

# Immunosuppressive effects of vascular endothelial growth factor (Review)

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**Abstract.** Vascular endothelial growth factor (VEGF) serves a critical role in vasculogenesis, angiogenesis, tumor, inflammatory angiogenesis and lymphangiogenesis. Since 2004, bevacizumab (Avastin), a humanized anti-VEGFA monoclonal antibody, has been approved for the treatment of non-small cell lung, breast, kidney and ovarian cancer in combination with standard chemotherapy. VEGF has been demonstrated to be important in the clinic as a therapeutic target in the anti-angiogenic approach to cancer therapy. The targeting of VEGF, together with immunotherapy, has been reported to be able to reverse the immunosuppressive effects of VEGF. A positive correlation between VEGF expression and the reduced survival rates of patients with cancer has also been demonstrated. Furthermore, increased VEGF expression can lead to immune suppression via the inhibition of dendritic cell maturation, the reduction of T-cell tumor infiltration and the promotion of inhibitory cell types in the tumor microenvironment.

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## 1. Introduction

The vascular endothelial growth factor (VEGF) family consists of five mammalian factors, VEGFA, VEGFB, VEGFC, VEGFD and placenta growth factor (PlGF). Genetic inactivation of VEGFA and VEGFC in mice results in embryonic death due to defects in the development of blood and lymphatic vessels, respectively. The VEGFs bind to three different but structurally related tyrosine kinase receptors, named VEGFR-1, VEGFR-2 and VEGFR-3 (Fig. 1). VEGFR-2 is preferentially expressed on blood vascular endothelial cells, whereas VEGFR-3 is primarily expressed on lymphatic endothelial cells. Moreover, VEGFR-1 is expressed on a range of non-endothelial cells and is essential for the regulation of leukocyte motility (1-3). All VEGFRs contain seven immunoglobulin (Ig) homology domains, which comprise the ligand-binding site, and an intracellular region endowed with tyrosine kinase (TK) activity, which transduces the signal. The downstream signaling includes activation of phospholipase C $\gamma$ 1, MAPK pathway via Ras/Raf1 activation and PI3K/Akt pathway. Phospholipase C $\gamma$ 1 regulates the concentration of intracellular Ca<sup>+2</sup> ions and formation of endothelial nitric oxide synthase (Fig. 1). The effect of all the cascades provides a balance of pro- and anti-angiogenic signals that maintain the vasculature and/or result in sprouting of new blood vessels, cell proliferation and cell migration. The VEGF/VEGFR signaling pathway is upregulated in many types of cancers, contributing to uncontrolled angiogenesis and metastatic spreading.

VEGFA serves a critical role in vasculogenesis, angiogenesis, tumors, inflammatory angiogenesis and lymphangiogenesis. VEGFA was initially discovered as a vascular permeability factor with a potency 50,000 times that of histamine (1) and extravascular fluid accumulation is prominent in tumor growth in body cavities, such as the peritoneum. A striking positive correlation between VEGFA expression levels, tumor progression and consequently reduced patient survival, has also been demonstrated (2).

## 2. Immunosuppressive effects of VEGF

VEGF modulates the function of T-cells, suppressive immune cells and the stroma in the tumor microenvironment, which results in an immunosuppressive state (Fig. 2). VEGF impairs

interactions between leukocytes and endothelial cells. It achieves this via the downregulation of the expression of adhesion molecules, including vascular cell adhesion molecule-1 (VCAM-1) and intercellular cell adhesion molecule-1 (ICAM-1), or via the inhibition of their clustering, which impairs the ability of immune cells to adhere and migrate across the endothelium into the tumor (3-7). The anergic phenotype of tumor endothelial cells can be reversed via antiangiogenic therapy, which upregulates the expression of endothelial adhesion molecules in the tumor vasculature (8).

The number of tumors infiltrating lymphocytes has been demonstrated to be markedly increased in animal tumor models and in humans following VEGF inhibition (4,9,10). VEGF signaling directly affects T-cell development, homing and cytotoxic functions (7). Moreover, VEGF negatively impacts T-cell migration from the lymph nodes into the tumor bed via the stimulation of abnormal tumor vasculature formation (11). VEGF/VEGFR-2 induces the production of pro-inflammatory molecules, including interferon- $\gamma$  and interleukin-2, and stimulates the migratory response in memory CD4<sup>+</sup> T-cells (12). Furthermore, VEGF induces regulatory T-cells (T-regs) (13). VEGF also impedes the differentiation of hematopoietic progenitor cells in the thymus to CD8<sup>+</sup> and CD4<sup>+</sup> T-cells (14).

VEGF/VEGFR-2 also suppresses CD3<sup>+</sup> T-cells and cytotoxic effects (15,16). Moreover, VEGFA contributes to CD8<sup>+</sup> T-cell exhaustion, which is characterized via the expression of negative immune checkpoints, such as programmed cell death protein-1 (PD-1) receptors (17), in a VEGFR-2 and nuclear factor of activated T-cells-dependent manner. In this context, VEGFA promotes the expression of check point molecules, including PD-1, cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), T-cell immunoglobulin mucin receptor 3 and lymphocyte activation gene 3 protein (18). VEGF can suppress effector T-cell functions via the induction of FAS-ligand expression on endothelial cells and by causing the apoptosis of infiltrating CD8<sup>+</sup> T-cells (19).

Dendritic cells are antigen-presenting cells that serve a critical role in T-cell priming and activation. VEGF inhibits dendritic maturation and antigen presentation (20). High levels of VEGF expression in human cancers have been associated with defective dendritic cell function and number (21-23). VEGFR-1 and neuropilin-1 are involved in the VEGF-inhibition of dendritic cell maturation (24,25). VEGF upregulates programmed death-ligand 1 (PD-L1) in dendritic cells, which results in the inhibition of T-cell expansion and function (26). Furthermore, it inhibits the function of human mature dendritic cells to stimulate T-cells, mediated by VEGFR-2 (27), via the inhibition of NF- $\kappa$ B activation (28).

Myeloid-derived suppressor cells (MDSCs) are a mixed cell population consisting mainly of neutrophils but also of macrophages and dendritic cells with immunosuppressive and tumor-promoting capacities (2,29). VEGF induces the differentiation of myeloid cells into immunosuppressive MDSCs (30) and the VEGF concentration has been demonstrated to be associated with the presence of MDSCs (31). In tumors resistant to anti-VEGF treatment, the increased mobilization and infiltration of MDSCs is distinguishable when compared with treatment-sensitive tumors (32).

Tumor-associated macrophages (TAMs) differentiate into anti-inflammatory M1 macrophages or pro-inflammatory and

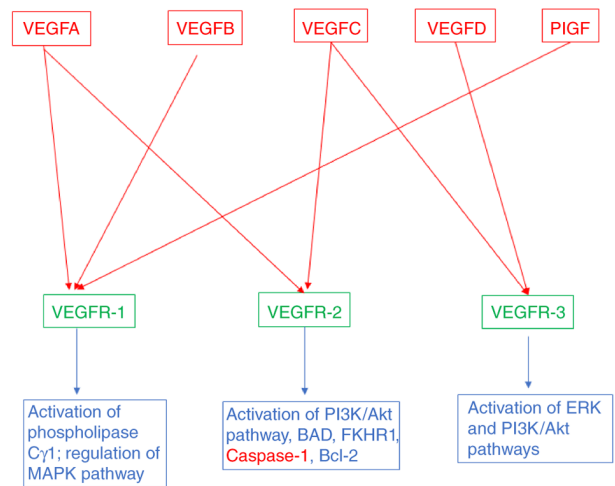


Figure 1. Schematic illustrating the VEGF family members and their respective receptors, and intracellular signaling pathways (pro-angiogenic in blue and anti-angiogenic in red) that are activated by VEGFRs. VEGF, vascular endothelial growth factor; PlGF, placental growth factor; FKHR1, forkhead transcription factor R1.

tumor promoting M2 macrophages, which is dependent on the local cues provided within the tumor microenvironment (33). M2 macrophages secrete immunosuppressive cytokines, including IL-10, chemokine (C-C) ligand (CCL)-7 and CCL-22 and angiogenic and tissue remodeling factors, including VEGF, PlGF and matrix metalloproteinase-9 (33). VEGF recruits TAMs and therefore contributes to the establishment of an immunosuppressive microenvironment (34) and induces the maturation of myeloid cells into the M2-like phenotype (35). A reduced recruitment of TAMs or the reprogramming of M2-like TAMs contributes towards anti-cancer M1 phenotype reversed immunosuppression (36).

The increased recruitment of neutrophils during anti-VEGF treatment promotes tumor progression and treatment resistance (32). Cancer-associated fibroblasts (CAFs) secrete several angiogenic factors, including epidermal growth factor, hepatocyte growth factor, insulin-like growth factor and fibroblast growth factor-2 (37). CAFs that are resistant to anti-VEGF therapy promote tumor growth in anti-VEGF treatment sensitive tumors (29).

VEGF may also contribute to the prevention of an uncontrolled, detrimental immune response to the microbiota in the lung. In this context, VEGF signaling in the alveolar microenvironment is responsible for endothelial cell-mediated tolerance to airborne pathogens and toxins via the interaction of VEGF released by alveolar type I cells and secretory epithelial cells with capillary endothelial cells (38). Moreover, VEGF is involved in fibrotic lung disease (39). Transfection of anti-VEGF gene therapy, in the form of the sFlt-1, resulted in the attenuation of pulmonary fibrosis with a reduction in lung collagen deposition and additional anti-inflammatory and anti-angiogenic effects.

### 3. Anti-angiogenic therapies reverse the immunosuppressive effects of VEGF

VEGF has served an important role in the clinic as a therapeutic target in the anti-angiogenic approach to tumor therapy.

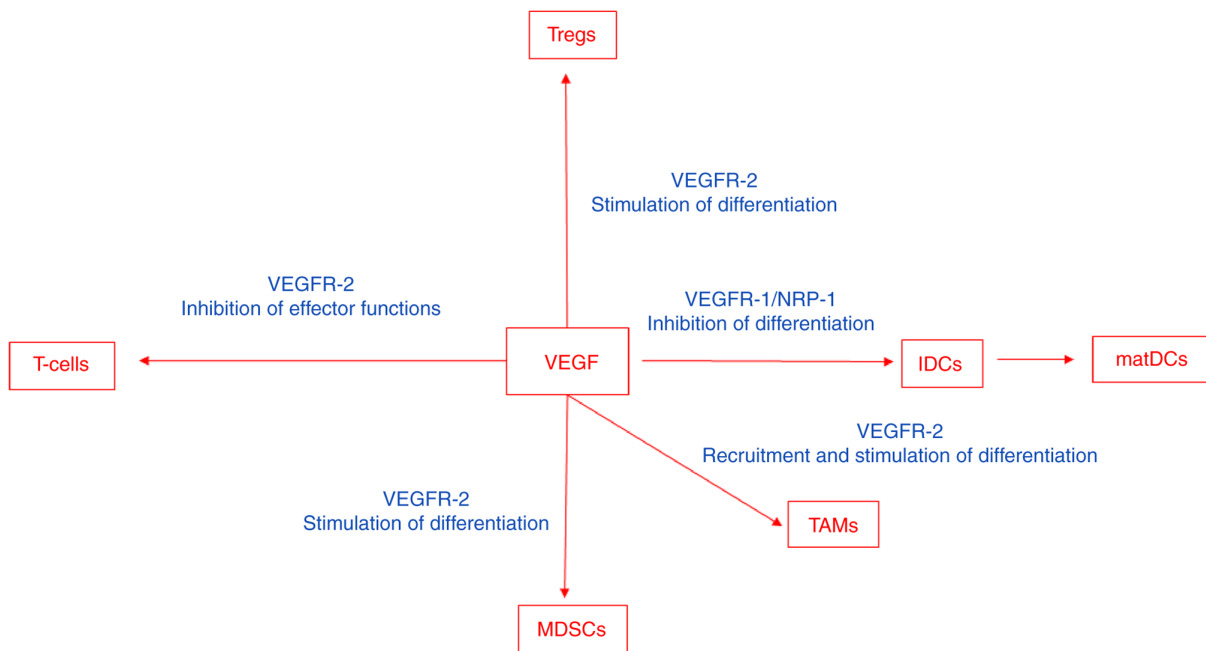


Figure 2. VEGF modulates the function of T-cells, suppressive immune cells and stroma in the tumor microenvironment, which results in an immunosuppressive state. VEGF, vascular endothelial growth factor; MDSC, myeloid-derived suppressor cell; IDC, immature dendritic cell; matDC, mature dendritic cell; TAM, tumor-associated macrophage; Treg, regulatory T-cell; NRP-1, neuropilin-1.

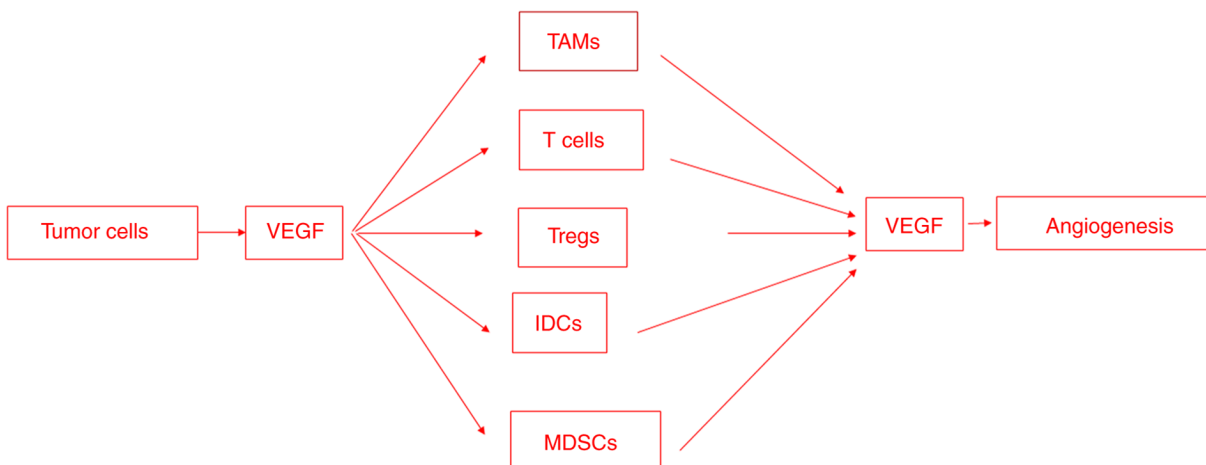


Figure 3. VEGF is involved in both angiogenesis and immunosuppression. Tumor cells secrete VEGF in order to recruit immunosuppressive cells to the tumor site. These cells, including TAMs, T cells, Treg cells, IDCs and MDSCs, secrete VEGF, which promotes endothelial cell proliferation and migration. VEGF, vascular endothelial growth factor; TAM, tumor-associated macrophage; Treg, regulatory T-cell; IDC, immature dendritic cell; MDSC, myeloid-derived suppressor cell.

Bevacizumab binds VEGFA, which prevents VEGFA from interacting with VEGFR-2 on vascular endothelial cells, endothelial progenitor cells and megakaryocytes. Bevacizumab treatment in patients with metastatic colorectal cancer induces an increase in B- and T-cells (40). Furthermore, the addition of bevacizumab to chemotherapy for patients with non-small cell lung cancer results in improved dendritic cell activation and T-cell cytotoxicity (41). Bevacizumab also reverses the VEGF-induced inhibition of monocyte differentiation into dendritic cells (42) and restores peripheral blood dendritic cell numbers in patients with cancer (43). In renal cell carcinoma, bevacizumab reduces the number of MDSCs (44). Furthermore, VEGF induces T-reg proliferation via VEGFR-2

activation. Bevacizumab also inhibits T-reg accumulation in the peripheral blood of patients with metastatic colorectal cancer (45). Moreover, ramucirumab (Cyramza) is a distinct monoclonal anti-VEGFR-2 antibody approved for the treatment of non-small cell lung cancer, colorectal cancer, hepatocellular carcinoma and gastric cancer (46).

Since VEGFRs possess a tyrosine kinase domain, several small ATP mimetics that can inhibit the activity of tyrosine kinase receptors involved in angiogenesis have been developed. Sorafenib and sunitinib were the first multi-kinase inhibitors approved for the treatment of metastatic renal cell and hepatocellular carcinomas. Sunitinib, a multi-tyrosine kinase inhibitor has been demonstrated to induce the upregulation of

ICAM-1 and VCAM-1 adhesion molecules on endothelial cells in tumor-bearing mice (47). These mice displayed increased Th1 responses via the reduction of inhibitory molecule expression, including transforming growth factor- $\beta$ , IL-10, forkhead box protein-3, PD-1 and CTLA-4 (48). Sunitinib reduces the immunosuppressive activity of MDSCs (49) and in human renal cell carcinoma increases tumor infiltrating lymphocytes and reduces MDSCs (50,51). Moreover, sunitinib in combination with CD40 immuno-stimulating immunotherapy induces dendritic cell activation, reduces MDSCs and enhances cytotoxic T-cell recruitment (47).

#### 4. Immunotherapies reverse the immunosuppressive effects of VEGF

PD-1, its ligand PD-L1 and CTLA-4 are negative regulators of T-cell immune function. Following ligand binding, PD-1 attenuates T-cell activation via recruiting SH2 domain-containing protein tyrosine phosphatase-2 and reducing cytokine production and T-cell proliferation (52). PD-L1 is constitutively expressed via T-cells, B-cells, dendritic cells, macrophages and non-hematopoietic cells, including endothelial cells, epithelial cells, hepatocytes and astrocytes (53). CTLA-4 is a cell surface receptor expressed on activated T-cells. Following T-cell receptor interactions with its cognate peptide/major histocompatibility complex, and co-stimulation via CD28, CTLA-4 suppresses this co-stimulation (54).

Numerous trials with anti-CTLA-4 and anti-PD-1/PD-L1 antibodies, which re-activate the anti-tumor processes of the human immune system, have resulted in durable responses in patients with cancer. In a previous study, VEGF expression decreased in patients with metastatic melanoma responding to anti-CTLA-4 and anti-PD-1 therapy, but increased in non-responders, which indicated therapeutic resistance (55).

Anti-angiogenic agents improve the effectiveness of immunotherapy by transiently restoring the abnormal tumor vasculature and increasing the infiltration of immune effector cells into the tumor microenvironment (56). This induces the formation of high endothelial venules that improve lymphocyte infiltration and improve anti-tumor immunity (57). A previous study demonstrated that patients with renal cell cancer or non-small cell lung cancer with a high dendritic cell number before treatment, exhibited an improved response to PD-L1 inhibition with atezolizumab (58).

#### 5. Conclusion

Angiogenesis and immunosuppression are closely associated and occur simultaneously in response to different stimuli (Fig. 3). The VEGF/VEGFR signaling pathway is recognized as the master regulator of tumor angiogenesis. VEGFRs may be targeted through monoclonal antibodies to inhibit VEGF binding to the extracellular domain of the receptor or, alternatively, using different small molecule tyrosine kinase inhibitors blocking the ATP binding in the kinase domain and phosphorylation of tyrosine residues, in clinical trials for the treatment of renal cell carcinoma, hepatocellular carcinoma, metastatic colorectal cancer and gastrointestinal stromal tumors. Another strategy concerns the use of small-molecule inhibitors targeting cell signaling pathways activated by

VEGFRs, approved for the treatment of metastatic melanoma and metastatic renal cell carcinoma.

Tumor cells secrete soluble factors, such as VEGFA, that recruit immunosuppressive cells, including TAMs, neutrophils, MDSCs, dendritic cells, T-regs and natural killer cells. The immunosuppressive cells, in turn, secrete VEGF, which promotes endothelial cell proliferation and migration and/or induces the release of MMPs. VEGFA exerts different immunosuppressive effects, including the inhibition of dendritic cell maturation, the induction of inhibitory molecule expression, such as PD-L1, on dendritic cells, and the activation of T-regs. The present review has demonstrated that VEGF can simultaneously promote angiogenesis and mediate immunosuppression. These overlapping activities may potentially explain the efficacy of anti-angiogenic and immunotherapies in reversing the immunosuppressive effects of VEGF.

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DR wrote, edited and revised the manuscript. Data authentication is not applicable. The author read and approved the final manuscript.

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Not applicable.

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Not applicable.

#### Competing interests

The author declares that they have no competing interests.

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