Structure and function of vascular endothelial growth factor and its receptor system

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Vascular endothelial growth factor and its receptor (VEGF-VEGFR) system play a critical role in the regulation of angiogenesis and lymphangiogenesis in vertebrates. Each of the VEGF has specific receptors, which it activates by binding to the extracellular domain of the receptors, and, thus, regulates the angiogenic balance in the early embryonic and adult stages. However, de-regulation of the VEGF-VEGFR implicates directly in various diseases, particularly cancer. Moreover, tumor growth needs a dedicated blood supply to provide oxygen and other essential nutrients. Tumor metastasis requires blood vessels to carry tumors to distant sites, where they can implant and begin the growth of secondary tumors. Thus, investigation of signaling systems related to the human disease, such as VEGF-VEGFR, will facilitate the development of treatments for such illnesses. [BMB Reports 2018; 51(2): 73-78]

INTRODUCTION

Angiogenesis, the physiological process through which new vessels form from pre-existing vessels, is responsible for most, if not all, blood vessel growth during development (1, 2). Various angiogenic proteins, including fibroblast growth factors (FGFs), vascular endothelial growth factors (VEGFs/VEGFRs), angiopoietin/Tie receptors, platelet-derived growth factors (PDGFs/PDGFRs), and EphrinB2/EphB4 (3-8) result in the stimulation of angiogenesis. This process is tightly regulated depending on the balance of pro- and anti-angiogenic factors (9). However, if the angiogenesis is not properly controlled, various diseases are induced. For example, excessive angiogenesis can lead to chronic disease states such as tumor growth and metastasis, and several disease, such as ulcers and ischemic heart disease, are the result of insufficient angiogenesis (10). Among the angiogenic proteins, VEGF-VEGFR is a crucial regulator of pathological angiogenesis such as in cancer as well as physiological vasculogenesis and angiogenesis in early embryonic and adult stages (11).

VEGFs bind to the VEGFRs on the cell surface, and stimulate cellular responses by causing the receptors to dimerize and become activated through transphosphorylation (12). When cells are deficient of oxygen, namely in hypoxia, the cell produces hypoxia-inducible factor (HIF), which can stimulate the release of VEGF. Thus, hypoxia may be an essential regulator of VEGF expression. Additionally, several diseases characterized by excess angiogenesis are associated with hypoxia-driven de-regulated VEGF expression (12, 13). Several antiangiogenic drugs target the VEGF-VEGFR system, including VEGF-neutralizing antibody (bevacizumab), small molecule kinase inhibitors (sunitinib, sorafenib, and apatinib), and humanized monoclonal antibody targeting the extracellular domain of the VEGFR (ramucirumab). However, the resistance mechanisms of cancer and the side effects of drugs limit the use of these drugs in chemotherapy (14). Consequently, a more detailed investigation focused on the pathological angiogenesis, as a therapeutic target, is required for the development of safe and continuously available drugs.

In this review, we describe the structural and functional information regarding the VEGF-VEGFR system to increase understanding of angiogenesis in physiological and pathological processes.

STRUCTURE AND BIOCHEMICAL PROPERTIES OF VEGFRs WITH ITS LIGANDS

Genes encoding novel tyrosine kinase receptors were isolated in the early 1990s, and the tyrosine kinase receptors that positively and negatively regulate the formation of blood and lymph vessels were denoted VEGFRs (15, 16). Three genes are encoding three full-length receptors (VEGFR-1, -2, and -3) and one soluble molecule (sVEGFR-1), and most VEGFRs show similar overall structures that comprise of three primary
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Fig. 1. Structure of the VEGFR-1 extracellular domain in complex with VEGF-A. (A) Schematic representation of the domain organization of VEGFR is shown. (B) Complex crystal structure of VEGFR-1 extracellular domain with VEGF-A (PDB ID: 5T89) is shown. We have shown the structure in a ribbon representation with each chain depicted by a different color. The chains of the VEGF-A homodimer are shown in light blue and gray, and the VEGFR-1 D1-D6 chains in deep blue and magenta.

VEGFRs are typically composed of an extracellular ligand-binding domain (ECD) with a seven immunoglobulin (Ig)-like domain, a transmembrane domain and a tyrosine kinase domain split by a kinase insert and a carboxy terminus (Fig. 1A) (11, 17). The kinase domains of VEGFRs are the most conserved region, with high sequence identities (78-80%). The VEGF-VEGFR system plays a central role in the regulation of tumor angiogenesis and can be a potential target for anti-angiogenic therapy. There are five VEGF family members (VEGF-A, VEGF-B, VEGF-C, VEGF-D, and placental growth factor) encoded from the mammalian genome (3, 18). Moreover, alternative splicing of primary RNA transcripts from the VEGF gene family generates various isoforms. For example, the isoforms of human VEGF-A are labeled as VEGF-A_{121}, VEGF-A_{145}, VEGF-A_{165}, VEGF-A_{189} and VEGF-A_{206}, and homodimeric VEGF-B exists as two different transcripts, VEGF-B_{167} and VEGF-B_{186} (19). Among them, VEGF-A (known as VEGF) is one of the most critical factors for blood vessel formation during early embryogenesis (11). VEGF-A binds to Ig domains 2 and 3 localized in the ECD of VEGFR-1 and VEGFR-2 (20, 21). Interestingly, the affinity of VEGF-A to VEGFR-1 is about one order of magnitude higher than that to VEGFR-2, but the tyrosine kinase activity of VEGFR-2 in response to VEGF-A is much higher than that of VEGFR-1 (17, 22). VEGF-B and placenta growth factor (PIGF) bind to VEGFR-1, but their mechanisms that activate the receptor are different (23). Specifically, VEGF-B stimulates Tyr1213 phosphorylation of VEGFR-1, whereas PIGF stimulates Tyr1309 phosphorylation (24). VEGF-C and VEGF-D are specific ligands for VEGFR-3, which plays a critical role in angiogenesis and lymphangiogenesis in adults (Fig. 2) (25).

To date, many structural studies of the VEGF/VEGFR complex based on single-particle electron microscopy, small-angle X-ray scattering, and X-ray crystallography show how the ligand binds to the membrane distal Ig domains. Moreover, studies of other Ig domains of the VEGFR suggest the possibility of receptor-receptor interactions (19, 26-29). The first complete and recently reported VEGF/VEGFR ECD complex structure provides insight information regarding the ligand binding and ligand-induced homotypic interactions of VEGFR (30). The structure of full-length VEGFR-1 ECD in complex with VEGF-A exists as two sets of 1:1 complexes in the asymmetric unit and two receptors linked by the dimeric VEGF-A bound to the Ig domains (Fig. 1B) (30). Unlike previous VEGFR-1 complex structures that contained only Ig domain 2, the recently reported complex structures include the complete ECD of the receptor with VEGF-A that interacts with both Ig domains 2 and 3 of VEGFR-1 (19, 30-32). The results of these studies also suggest that the homotypic receptor-receptor contacts in Ig domains 4-7 increase the binding affinity of VEGFR-1 ECD for VEGF-A based on the finding that the binding affinity is 20 times higher in the...
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Fig. 3. Signaling pathways activated by VEGFR2. The phosphorylation of tyrosine residues creates docking sites for the recruitment of downstream signaling effectors. Subsequently, signaling cascades activated by VEGFR2 can regulate gene expression, cell proliferation, survival, and migration.
apoptosis, limitless replicative potential, sustained angiogenesis, tissue invasion and metastasis, abnormal metabolic pathways, evading the immune system, and genome instability (49). Blood vessel growth is essential for the growth and metastasis of solid tumors; thus, angiogenesis is considered one of the most critical targets for investigation of tumor therapy (50). The VEGF-VEGFR system is known as a primary regulator of tumor angiogenesis, and inactivation of the system has been reported in a variety of human diseases such as tumor angiogenesis, tumor-dependent ascites formation, metastasis, and inflammatory diseases including rheumatoid arthritis, rheumatoid psoriasis, hyperthyroidism and atherosclerosis (3, 18). VEGFR-1 may contribute to pathological angiogenesis by stimulating the activation of endothelial cells and the recruitment of bone marrow progenitor cells (51, 52). Additionally, sVEGFR-1 expressed in the trophoblast layer is a splice variant of VEGFR-1, and may play a critical role in the formation of a regulatory barrier against abnormal vascular permeability and abnormal angiogenesis (11). The finding that artificial overexpression of sVEGFR-1 in a pregnant rat model induces hypertension and proteinuria strongly suggests that increased sVEGFR-1 is a crucial causative factor of the preeclampsia symptoms (hypertension and proteinuria) (53). VEGFR-2 has also been directly linked to tumor angiogenesis and blood vessel-dependent metastasis. Specifically, VEGFR-2 is up-regulated under the hypoxic stress that occurs during the rapid growth of tumor cells (11). Either dysfunction or increased activation of VEGFR-3 can be involved in human pathological conditions. Inactivation of VEGFR-3 can aggravate congenital lymphedema that results from decreased transport capacity of the lymphatic vessels and features chronic and disabling swelling of tissues (54, 55). Another lymphedema caused by filariasis, trauma or infection may be treated with VEGF-C, alleviating the increased activation of VEGFR-3 (17).

The VEGF-VEGFR system has been confirmed to be useful as a target of new drugs to suppress a range of diseases, particularly malignancies. There are several anti-angiogenic compounds including VEGF-neutralizing antibody (bevacizumab) and tyrosine kinase inhibitor (sunitinib and sorafenib), which inhibit growth and metastasis of tumors. When tumors show drug-resistance to standard cytotoxic therapy, anti-angiogenic compounds may be the ideal drugs for treating cancer patients (11). Bevacizumab is a humanized monoclonal antibody targeting VEGF-A that can selectively neutralize VEGF-A, but not other VEGF family members. The FDA approved Bevacizumab in 2004 for the treatment of cancer. However, it was withdrawn in late 2011 because it has no clear efficacy data on overall survival in large-scale phase III clinical researches such as E2100, AVADO and RIBBON-1 clinical trials (11, 56). Bevacizumab has some adverse effects that can be life-threatening, including hypertension, proteinuria, rhinorrhagia, thrombosis and bleeding (57). Additionally, certain cancers are resistance to bevacizumab through several mechanisms, such as the enhancement of alternative pro-angiogenic signaling pathways, recruitment of bone marrow-derived pro-angiogenic cells to the tumor, and increasing of pericyte in tumor (58). Sunitinib malate and sorafenib tosylate can selectively target some protein receptors, including VEGFRs, and inhibit their kinase activity. Moreover, they can be widely applied because they cause few adverse reactions (59). In addition, the development of other anti-VEGF-VEGFR drugs such as VEGF-Trap and humanized anti-VEGF antibodies is consistently ongoing to overcome adverse drug effects. Recent studies suggest that VEGF pathway appears to be useful for prognosis of several cancers patients including breast cancer and is also conducted as the most critical pathway regulating liver and lymph node metastasis of breast cancer (60-62). Therefore, we can use VEGF-VEGFR system as a potential target of new drugs, and more detailed structure-based insightful information regarding the VEGF-VEGFR system is essential to improve the anti-angiogenic therapy for the improved quality of life of cancer patients.

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CONFLICTS OF INTEREST

The authors have no conflicting interests.

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