Current Topics

Target Therapy for Cancer: Anti-cancer Drugs Targeting Growth-Factor Signaling Molecules

Vascular Endothelial Growth Factor (VEGF), VEGF Receptors and Their Inhibitors for Antiangiogenic Tumor Therapy

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Vascular endothelial growth factor (VEGF) and its receptors (VEGFRs) have crucial roles in both physiological and pathological angiogenesis. The VEGF family consists of VEGF-A (generally called VEGF), VEGF-B, VEGF-C, VEGF-D, and placental growth factor (PlGF). These peptides show different affinities for VEGFR subtypes. VEGFR exists as three subtypes, VEGFR-1, VEGFR-2, and VEGFR-3, and is structurally related to platelet-derived growth factor receptors. All subtypes possess seven immunoglobulin-like domains in the extracellular region and a tyrosine kinase domain in the intracellular region. VEGF-A activates VEGFR-1 and VEGFR-2, whereas VEGF-B and PlGF bind to only VEGFR-1. VEGF-C and VEGF-D only bind to VEGFR-3. VEGFR-1 (fms-like tyrosine kinase-1, Flt-1) negatively regulates embryonic vasculogenesis and is involved in tumor angiogenesis *via* activation of monocytes and macrophages. VEGFR-2 (KDR in humans or Flk-1 in mice) is predominantly responsible for both embryonic vasculogenesis and tumor angiogenesis. In contrast, VEGFR-3 (Flt-4) regulates lymphangiogenesis. Consequently, VEGF-A and VEGFR-2 are currently the main targets for antiangiogenic therapy. Bevacizumab is a humanized monoclonal antibody against VEGF-A, and aflibercept (VEGF-Trap) is a soluble fusion protein of the extracellular domain of VEGFR-1 and VEGFR-2 and the Fc region of immunoglobulin G (IgG). They neutralize VEGF-A, resulting in prevention of tumor angiogenesis. VEGFR tyrosine kinase inhibitors such as sunitinib and sorafenib are also effective in antiangiogenic tumor therapy by inhibiting VEGFR signaling. Anti-VEGF drugs are a promising therapy for cancer patients.

Key words vascular endothelial growth factor; angiogenesis; tumor

1. INTRODUCTION

A network of blood vessels is essential for the development and maintenance of all tissues in the human body. The blood vessels provide oxygen and nutrition to tissues, remove carbon dioxide and metabolites from them, and deliver signaling molecules such as hormones and autacoids. Vascular endothelial growth factor (VEGF) plays a crucial role in building up the circulatory system under physiological conditions.^{1,2)} Furthermore, angiogenesis is a requisite for the growth and persistence of solid tumors and their metastases, and severe inflammatory diseases progress to a malignant stage associated with angiogenesis.^{3–5)} VEGF serves as a major stimulator of pathological angiogenesis.⁵⁾ Thus, VEGF is a focus of interest with respect to vascular research and oncology. The following section describes VEGF, VEGF receptors (VEGFRs), and their inhibitors for antiangiogenic tumor therapy.

2. VEGF, VEGFR AND THEIR INHIBITORS

The VEGF family is divided into five members having a homodimer structure: VEGF-A, VEGF-B, VEGF-C, VEGF-D, and placental growth factor (PlGF). These peptides are encoded by individual genes. In addition, VEGF-A exists in four isoforms. VEGF₁₂₁, VEGF₁₆₅, VEGF₁₈₉, and

VEGF₂₀₆ are generated by alternative mRNA splicing.^{6,7)} VEGF-A is generally called VEGF, because VEGF-A is a key regulator of developmental vasculogenesis, angiogenesis, and differentiation of progenitor endothelial cells. Among VEGF-A isoforms, VEGF₁₆₅ is dominant from the aspect of amount and biological activity. VEGF₁₆₅ is overexpressed in a variety of human tumors, and the overexpression is correlated with progression, invasion, and metastasis of tumors.^{8–10)} Gene targeting studies revealed the importance of VEGF-A in developmental vasculogenesis. A defect of even a single VEGF-A allele results in embryonic lethality.^{11,12)} VEGF-B and PlGF also stimulate angiogenesis in normal tissues, but their activities are much weaker than that of VEGF-A.¹³⁾ However, an increase in PlGF promotes pathological angiogenesis in tumors and inflammatory lesions.^{14,15)} VEGF-C and VEGF-D are generated from their precursors by proteolytic cleavage.¹⁶⁾ The lack of lymph vessels results in prenatal death due to fluid accumulation in tissues in both homozygotes and heterozygotes of VEGF-C gene knockout mice, indicating that VEGF-C plays an important role in embryonic lymphangiogenesis.¹⁷⁾

These VEGF members interact with cell membrane receptors. VEGFRs consist of three subtypes, VEGFR-1, VEGFR-2, and VEGFR-3, and are structurally related to platelet-derived growth factor receptors. All subtypes possess seven immunoglobulin-like domains in the extracellular region and a

Fig. 1. VEGF Members and Their Receptors

tyrosine kinase domain in the intracellular region.^{18–22)} VEGFR-1 and VEGFR-2 are expressed in vascular endothelial cells and hematopoietic stem cells. Some tumor cells express VEGFR-1 and VEGFR-2. VEGFR-1 is also expressed in monocytes and macrophages. In contrast, the expression of VEGFR-3 is largely restricted to lymphatic endothelial cells. These VEGF members have different affinities for one of three VEGFR subtypes (Fig. 1). VEGF-A activates VEGFR-1 and VEGFR-2, whereas VEGF-B and PlGF bind only to VEGFR-1. VEGF-C and VEGF-D bind only to VEGFR-3. Furthermore, heparin and neuropilin-1 are able to bind VEGF-A165 and are involved in VEGF-A-induced activation of VEGFRs.^{21–23)}

VEGFR-1 is otherwise known as fms-like tyrosine kinase-1 (Flt-1). Tyrosine kinase is poorly activated in response to VEGF-A, although VEGFR-1 binds VEGF-A with about 10-fold higher affinity than VEGFR-2. It was reported that VEGFR-1 mediates proliferation of endothelial cells,²⁴⁾ migration of monocytes and macrophages,^{25,26)} and recruitment of bone marrow-derived endothelial and hematopoietic precursor cells.²⁷⁾ Surprisingly, a study in VEGFR-1 knockout mice demonstrated that VEGFR-1 negatively regulates physiological vasculogenesis during embryogenesis.²⁸⁾ Overgrowth of impaired blood vessels results in the death of the knockout mice. Mice expressing VEGFR-1 lacking the tyrosine kinase domain possess normal vasculature, indicating that VEGFR-1 is weakly involved in mediating the physiological angiogenic response to VEGF.²⁹⁾ In contrast, VEGFR-1 signaling has been implicated in tumor growth and metastasis. Stimulation of VEGFR-1 indirectly induces tumor angiogenesis *via* activation of monocytes and macrophages. These cells migrate into tumor and inflammatory lesions to produce VEGF-A, VEGF-C, and cytokines, leading to tumor angiogenesis and lymphoangiogenesis *via* VEGFR-2 and VEGFR-3.^{30,31)} In addition, matrix metalloproteinase-9 is induced *via* VEGFR-1 in endothelial cells and macrophages, contributing to lung metastasis.³²⁾

VEGFR-2 is otherwise termed KDR in humans or Flk-1 in mice and has more potent tyrosine kinase activity than VEGFR-1 despite a lower affinity for VEGF-A. A growing body of evidence demonstrates that VEGFR-2 is predominantly responsible for responses of vascular endothelial cells to VEGF under both physiological and pathological conditions. Stimulation of VEGFR-2 promotes growth, migration, and tubular formation of endothelial cells and enhances vascular permeability. Failure to form blood vessels results in embryonic lethality in flk-1-deficient mice, indicating that VEGFR-2 plays an essential role in establishing circulatory systems during this stage of development.³³⁾ Anti-VEGFR-2 antibody inhibits primary and metastatic tumor growth in mouse models, indicating the crucial role of VEGFR-2 in tumor angiogenesis.³⁴⁾

VEGFR-3 (Flt-4) is expressed predominantly in lymphatic endothelial cells and regulates lymphangiogenesis in response to VEGF-C and VEGF-D.^{35,36)} In addition, the ligand-binding domain alone of VEGFR-1 exists as soluble VEGFR-1 (sFlt-1).^{37,38)} The mRNA encoding the ligand-binding domain of VEGFR-1 is transcribed as well as full-length VEGFR-1 mRNA. sFlt-1 exerts an antiangiogenic effect by neutralizing VEGF.

VEGFR stimulation with VEGF or PlGF causes receptor dimerization, leading to activation of intrinsic tyrosine kinase. The signals generated from different VEGFRs are various, despite the high homology within the tyrosine kinase domain. The tyrosine kinase domains of VEGFR-1 and VEGFR-2 are split by an insertion of a 65–97 hydrophobic region. This insert functions as an important recognition site for kinase substrates.³⁹⁾ The complex signaling from VEGFRs uses multiple factors to determine the biological responses to VEGF. The factors form a network of pathways with various crosstalk and overlapping functions, as well as distinct functions. It has been difficult to link endogenous VEGFR activation to the biological responses in endothelial and other cells. VEGFR-1 is poorly autophosphorylated by

VEGF-A in endothelial cells. It was suggested that phospholipase C- γ , phosphatidylinositol 3-kinase, Src homology phosphatase-2, and growth factor-bound protein 2 (Grb2) are potential interacting proteins with VEGFR-1.⁴⁰⁾ In contrast, VEGFR-2 signaling is relatively well understood. VEGFR-2 is more efficiently autophosphorylated than VEGFR-1, and Tyr1175 and Tyr1214 residues are identified as a major autophosphorylation site in VEGFR-2.^{40,41)} Phosphorylation at Tyr1175 is crucial to initiate phospholipase C- γ activation as well as to transduce mitogen-activated protein kinase-mediated signals for DNA synthesis in endothelial cells. Furthermore, Tyr1175 is a binding site for other adaptor proteins such as Shb, which is responsible for the activation of phosphatidylinositol 3-kinase and the subsequent cell migration.⁴²⁾ Phosphorylation of Tyr1214 is involved in the activation of Cdc42 and stress-activated protein kinase p38, which may regulate endothelial cell motility.⁴³⁾

Since Folkman³⁾ proposed that antiangiogenesis would be a novel antitumor strategy, angiogenesis has become an attractive drug target.^{44,45)} Especially, VEGF-A and VEGFR-2 are currently the main targets for antiangiogenic therapy (Fig. 1). Bevacizumab (Avastin) is a humanized monoclonal antibody against VEGF-A, and binding of the antibody to VEGF-A results in the prevention of VEGFR activation and the subsequent signaling cascades. Targeting VEGF-A by anti-VEGF-A antibody inhibited the growth of transplantable human tumors in mice, leading to the clinical development of bevacizumab.⁴⁶⁾ Bevacizumab significantly improved survival rates in colorectal cancer patients, and then was approved by the U.S. Food and Drug Administration (FDA) for the treatment for colorectal cancer in combination with chemotherapy.⁴⁷⁾ Bevacizumab was also approved by the Health, Labor and Welfare Ministry of Japan and has been clinically used in the therapy for colorectal cancer. Synthetic compounds to inhibit VEGFR tyrosine kinase activity are effective against VEGF-induced angiogenesis and tumor growth. Sunitinib and sorafenib have been approved by the FDA and Health, Labor and Welfare Ministry of Japan. Sunitinib (SU11248) is an orally available drug that inhibits several tyrosine kinases including VEGFR, platelet-derived growth factor receptor and c-kit.^{48,49)} Sunitinib is used for the treatment of renal carcinoma and gastrointestinal stromal tumors. Sorafenib (BAY 43-9006) is also an inhibitor of VEGFR-2, VEGFR-3, Raf kinase, and extracellular signal-regulated kinases^{48,50)} and used in the treatment of renal carcinoma. In addition, vatalanib (PTK787, ZK222584) suppresses the kinase activities of VEGFR, platelet-derived growth factor receptor and c-kit, and is under clinical investigation for the treatment of metastatic colorectal cancer.^{48,51)} Aflibercept (AVE0005) is a soluble fusion protein of the extracellular domain of human VEGFR-1 and VEGFR-2 and the Fc region of IgG, and is also called VEGF-Trap.⁵²⁾ Aflibercept prevents VEGFR activation and downstream signaling because it efficiently binds VEGF-A and PlGF with high affinity. Aflibercept is being evaluated clinically.

3. CONCLUSION AND PERSPECTIVE

It has been established that VEGF and VEGFRs play key roles in both physiological and pathological angiogenesis. VEGF inhibitors have been developed as desirable anticancer

drugs with little concern about their adverse effects, because angiogenesis is essential for growth of solid tumors but is limited in normal adult tissues. Classical antitumor drugs act on transformed cells, whereas VEGF inhibitors suppress proliferative, migratory, and morphogenic responses to normal endothelial cells. Consequently, resistance to antiangiogenic drugs is unlikely to occur. Bevacizumab, sunitinib, and sorafenib are widely used for the treatment of colorectal and kidney cancers, and clinical trials are also being conducted to expand the use of these drugs against other tumors. Overall, the findings obtained so far have demonstrated that the blockade of VEGF signaling is a promising therapy for cancer patients, although several issues remain to be improved.

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