

Pivotal role of vascular endothelial growth factor pathway in tumor angiogenesis

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The shaping of new blood vessels is a significant event in cancer growth and metastasis. Therefore, the molecular system of cancer angiogenesis has garnered considerable interest in cancer research. The vascular endothelial growth factor (VEGF) and VEGF receptor pathway are recognized as the key regulators of the angiogenic process. Activation of the VEGF/VEGF-receptor pathway initiates signaling cascades that promote endothelial cell growth, migration, and differentiation. Recently, VEGF was shown to play a role in the recruitment of bone marrow-derived endothelial progenitor cells to neovascularization sites. The role of VEGF in promoting tumor angiogenesis and the occurrence of human cancers has led to the rational design and development of agents that selectively target this pathway. Moreover, these anti-VEGF/VEGF receptor agents show therapeutic potential by inhibition of angiogenesis and tumor growth in preclinical models. In this review, we summarize the role of the VEGF pathway during tumor angiogenesis.

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Key Words: Angiogenesis inhibitors, Vascular endothelial growth factor receptors, Cell hypoxia, Tumor microenvironment

INTRODUCTION

The formation of new blood vessels from pre-existing vessels, known as angiogenesis, is an essential process in malignant tumor growth. In addition, this process is regulated by proangiogenic or angiostatic factors; when tumor cells are switched to the angiogenic phenotype, tumor growth and progression occur [1]. The angiogenesis regulators have been discovered owing to the increase in angiogenesis research after the early 1990s. For example, basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF) are identified as positive regulators of angiogenesis. The first generation of angiogenesis inhibitors includes interferon- α , angiostatin, and endostatin, whereas compounds such as bevacizumab, sunitinib, and erlotinib are currently used in

clinical settings [2].

Tumor endothelial cells play an essential role in angiogenesis. Therefore, they have been demonstrated as suitable targets for cancer therapy from basic research and clinical applications [3]. There is substantial evidence that the proinflammatory response at the tumor stroma can be rerouted in a tumor-promoting direction by the stimulation of angiogenesis and tissue remodeling [4]. In this review, we discuss the current literature regarding the molecular mechanisms by which tumor angiogenesis is regulated.

VASCULAR ENDOTHELIAL GROWTH FACTOR

VEGF and VEGF receptors (VEGFRs) are recognized as components of one of the main signaling pathways in angio-

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genesis [5]. The members of the VEGF family are five VEGF glycoproteins (VEGF-A, VEGF-B, VEGF-C, VEGF-D, and VEGF-E), and placental growth factors 1, 2. In particular, VEGF-A is the best-characterized member of the VEGF family. VEGF-A and its receptor VEGFR-2 are the main targets of the current antiangiogenic agents. The main targets of VEGF are endothelial cells, and VEGF is released by cancer cells to induce tumor angiogenesis [6]. The increase of VEGF is induced by platelet-derived growth factor (PDGF) B and hypoxia-inducible factor (HIF)-1 α . In addition, it can also be secreted from the extracellular matrix (ECM) by matrix metalloproteinase-9 to start an angiogenic switch that promotes tumor growth (Fig. 1). The decrease of VEGF expression in hepatocellular carcinomas (HCCs) occurs by disruption of c-Jun N-terminal kinase (JNK), a member of the mitogen-activated protein kinase (MAPK) family [7]. JNK regulates VEGF transcription via activator protein 1.

However, this effect may be indirect and mediated via factors such as interleukin (IL)-1 β and oxygen tension. In epithelial cancers, E-cadherin is downregulated by an HIF-1 α -dependent mechanism through the transcription factor Snail, therefore enhancing epithelial-mesenchymal transition [8]. VEGF elicits epithelial-mesenchymal transition via an autocrine loop [9], suggesting that it is involved in not only tumor angiogenesis but also in the early propagation of malignant cells outside the epithelial layer.

VEGFRs are key element of the ECM and contain an intracellular domain with a consensus tyrosine kinase sequence. VEGFR-1 and VEGFR-2 are mostly expressed in endothelial cells. However, VEGFR-3 was found to be mostly associated with lymphangiogenesis [10]. The VEGF family members bind to receptors with different affinities. VEGFR-1 is a receptor for VEGF-B and placental growth factor. In addition, during

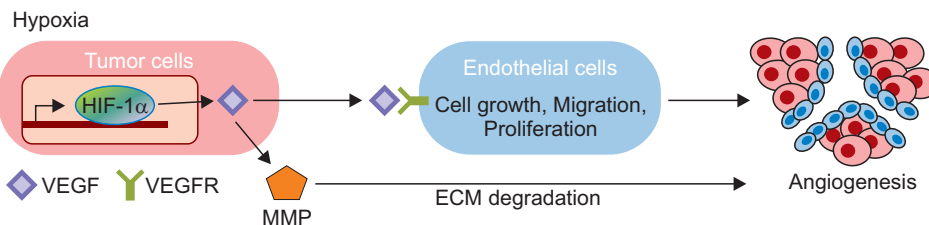


Fig. 1. VEGF/VEGFR induces tumor angiogenesis with endothelial cells. When hypoxic stress occurs in tumor cells, transcription of hypoxia-inducible factor (HIF)-1 α is activated, which promotes VEGF expression. The secreted VEGF binds to the VEGFR on the surface of endothelial cells, and increases MMP expression in tumor cells. As a result, tumor angiogenesis is increased leading to endothelial cell growth, proliferation, and migration. VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor; MMP, matrix metalloproteinase; ECM, extracellular matrix.

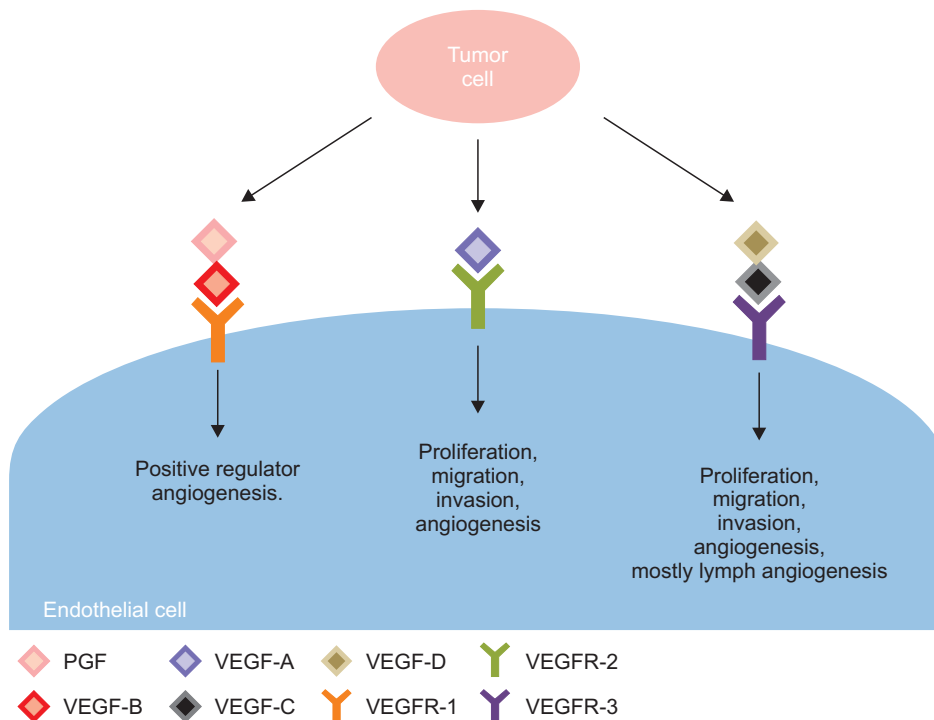


Fig. 2. Role of the VEGFRs. VEGFR-1 and -2 are expressed on the cell surface of most blood endothelial cells. By contrast, VEGFR-3 is mostly expressed on lymphatic endothelial cells. VEGFR-1 binds to VEGF-B and PGF, and functions as a positive-angiogenesis regulator. VEGFR-2 binds to VEGF-A, and mostly has a proangiogenesis effect such as by increasing microvascular permeability, endothelial cell proliferation and migration, and invasion. VEGFR-3 binds to VEGF-C and -D, and has a similar function to VEGFR-2, but is expressed in different endothelial cells. VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor; PGF, placental growth factor.

pathological conditions such as tumor occurrence, it acts as a positive regulator of angiogenesis. VEGFR-2 mostly acts as a mediator of the cellular effects of VEGF-A during angiogenesis, and is involved in microvascular permeability, endothelial cell proliferation, migration, and invasion [5]. VEGFR-3 has highest affinities to VEGF-C and VEGF-D, and induces lymphangiogenesis (Fig. 2). In addition, VEGFR-3 is similar to VEGFR-2, in contributing to angiogenesis and both are expressed on tumor blood vessels as well as on lymphatics. Neuropilins 1 and 2 serve as coreceptors for VEGF, and increase the binding affinity of ligands to VEGFRs [11]. Targeting of the tumor vasculature is an especially interesting therapeutic strategy because of the supposed genetic stability of endothelial cells [12]. Indeed, current antiangiogenic pathway agents were approved by the U.S. Food and Drug Administration (FDA). These agents that target VEGFR-2 [13] include bevacizumab, a humanized anti-VEGF-A monoclonal antibody [14], and sorafenib and sunitinib, small-molecule inhibitors. In combination with other anticancer agents, the addition of bevacizumab significantly increased the progression-free survival and median overall survival in non-small cell lung cancer (NSCLC) and colorectal cancer [15,16]. It has been demonstrated that systemic administration of sorafenib, a tyrosine kinase inhibitor targeting the VEGF and extracellular-signal related kinase pathways, significantly increased the survival of patients with late-stage HCC [17]. However, antiangiogenic treatments targeting a single pathway, such as VEGF-A, rarely induce durable tumor responses both in mice and in patients with cancer [18], and may also favor metastasis in selected tumor models [19]. The mechanism for this acquired resistance is not well reported but may partially result from the expansion or expression of repetitive changes in the maturing vasculature [20], and epigenetic mechanisms [21]. Recently, tumor resistance or recurrence after antiangiogenic therapy was found to be caused by the relapse of bone marrow-derived myeloid cells [22]. Indeed, damaging the tumor vasculature induces tumor hypoxic conditions, which in turn upregulate the expression of some myeloid cell chemoattractants that enhance the influx of myeloid cells to treated tumors [18]. The myeloid cells enhance angiogenesis by secreting angiogenic and tissue-remodeling factors [23], in addition to stimulating tumor cell intravasation, dissemination, and metastasis [24]. Therefore, identification of new antiangiogenesis targets is important and further research is needed for their discovery.

REGULATORS OF VEGF AND VEGFR EXPRESSION

Hypoxia

VEGF expression is influenced by hypoxia. Recent research has shown that HIF-1 is the key factor of the hypoxic response,

and also plays an important role in the product of the von Hippel Lindau (vHL) tumor suppressor gene [5,25,26]. Under normoxic conditions, HIF-1 α is rapidly degraded by the ubiquitin-proteasome pathway; a process that is controlled by the vHL tumor suppressor gene product [25,27]. VEGF transcription is induced under hypoxia conditions, when vHL is mutated or absent, or when the HIF-1 α complex is bound to the VEGF promoter.

Growth factors and cytokines

The cytokines and growth factors are known through several studies that can control the expression of angiogenic factors in tumor cells and thereby induce angiogenesis indirectly. The importance of the epidermal growth factor receptor (EGFR, ErbB1) and HER-2/ErbB2 systems in VEGF regulation and angiogenesis has been verified in several tumor systems, including colon cancer [28], pancreatic cancer [29], gastric cancer [30], breast cancer [31,32], glioblastoma multiforme [33], NSCLC [34], and renal cell carcinoma [35]. The insulin-like growth factor I receptor (IGF-IR) is often overexpressed in human cancers, and has been associated with fatal disease and metastases [36]. Recently, research model systems have shown the significance of the activation of the IGF-IR system in arbitrating angiogenesis by upregulating VEGF expression in several types of cancers [37]. VEGF expression is possibly induced by hepatocyte growth factor via activation of mesenchymal epithelial transition factor in normal and cancer cells [38]. PDGFs were shown to induce angiogenesis via regulating the survival of endothelial cells and pericyte/vascular smooth muscle cell recruitment *in vivo*, and were also found to induce VEGF in several model system [39]. Dong et al. [40] recently reported the PDGF has a significant role in regulating stromal-derived VEGF-A. PDGF-AA expression was shown to promote recruitment of tumor-associated fibroblasts and VEGF production by researchers using VEGF-knockdown cancer cells. Angiogenesis and tumor growth were significantly inhibited by obstruction of paracrine PDGF receptor-alpha (PDGFR- α) signaling among tumor cells and stromal fibroblasts. Therefore, these results show that PDGFR- α signaling is vital for the recruitment of VEGF-expressing stromal fibroblasts, and secreted VEGF of host cells is important for maintaining tumor angiogenesis.

The prostaglandins have important functions in many biological processes. In addition, particular prostaglandins were recently shown to be related to tumor angiogenesis via the upregulation of VEGF expression [41]. The function of the rate-limiting enzyme prostaglandin-endoperoxide synthase (also known as cyclooxygenase or COX) is involved in the oxidative transformation of arachidonic acid into various prostaglandin compounds [42]. In the last 10 years, many studies have demonstrated a relationship between COX-2 overexpression, tumor growth, and increasing VEGF expression for angiogenesis

in many solid tumors, including gastric [43], colon [44], prostate [45], breast [46], and pancreatic [47] cancers. Moreover, many *in vivo* research showed the VEGF expression by COX-2 in many cell lines [41]; however, COX-2 inhibitors do not affect VEGF expression in all tumors, indicating that this effect may be tumor-dependent.

Oncogenes and tumor suppressor genes

Generally, oncogenes have the capacity to induce proangiogenic factors and growth factors such as VEGF, and are associated with the angiogenesis system of solid tumors [48]. The tyrosine kinase pp60src is encoded by the *c-Src* proto-oncogene; it has been associated with the regulation of VEGF expression, and to enhance angiogenesis in growing tumors [49]. In addition, several factors that regulate VEGF expression are dependent on *c-Src*-mediated signal transduction in tumor systems [49]. Similarly, the *BCR-ABL* oncogene has been demonstrated to play an important role for angiogenesis-dependent tumors in the molecular pathogenesis of leukemias [50]. Recent research has shown that VEGF levels were reduced in *BCR-ABL*-positive chronic myeloid leukemia cells by the use of STI-571 to target *BCR-ABL* [51]. In addition, transfection of *BCR-ABL* promoted expression of VEGF in mouse myeloid cells and human megakaryocytes.

Mutation in the *Ras* oncogene is a common genetic change observed in human cancers. In addition, the mutation of H- or K- *Ras* oncogenes induces VEGF expression in various cancers such as pancreatic cancer, colon cancer, and NSCLC cells [52-54]. In addition, genetic change of the mutant K-*Ras* allele in human colon cancer cells was related to a decrease in VEGF activity [55]. The activation of *Ras* or similar signaling molecules forms part of signaling cascade started by specific growth factor receptors such as EGFR [56,57], which may be one of the important signaling pathways for VEGF expression and growth factor-dependent angiogenesis.

p53 is a tumor suppressor gene related to the molecular pathology of many types of solid tumors. In addition, several studies have demonstrated that *p53* plays an important role in the regulation of VEGF in tumors. Breast cancer cells inhibit transcriptional activation of the VEGF promoter via interaction between *p53* protein and the transcription factor Sp1 [58], and this inhibition results in hypoxic induction of *Src* kinase. Stable transfection of wild-type *p53* resulted in decreased VEGF expression in colon and endometrial cancer cells [59,60]. Several studies have also demonstrated that increased HIF-1 activity in cancer tissues led to increases in VEGF levels via genetic changes of tumor suppressor genes such as *vHL*, *p53*, and Phosphatase and tensin homolog [61,62].

VEGFR EXPRESSION IN TUMOR CELLS

The role of VEGF in tumor angiogenesis was established via stimulation of VEGFRs on the tumor endothelium. However, there is increasing evidence that VEGF may have an additional role in cancer via stimulation of VEGFRs in tumor cells.

Several studies have demonstrated the presence of VEGFRs in liquid and solid tumor cells, such as NSCLC, melanoma, prostate cancer, leukemia, mesothelioma, and breast cancer [50,63-67]. The relevance of this expression pattern is still under investigation. These results must also be construed with discretion, because many of these studies have using immunohistochemical staining, which can lead to high false positive rates. Nonetheless, it could be hypothesized that diverse VEGF ligands support tumor growth, not only by inducing angiogenesis but also by direct action via VEGFRs expression by tumor cells. In addition, because most solid tumors and various hematologic tumors have the ability to express VEGF, expression of VEGFRs by tumor cells involves the latent role of VEGF/VEGFR autocrine signaling in these tumors. Corresponding with this hypothesis, recent research has demonstrated that for specific leukemias, VEGFRs may be vital for tumor cell growth by enhancing a VEGF/VEGFR autocrine signal, which induces tumor growth arrest and apoptosis when interrupted. For example, Dias et al. [68] have demonstrated that functional VEGF/VEGFR-2 autocrine signals exist in subsets of human leukemias and support *in vivo* leukemic cell survival and migration [50]. Treatment of mice bearing transplanted human leukemia cells with a function-blocking VEGFR-2 antibody has been shown to result in decreased tumor growth and survival. Price et al. [67] have shown that VEGFR-1 is expressed in various breast cancer cell lines and that stimulation of breast cancer cells with VEGF induced invasion and signaling *in vitro*, suggesting a possible autocrine pathway that leads to increased tumorigenesis. Recently, VEGFR-1 expression has been detected in many colon, breast, and pancreatic cancer cell lines [67,69]. *Ex vivo* studies have also shown that VEGFR-1 activation by VEGF-A or VEGF-B induces increased cell invasion and migration, as well as phenotypic changes associated with activation of the MAPK pathway in tumor cells [70]. Thus, VEGF appears to enhance cell growth in human tumors by direct action with VEGFRs through an endothelial cell-independent pathway, and therefore may serve as a useful target for cancer therapy.

SUMMARY OF THERAPEUTIC STRATEGIES TO INHIBIT THE VEGF PATHWAY

As an important regulator of tumor angiogenesis, there has been increasing interest in and efforts made to utilize the capacity of the VEGF pathway for therapeutic strategies

in oncology. Thus, strategies focused on antiangiogenesis therapy via inhibition of the VEGF pathway are currently in preclinical and clinical development. Some of the current anti-VEGF strategies include development of antibodies to VEGF or VEGFRs, tyrosine kinase inhibitors of VEGFRs, and soluble VEGFR/VEGFR hybrids [71-75].

Many anti-VEGF agents are progressing toward testing in clinical trials. Among the anti-VEGF antibodies, bevacizumab (in combination with chemotherapy) is the first of these agents to be permitted for use in tumor therapy by the FDA.

The use of neutralizing antibodies to VEGF was one of the earliest strategies used to inhibit VEGF activity. In preclinical research, the anti-VEGF monoclonal antibody of mice was found to inhibit angiogenesis and growth of transplanted human tumors [73]. Preclinical data of anti-VEGFR-2 antibodies have shown successful inhibition of the VEGF-induced signaling pathway, antiangiogenesis, and decreased primary and metastatic growth in variety tumor systems [76,77]. The other main approach to obstruct VEGF-mediated angiogenesis is the use of small-molecule inhibitors of VEGFR tyrosine kinase activity. The function of some tyrosine kinase inhibitors selectively inhibit VEGFR-2 but also show activity on other VEGFRs and other diverse tyrosine kinase receptors, including the PDGFR- α , PDGFR- β , bFGF receptor, EGFR family members, c-kit, and Flt3.

A successful strategy that can indirectly regulate the VEGF pathway would result in antiangiogenic effects such as inhibition of diverse regulators of VEGF expression, and production of EGFR, HER-2, COX-2, or HIF-1 α . However, each of the angiogenic factors produced, such as VEGF, is associated with multiple regulatory factors and intracellular signaling pathways. Thus, targeting one pathway through the inhibition may not induce complete inhibition of angiogenesis or VEGF, which may still be activated by many other pathways. In this regard, drug resistance is an important consideration in the development of VEGF-targeted therapies, and is need of mention. One of the advantages of antiangiogenic agents is their ability to evade acquired resistance to therapy and drugs; the reader is referred to several excellent reviews on this topic [78]. This hypothesis is based on the supposition of genetically stable host vasculature in the targets of anti-VEGF therapy. Nonetheless, tumors can be exceptionally heterogeneous with respect to their dependence on microenvironmental factors. Therefore, other tumors may be intrinsically resistant to certain environmental conditions. For example, several tumors may be highly sensitive to hypoxia conditions and therefore more reactive in inhibiting VEGF. To date, there is a lack of evidence of the acquired resistance for VEGF-targeted agents. However, several preclinical studies have shown the prevention of relapse tumor growth after an evident initial phase of inhibition

during chronic VEGF/VEGFR blockade [79]. One possible mechanism underlying this relapse growth is the compensatory upregulation of other proangiogenic factors (or down-regulation of antiangiogenic factors) by cancer or stromal cells, which results in the remodeling and increased efficiency of the tumor vasculature. Recently, the results of preclinical research have provided support for this hypothesis [20]. Long-term anti-VEGF treatment after Wilms' tumor transplantation has been shown to significantly increase the tumor vessel radius with active proliferation of vascular mural cells and stromal cell recruitment. Furthermore, increased tumor perfusion and relapse tumor growth accompanied these morphological changes in tumor vessels. Additional research of the resistance mechanisms against VEGF-targeted agents is warranted in the future.

CONCLUSIONS

The complex molecular pathways that regulate tumor angiogenesis are major targets for pharmacological development, owing to their important roles in the promotion and regulation in the growth and development of cancers. Tumor cells are genetically unstable, which is considered the main cause of the failure of systemic chemotherapies. Endothelial cells in the tumor stroma are considered to be genetically stable, and it is believed that these cells will not become drug-resistant in response to antivascular therapy. However, recent research has demonstrated that endothelial cells are aneuploid and tumor markers [80]. Signals from some other stromal cell types have been demonstrated to regulate tumor growth and their responsiveness to therapies in diverse models, raising the possibility that drugs interfering with these pathways could supply additional therapeutic strategies. Future research focused on altering the tumor microenvironment involved in tumor angiogenesis may lead to new therapeutic applications via regulation of the critical mediators of tumor growth.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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