (Fig. 4).[12] Hypoxia is known to be one of the most important triggers of angiogenesis, and induces the expression of VEGF in various cell types. VEGF acts on ECs and induces Ang-2.[13,14] Hypoxia also induces Ang-2 in various cell types, including ECs.[13] Ang-2 is an antagonistic ligand of TIE-2, and its action results in the detachment of pericytes from the vascular wall and vessel destabilization. Consecutively, VEGF stimulates ECs in destabilized vessels to form neovessels by promoting protease synthesis for ECM degradation, and by promoting EC migration and proliferation. Ang-2 does not stimulate angiogenesis, but enhances these angiogenic activities of VEGF when combined with VEGF. ECs of neovessels produce platelet-derived growth factor (PDGF), which attracts pericytes to these neovessels. Pericytes produce Ang-1.[15] When Ang-1

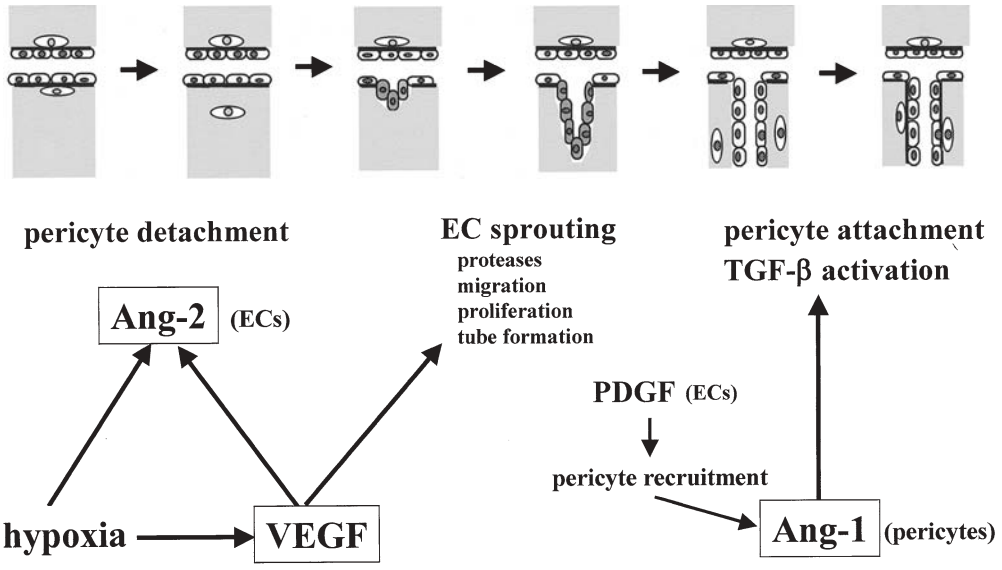

Fig. 4. Process of angiogenesis. Angiogenesis includes the following sequential steps: (i) detachment of preexisting pericytes for vascular destabilization, (ii) extracellular matrix (ECM) degradation by endothelial proteases, (iii) migration of ECs, (iv) proliferation of ECs, (v) tube formation by ECs, and (vi) attachment of pericytes for vascular restabilization/maturation. The balance of various factors, including VEGF, angiopoietin-1 (*Ang-1*), Ang-2, platelet-derived growth factor (*PDGF*), and transforming growth factor (*TGF*)-β is important for the regulation of angiogenesis

synthesized from pericytes dominates Ang-2, pericytes attach to and stabilize the neovessels. In addition, Ang-1 can directly stimulate the migration of ECs. When pericytes attach to ECs of neovessels in this manner, latent transforming growth factor-β (TGF-β) is efficiently activated, the action of which causes the maturation of neovessels.[16]

Gene expression in ECs during angiogenesis

During the course of angiogenesis, a number of molecules are expressed in ECs. They include matrix metalloproteinases (MMPs) and plasminogen activators (PAs) for matrix degradation, integrins for cell adhesion and migration, and vascular endothelial (VE)-cadherin for three-dimensional tube formation. The regulated expression of these molecules in ECs reveals that endothelium-specific gene regulation is an important issue for understanding the molecular mechanisms of angiogenesis. Indeed, several transcription factors are expressed in ECs and play an important role in angiogenesis.[17]

My laboratory has focused on one of such transcription factors, namely, E-twenty six (ETS)-1. Representative angiogenic factors such as VEGF, acidic fibroblast growth factor (aFGF), and basic fibroblast growth factor (bFGF) induce the expression of ETS-1 in ECs.[18,19] Hypoxia also induces the expression of ETS-1 via hypoxia inducible factor-1 (HIF-1) activation.[20] The finding that a nonproliferative adenovirus encoding dominant-negative ETS-1 inhibited in vivo angiogenesis indicates that ETS-1 in ECs is required for angiogenesis.[21] Possible downstream targets of ETS-1 for angiogenesis in ECs are MMPs, urokinase-type PA (uPA), integrin β3, VEGFR-2, neuropilin-1, and Ang-2.[18,22–24]

Tumor angiogenesis

Regulators of tumor angiogenesis

Increased synthesis of VEGF in tumor tissue is one of the most important features of tumor angiogenesis. The hypoxic condition is the most important inducer of VEGF, and the microenvironment of solid tumors contains regions of poor oxygenation. Hypoxia-mediated gene expression is regulated by transcription factor HIF-1, which is a heterodimer of an oxygen-regulated HIF-1α subunit and a constitutively expressed HIF-1β subunit. HIF-1α is promptly degraded by the ubiquitin-proteasome pathway under the normoxic condition, but becomes stable under a hypoxic condition.[25] The stability of HIF-1α is regulated by the interaction of this subunit with various proteins, as well as by posttranslational modifications such as hydroxylation. Tumor suppressor *von Hippel Lindau* (*VHL*) gene product (pVHL), which is found in a multiprotein complex with elongins B/C, forming an E3 ubiquitin ligase complex, regulates HIF-1α ubiquitination for degradation.[26,27] Thus, alteration of the *VHL* gene results in an increased amount of HIF-1α and HIF-1-mediated upregulation of the *VEGF* gene.[28] p53 is also involved in the degradation of HIF-1α, and, thus, alteration of the *p53* gene enhances the transcriptional activity of HIF-1.[29] Phosphatase and tensin homolog (PTEN) selectively blocks the PI3-kinase-Akt signaling pathway, and Akt is involved in the stabilization of HIF-1α; thus, alteration of the *PTEN* gene augments HIF-1-mediated VEGF expression in tumor cells.[30]

Decreased expression of endogenous angiogenesis inhibitors is another important feature of tumor angiogenesis. p53 is involved in the expression of angiogenesis inhibitors such as thrombospondin-1[31] and brain-specific angiogenesis inhibitor 1 (BAI-1).[32] Therefore, alteration of the *p53* gene decreases the expression of thrombospondin-1 or BAI-1 and shifts the balance to the pro-angiogenic state. More-

over, a recent report suggests that alteration of the *p53* gene decreases the sensitivity of tumor cells to anti-angiogenesis therapy.[33]

Abnormal architecture of tumor vessels

Unlike normal blood vessels, tumor blood vessels are dilated, tortuous, and leaky, and they contain multiple bifurcations, loops, and deadend sprouts. These abnormal architectural features of tumor vessels may be the consequence of peculiar EC-pericyte interaction. Pericytes are the cells that control the migration and proliferation of ECs, as well as the maturation and permeability of small vessels. Pericytes attach firmly to ECs in normal vessels but not in tumor vessels. Morikawa et al.[34] showed that pericytes associate with ECs but have multiple processes that extend away from the vessel wall in the tumor.

Although the primary cause of these abnormalities is not well characterized, recent data suggest that an imbalance of angiopoietins may be a factor. Ang-2, which antagonizes Ang-1 and dissociates pericytes from vessels, is upregulated in tumor cells, as well as in ECs of tumor vessels.[35,36] In this situation, the absence of VEGF causes the apoptosis of ECs and vascular regression, whereas the presence of VEGF accelerates angiogenesis. Also of interest, the gene transfer of *Ang-1* in tumor cells enhanced the coverage of vessels with pericytes and inhibited tumor growth.[37,38] Thus, the correction of angiopoietin imbalance in tumors can be an additional option for anti-angiogenic cancer treatment.

Tumor vessel markers for anti-vascular therapy

Destruction of the tumor vasculature or anti-vascular therapy will provide an additional approach for cancer therapy. Several markers have been described for the identification of tumor vessels for the targeting. They include EN 7/44, endosialin, and endoglin (CD105).[39–41] Endoglin, a plasma membrane glycoprotein, is emerging as a powerful marker to quantify tumor angiogenesis. Endoglin is an accessory component of the TGF-β receptor complex. In normal human tissues, endoglin is weakly expressed in erythroid precursors, stromal cells, and monocytes, whereas it is highly expressed in proliferating ECs. In various human tumors, endoglin is mainly present on ECs of tumor vessels, but is weakly expressed or absent on neoplastic cells. In animal models, administration of radiolabeled anti-endoglin monoclonal antibodies efficiently images primary tumors, and naked or conjugated anti-endoglin monoclonal antibody suppresses angiogenesis and tumor growth.[42,43]

Tumor vessels may show an aberrant gene expression profile. St. Croix et al.[44] compared the gene expression profiles of ECs derived from blood vessels of normal and malignant colorectal tissues by using the strategy of serial analysis of gene expression (SAGE). Of over 170 transcripts predominantly expressed in the endothelium, 79 were differentially expressed, including 46 that were specifically elevated in tumor-associated endothelium. They designated those 46 transcripts as tumor endothelial markers (TEMs). Most of these TEMs were expressed in a wide range of tumor types, as well as in normal vessels associated with physiological angiogenesis in wound healing and corpus luteum formation. Among these TEMs, TEM1, TEM5, TEM7, and TEM8 contain putative transmembrane domains and are associated with the cell surface membrane.[45] Examination of mouse counterparts of TEMs in mouse tumors, embryos, and adult tissues demonstrated that mTEM1, mTEM5, and mTEM8 were abundantly expressed in tumor vessels as well as in the vasculature of the developing embryo. These studies suggest that TEM1, TEM5, and TEM8 on tumor endothelium are attractive targets for the development of anti-vascular therapies.

Lymphangiogenesis and lymph node metastasis

Tumors metastasize through blood vessels and lymphatic vessels. Clinicopathological data suggest that the lymphatic vessels are the initial route for the spread of tumors. Indeed, the detection of sentinel lymph nodes provides significant information for staging and for designing therapeutic regimens. Lymphangiogenesis—the growth of new lymphatic vessels—has long been regarded as a putative pathway to lymph node metastasis. However, the molecular mechanism of lymphangiogenesis has been unclear. The recent characterization of VEGF-C and VEGF-D, and their cognate receptor VEGFR-3 on lymphatic endothelial cells (LECs), as well as the development of reliable LEC-specific markers such as lymphatic vessel endothelium HA receptor 1 (LYVE-1), have changed this situation.[46] Studies in animal models have shown that VEGF-C or VEGF-D and VEGFR-3 are critical regulators of lymphangiogenesis and lymph node metastasis of tumors. Thus, VEGF-C or VEGF-D and VEGFR-3 can be considered targets for the treatment of lymph node metastasis of various tumors.[47–49]

Anti-angiogenic therapy

Anti-angiogenic therapy offers several potential advantages as an approach to cancer treatment; notably, the physical accessibility and genetic stability of ECs. Two categories used in the development of anti-angiogenic therapy involve the inhibition of angiogenic factors and the application of endogenous angiogenesis inhibitors. Although preclinical trials of anti-angiogenic therapy have been very successful, the initial clinical data have not been satisfying. This has led to the reassessment of anti-angiogenic therapy for cancer. Increased understanding of the process of angiogenesis, the diversity of its regulators, appropriate drug schedules, and the use of anti-angiogenic agents with other modalities may lead to radically new treatment regimens for many of these conditions.

Table 1 summarizes representative anti-angiogenic agents in the National Cancer Institute (NCI) database of recent clinical trials. Among them, agents that block the VEGF-mediated signals are thought to be most promising.

Table 1. Anti-angiogenic agents used in clinical trials in the NCI clinical trials database

Drugs that block matrix breakdown
BMS-275291 (MMP inhibitor)
Dalteparin (Fragmin, Pharmacia and Upjohn) (low-molecular-weight heparin)
Suramin
Drugs that inhibit endothelial cells directly
2-Methoxyestradiol
LY317615 (PKC-β inhibitor)
Soy isoflavone (soy protein isolate)
Thalidomide
CC-5013 (thalidomide analog)
Drugs that block activators of angiogenesis
Anti-VEGF antibody (bevacizumab)
VEGF-Trap (soluble VEGFR-1)
ZD6474 (VEGFR tyrosine kinase inhibitor)
Neovastat (product exracted from cartilage)
Interferon-α
Drugs that inhibit endothelial integrin
EMD 121974 (inhibitor of αvβ3 and αvβ5)
Medi-522 (Vitaxin, MedImmunl) (anti-αvβ3 antibody)
Drugs with nonspecific mechanism of action
Carboxyamidotriazole (CAI)
Celecoxib (Celebrex, Pharmacia Canada) (COX-2 inhibitor)
Rofecoxib (Vioxx, Merck) (COX-2 inhibitor)
Halofuginone hydrobromide (a collagen type I inhibitor)
Interleukin-12

NCI, National Cancer Institute; MMP, matrix metalloproteinase; PKC, protein kinase C; VEGF, vascular endothelial growth factor; VEGFR-1, VEGF receptor-1; COX-2, cyclooxygenase-2

Blockade of VEGF-mediated signals

Insights into the biology of tumor angiogenesis have led to the identification of various molecules that are important for the progression of angiogenesis. Of particular interest is the VEGF family, because VEGF is expressed in almost all tumors. Several different strategies have been used to inhibit VEGF-mediated signals, including anti-VEGF antibody, agents that inhibit the VEGF receptor tyrosine kinase, soluble VEGFR-1 that traps VEGF, and so forth. The most promising agents appear to be the monoclonal anti-VEGF antibodies and the receptor tyrosine kinase (RTK) inhibitors, as these have demonstrated broadspectrum antitumor activity in vivo and single-agent activity in early-phase clinical trials in patients with advanced pretreated breast and colorectal carcinoma and Kaposi's sarcoma.

Anti-VEGF antibody

The recent success of anticancer antibodies such as rituximab (anti-CD20 monoclonal antibody) and trastuzumab (anti-HER2/Neu antibody) has created great interest in antibody-based therapeutics for malignant hematopoietic neoplasms and solid tumors. Bevacizumab, a recombinant humanized monoclonal anti-VEGF antibody, is currently being tested clinically against a number of tumor types, including nonsmall-cell lung cancer, prostate cancer, renal cell cancer, and colorectal cancer.[50] The efficacy of bevacizumab on metastatis colorectal cancer in phase III trial was shown in the meeting of American Society of Clinical Oncology 2003.[51]

VEGFR tyrosine kinase inhibitors

In recent years, a number of promising new low-molecular-weight anticancer drugs targeting intracellular pathways or extracellular molecules have been developed. Such compounds are tyrosine kinase inhibitors, and include imatinib (Gleevec, Novartis Pharmaceuticals), which inhibits the Breakpoint cluster region-Abelson (BCR-ABL) tyrosine kinase, and gefitinib (Iressa, AstraZeneca), which inhibits the EGF-receptor tyrosine kinase. In the past few years, chemists have expanded the kinase selectivity profile and identified novel classes of VEGFR inhibitors. SU-5416 is the prototype of a selective tyrosine kinase inhibitor of VEGFR-2.[52] PTK787/ZK222584 is also a selective tyrosine kinase inhibitor of VEGFR-2[53], whereas SU6668 and ZD6474 are nonselective multitarget tyrosine kinase inhibitors.[54,55]

Soluble VEGFR-1

Previous studies have revealed that one of the most effective ways to block the VEGF signaling pathway is to prevent VEGF from binding to its receptors by the application of decoy-type soluble VEGFR. VEGFR-1 exhibits about ten times higher affinity than VEGFR-2 for its ligand, VEGF. VEGF-Trap was created by fusing the first three Ig domains of VEGFR-1 to the Fc region of human IgG. A preclinical study revealed that VEGF-Trap effectively suppressed tumor growth and neovascularization in vivo, resulting in stunted and almost completely avascular tumors.[56]

Neovastat

Neovastat is a cartilage-derived naturally occurring product that has anti-angiogenic activities.[57] Neovastat inhibits several steps of angiogenesis, including VEGF signaling pathways and MMP activities. Moreover, neovastat induces tissue-type plasminogen activator activity and EC apoptosis. Therefore, it is a multifunctional anti-angiogenic agent. Results from phase I/II clinical trials indicate that neovastat, given orally, is well tolerated. It has reached phase III clinical trial evaluation for the treatment of solid tumors, including nonsmall-cell lung cancer and renal cell carcinoma.[58] The median survival time of patients with renal cell carcinoma and nonsmall-cell lung cancer was significantly longer in patients receiving high doses of neovastat than in those receiving low doses.

Concluding remarks

Anti-angiogenesis is regarded as a promising strategy for cancer treatment. Anti-angiogenic agents may be

valuable for longterm administration to maintain tumor dormancy, because the development of drug resistance is rare, and these agents should have a sustained effect on ECs.

In order to achieve the benefit of anti-angiogenic agents for cancer treatment, strategies that enhance the anti-angiogenic activity need to be considered. Several anti-angiogenic agents might be used in combination for the complete inhibition of angiogenesis. Anti-angiogenic treatment is likely to attain an important role in the adjuvant setting, and thus may be useful after surgical resection of a primary tumor. Also, anti-angiogenic agents may be used either sequentially or concurrently with chemotherapy or radiotherapy. The report of the efficacy of anti-VEGFAb is a such case. Anti-angiogenic treatment may prolong the time to progression, the symptom-free interval, and the overall survival of cancer patients. In any case, careful setting and assessment of clinical trials is most warranted.

References

1. Folkman J (1971) Tumor angiogenesis: therapeutic implications. N Engl J Med 285:1182–1186
2. Risau W (1997) Mechanisms of angiogenesis. Nature 386:671–674
3. Lyden D, Hattori K, Dias S, et al. (2001) Impaired recruitment of bone-marrow-derived endothelial and hematopoietic precursor cells blocks tumor angiogenesis and growth. Nat Med 7:1194–1201
4. Hill JM, Zalos G, Halcox JP, et al. (2003) Circulating endothelial progenitor cells, vascular function, and cardiovascular risk. N Engl J Med 348:593–600
5. Hanahan D, Folkman J (1996) Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. Cell 86:353–364
6. Kawasaki T, Kitsukawa T, Bekku Y, et al. (1999) A requirement for neuropilin-1 in embryonic vessel formation. Development 126:4895–4902
7. Yuan L, Moyon D, Pardanaud L, et al. (2002) Abnormal lymphatic vessel development in neuropilin 2 mutant mice. Development 129:4797–4806
8. Shibuya M (2001) Structure and function of VEGF/VEGF-receptor system involved in angiogenesis. Cell Struct Funct 26:25–35
9. Davis S, Aldrich TH, Jones PF, et al. (1996) Isolation of angiopoietin-1, a ligand for the TIE2 receptor, by secretion-trap expression cloning. Cell 87:1161–1169
10. Maisonpierre PC, Suri C, Jones PF, et al. (1997) Angiopoietin-2, a natural antagonist for Tie2 that disrupts in vivo angiogenesis. Science 277:55–60
11. Valenzuela DM, Griffiths JA, Rojas J, et al. (1999) Angiopoietins 3 and 4: diverging gene counterparts in mice and humans. Proc Natl Acad Sci USA 96:1904–1909
12. Hanahan D (1997) Signaling vascular morphogenesis and maintenance. Science 277:48–50
13. Mandriota SJ, Pepper MS (1998) Regulation of angiopoietin-2 mRNA levels in bovine microvascular endothelial cells by cytokines and hypoxia. Circ Res 83:852–859
14. Abe M, Sato Y (2001) cDNA microarray analysis of the gene expression profile of VEGF-activated human umbilical vein endothelial cells. Angiogenesis 4:289–298
15. Sundberg C, Kowanetz M, Brown LF, et al. (2002) Stable expression of angiopoietin-1 and other markers by cultured pericytes: phenotypic similarities to a subpopulation of cells in maturing vessels during later stages of angiogenesis in vivo. Lab Invest 82:387–401
16. Folkman J, D'Amore PA (1996) Blood vessel formation: what is its molecular basis? Cell 87:1153–1155
17. Sato Y (2000) Molecular mechanism of angiogenesis transcription factors and their therapeutic relevance. Pharmacol Ther 87:51–60
18. Iwasaka C, Tanaka K, Abe M, et al. (1996) Ets-1 regulates angiogenesis by inducing the expression of urokinase-type plasminogen activator and matrix metalloproteinase-1 and the migration of vascular endothelial cells. J Cell Physiol 169:522–531
19. Kanno S, Oda N, Abe M, et al. (2000) Roles of two VEGF receptors, Flt-1 and KDR, in the signal transduction of VEGF effects in human vascular endothelial cells. Oncogene 19:2138–2146
20. Oikawa M, Abe M, Kurosawa H, et al. (2001) Hypoxia induces transcription factor ETS-1 via the activity of hypoxia-inducible factor-1. Biochem Biophys Res Commun 289:39–43
21. Nakano T, Abe M, Tanaka K, et al. (2000) Angiogenesis inhibition by transdominant mutant Ets-1. J Cell Physiol 184:255–262
22. Oda N, Abe M, Sato Y (1999) ETS-1 converts endothelial cells to the angiogenic phenotype by inducing the expression of matrix metalloproteinases and integrin β3. J Cell Physiol 178:121–132
23. Teruyama K, Abe M, Nakano T, et al. (2001) Neurophilin-1 is a downstream target of transcription factor Ets-1 in human umbilical vein endothelial cells. FEBS Lett 504:1–4
24. Sato Y (2003) The ETS family of transcription factors. In: Hoying JB (ed) Genetics of angiogenesis. BIOS Scientific, Oxford, pp 55–68
25. Huang LE, Gu J, Schau M, et al. (1998) Regulation of hypoxia-inducible factor 1alpha is mediated by an O2-dependent degradation domain via the ubiquitin-proteasome pathway. Proc Natl Acad Sci USA 95:7987–7992
26. Masson N, Willam C, Maxwell PH, et al. (2001) Independent function of two destruction domains in hypoxia-inducible factor-α chains activated by prolyl hydroxylation. EMBO J 20:5197–5206
27. Hon WC, Wilson MI, Harlos K, et al. (2002) Structural basis for the recognition of hydroxyproline in HIF-1α by pVHL. Nature 417:975–978
28. Maxwell PH, Wiesener MS, Chang GW, et al. (1999) The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. Nature 399:271–275
29. Ravi R, Mookerjee B, Bhujwalla ZM, et al. (2000) Regulation of tumor angiogenesis by p53-induced degradation of hypoxia-inducible factor 1α. Genes Dev 14:34–44
30. Zundel W, Schindler C, Haas-Kogan D, et al. (2000) Loss of PTEN facilitates HIF-1-mediated gene expression. Genes Dev 14:391–396
31. Dameron KM, Volpert OV, Tainsky MA, et al. (1994) Control of angiogenesis in fibroblasts by p53 regulation of thrombospondin-1. Science 265:1582–1584
32. Nishimori H, Shiratsuchi T, Urano T, et al. (1997) A novel brain-specific p53-target gene, BAI1, containing thrombospondin type 1 repeats inhibits experimental angiogenesis. Oncogene 15:2145–2150
33. Yu JL, Rak JW, Coomber BL, et al. (2002) Effect of p53 status on tumor response to antiangiogenic therapy. Science 295:1526–1528
34. Morikawa S, Baluk P, Kaidoh T, et al. (2002) Abnormalities in pericytes on blood vessels and endothelial sprouts in tumors. Am J Pathol 160:985–1000
35. Tanaka S, Mori M, Sakamoto Y, et al. (1999) Biologic significance of angiopoietin-2 expression in human hepatocellular carcinoma. J Clin Invest 103:341–345
36. Holash J, Maisonpierre PC, Compton D, et al. (1999) Vessel cooption, regression, and growth in tumors mediated by angiopoietins and VEGF. Science 284:1994–1998
37. Ahmad SA, Liu W, Jung YD, et al. (2001) The effects of angiopoietin-1 and -2 on tumor growth and angiogenesis in human colon cancer. Cancer Res 61:1255–1259
38. Hawighorst T, Skobe M, Streit M, et al. (2002) Activation of the tie2 receptor by angiopoietin-1 enhances tumor vessel maturation and impairs squamous cell carcinoma growth. Am J Pathol 160:1381–1392
39. Hagemeier HH, Vollmer E, Goerdt S, et al. (1986) A monoclonal antibody reacting with endothelial cells of budding vessels in tumors and inflammatory tissues, and non-reactive with normal adult tissues. Int J Cancer 38:481–488
40. Rettig WJ, Garin-Chesa P, Healey JH, et al. (1992) Identification of endosialin, a cell surface glycoprotein of vascular endothelial cells in human cancer. Proc Natl Acad Sci USA 89:10832–10836

41. Burrows FJ, Derbyshire EJ, Tazzari PL, et al. (1995) Up-regulation of endoglin on vascular endothelial cells in human solid tumors: implications for diagnosis and therapy. Clin Cancer Res 1:1623–1634
42. Bredow S, Lewin M, Hofmann B, et al. (2000) Imaging of tumour neovasculature by targeting the TGF-β binding receptor endoglin. Eur J Cancer 36:675–681
43. Fonsatti E, Jekunen AP, Kairemo KJ, et al. (2000) Endoglin is a suitable target for efficient imaging of solid tumors: in vivo evidence in a canine mammary carcinoma model. Clin Cancer Res 6:2037–2043
44. St Croix B, Rago C, Velculescu V, et al. (2000) Genes expressed in human tumor endothelium. Science 289:1197–1202
45. Carson-Walter EB, Watkins DN, Nanda A, et al. (2001) Cell surface tumor endothelial markers are conserved in mice and humans. Cancer Res 61:6649–6655
46. Alitalo K, Carmeliet P (2002) Molecular mechanisms of lymphangiogenesis in health and disease. Cancer Cell 1:219–227
47. Mandriota SJ, Jussila L, Jeltsch M, et al. (2001) Vascular endothelial growth factor-C-mediated lymphangiogenesis promotes tumour metastasis. EMBO J 20:672–862
48. Skobe M, Hawighorst T, Jackson DG, et al. (2001) Induction of tumor lymphangiogenesis by VEGF-C promotes breast cancer metastasis. Nat Med 7:192–198
49. He Y, Kozaki K, Karpanen T, et al. (2002) Suppression of tumor lymphangiogenesis and lymph node metastasis by blocking vascular endothelial growth factor receptor 3 signaling. J Natl Cancer Inst 94:819–825
50. Chen HX, Gore-Langton RE, Cheson BD (2001) Clinical trials referral resource: current clinical trials of the anti-VEGF monoclonal antibody bevacizumab. Oncology (Huntingt) 15:1017, 1020, 1023–1026
51. Hurwitz H, Fehrenbacher L, Cartwright T, et al. (2003) Bevacizumab (Avastin, a monoclonal antibody to vascular endothelial growth factor) prolongs survival in first-line colorectal cancer (CRC): Results of a phase III trial of bevacizumab in combination with bolus IFL (irinotecan, 5-fluorouracil, leucovorin) as first-line therapy in subjects with metastatic CRC. ASCO abstract no. 3646
52. Mendel DB, Laird AD, Smolich BD, et al. (2000) Development of SU5416, a selective small molecule inhibitor of VEGF receptor tyrosine kinase activity, as an anti-angiogenesis agent. Anticancer Drug Des 15:29–41
53. Wood JM, Bold G, Buchdunger E, et al. (2000) PTK787/ZK 222584, a novel and potent inhibitor of vascular endothelial growth factor receptor tyrosine kinases, impairs vascular endothelial growth factor-induced responses and tumor growth after oral administration. Cancer Res 60:2178–2189
54. Laird AD, Vajkoczy P, Shawver LK, et al. (2000) SU6668 is a potent antiangiogenic and antitumor agent that induces regression of established tumors. Cancer Res 60:4152–4160
55. Wedge SR, Ogilvie DJ, Dukes M, et al. (2002) ZD6474 inhibits vascular endothelial growth factor signaling, angiogenesis, and tumor growth following oral administration. Cancer Res 62:4645–4655
56. Holash J, Davis S, Papadopoulos N, et al. (2002) VEGF-Trap: a VEGF blocker with potent antitumor effects. Proc Natl Acad Sci USA 99:11393–11398
57. Gingras D, Batist G, Beliveau R (2001) AE-941 (Neovastat): a novel multifunctional antiangiogenic compound. Expert Rev Anticancer Ther 1:341–347
58. Falardeau P, Champagne P, Poyet P, et al. (2001) Neovastat, a naturally occurring multifunctional antiangiogenic drug, in phase III clinical trials. Semin Oncol 28:620–625