



## REVIEW

# Relationship of VEGF/VEGFR with immune and cancer cells: staggering or forward?

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### ABSTRACT

Vascular endothelial growth factor (VEGF) is primarily known as a proangiogenic factor and is one of the most important growth and survival factors affecting the vascular endothelium. However, recent studies have shown that VEGF also plays a vital role in the immune environment. In addition to the traditional growth factor role of VEGF and VEGF receptors (VEGFRs), they have a complicated relationship with various immune cells. VEGF also reportedly inhibits the differentiation and function of immune cells during hematopoiesis. Dendritic cells (DCs), macrophages, and lymphocytes further express certain types of VEGF receptors. VEGF can be secreted as well by tumor cells through the autocrine pathway and can stimulate the function of cancer stemness. This review will provide a paradigm shift in our understanding of the role of VEGF/VEGFR signaling in the immune and cancer environment.

### KEYWORDS

Vascular endothelial growth factor (VEGF); VEGF receptors (VEGFRs); dendritic cell (DC); macrophage; T lymphocyte; tumor

## Introduction

Vascular endothelial growth factor (VEGF) was primarily identified and isolated as an endothelial mitogen that can induce angiogenesis both in the physiological and pathological states<sup>1</sup>. VEGF has become prominent, showing that its function is significantly beyond promoting angiogenesis and vascular permeability<sup>2</sup>. For example, VEGF can profoundly influence the early development and differentiation of hematopoietic progenitors<sup>3</sup>. Furthermore, some of the earliest hematopoietic progenitors are found to express VEGF receptors<sup>4</sup>. For immune cells, VEGF can cause a defect in the functional maturation of dendritic cells (DCs) from progenitors and can interact with macrophages and T lymphocytes depending on a certain pathological state. The expression of VEGF receptors in different immune cells has also been reported<sup>5</sup>. VEGF can also be a tumor-growth stimulator, which is independent of its proangiogenic effect<sup>6</sup>. Autocrine and paracrine VEGF signaling have been revealed

to promote cancer cell stemness. These findings have extended our traditional concept about the biological function of VEGF/VEGFR signaling and have encouraged us to explore this relatively new field<sup>7, 8</sup>.

## Biology of VEGF

VEGF is a large family of growth factors that include VEGF-A, VEGF-B, VEGF-C, VEGF-D, and placental growth factor. These family members differ in their expression patterns, receptor specificities, and biological functions. VEGF-A, which is often referred to as VEGF, has been more extensively studied than the other members and has several distinct variants (VEGF<sub>121</sub>, VEGF<sub>145</sub>, VEGF<sub>148</sub>, VEGF<sub>165</sub>, VEGF<sub>183</sub>, VEGF<sub>189</sub>, and VEGF<sub>206</sub>). These variants exist because of alternative splicing and thus differ in receptor specificity and function<sup>9</sup>. VEGF can promote the growth of vascular endothelial cells (ECs) derived from arteries, veins, and lymphatics and can act as a survival factor for ECs by preventing apoptosis. VEGF is also known as vascular permeability factor because it can induce endothelial fenestration in some vascular beds<sup>10</sup>. Many cytokines and growth factors could upregulate VEGF mRNA expression or induce VEGF release. These cytokines and growth factors include placental derived growth factor (PDGF), tumor

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necrosis factor- $\alpha$  (TNF- $\alpha$ ), fibroblast growth factor (FGF), epithelial growth factor (EGF), transforming growth factor (TGF) and insulin growth factor-1 (IGF-1)<sup>11</sup>. Increasing evidence shows that VEGF also has direct biological effects on immune and cancer cells. VEGF can inhibit the differentiation of DCs, promote the infiltration and migration of macrophages, and stimulate cancer-cell stemness<sup>12</sup>. However, the mechanism(s) and basis for these interactions are poorly understood.

## Biology of VEGFRs

The classical VEGFRs are the receptor tyrosine kinases (RTKs): VEGFR-1 (also known as FLT1), VEGFR-2 (also known as FLK1 and KDR), and VEGFR-3 (also known as FLT4)<sup>13</sup>. They are characterized by seven extracellular immunoglobulin (Ig)-like domains that consist of a membrane-spanning region and a conserved intracellular tyrosine kinase domain interrupted by a kinase insert sequence<sup>14</sup>. VEGFR-1 and VEGFR-2 are both high-affinity receptors for VEGF that, along with VEGFR-3, form the Flt subfamily of RTKs. VEGFR-1 binds to VEGF with a higher affinity than VEGFR-2, but the tyrosine phosphorylation of VEGFR-1 in response to VEGF is weaker<sup>9</sup>. VEGFR-2 is the predominant RTK that mediates VEGF signaling in ECs and that drives VEGF-mediated angiogenesis<sup>13</sup>. VEGFR-3 is not a receptor for VEGF, binding instead to VEGF-C and VEGF-D. In addition to these RTKs, VEGF interacts with a family of coreceptors—the neuropilins (NRP-1 and NRP-2). Lacking an intrinsic signaling capability, NRPs form complexes with VEGF RTKs (VEGFR-1 and VEGFR-2) and increase the affinity of these receptors for VEGF<sup>15</sup>. In addition, CD146 is a new coreceptor for VEGFR-2<sup>16</sup>. The soluble form of VEGFR-1 and -2 can form non-signaling heterodimers with their cognate receptor to inhibit VEGF activity<sup>17</sup>. Recently, Singh et al.<sup>18</sup> established the existence of a new soluble isoform of VEGFR-3 (sVEGFR-3) in the corneal epithelial cells. Similarly, it also has an inhibitory effect on VEGFR-3 by impounding VEGF-C. Although the expression of these receptors was initially thought to be limited to ECs, most of them can be expressed by many immune and cancer cells, and their expression correlates with clinical parameters<sup>19</sup>. The expression of VEGFRs by non-epithelial cells has gained significant attention, and their biological function has broadened in recent years. In this review, we will especially discuss the expression of VEGFR-3 in DCs and macrophages and generalize an overall expression of VEGFR-1 and -2 in

immune and cancer cells.

## Relationship between VEGF/VEGFR and DCs

### Expression of VEGF receptors in DCs

DCs are specialized antigen presenting cells that acquire, process, and present antigens to T cells for the adaptive immune responses<sup>20</sup>. The expression of VEGF receptors in human hematopoietic cells paved the way for the identification of their expression in DCs. Initially, Hoehn et al.<sup>21</sup> reported the presence of Flt1- but not of Kdr-specific mRNA in CD34<sup>+</sup> human umbilical cord blood cells; Katoh et al.<sup>22</sup> reported the presence of both Kdr- and Flt1-specific mRNA in CD34<sup>+</sup> cells.

Thereafter, a follow-up study took a long time to testify the expression of VEGF receptors in DCs. VEGFR-3 was primarily identified as the first specific lymphatic endothelium marker in adult tissues<sup>23</sup>. Subsequently, VEGFR-3 was found to be expressed in a subset of capillary endothelia, whereas it was absent in all large blood vessels<sup>24</sup>. In addition, Wilting et al.<sup>25</sup> was the first group to observe the expression of VEGFR-3 in non-ECs, and they testified the expression of VEGFR-3 in podocytes of kidney glomeruli of quail embryos. More recently, Mimura et al.<sup>26</sup>, Cursiefen et al.<sup>27</sup>, and Hamrah et al.<sup>28</sup> reported the VEGFR-3 expression by non-ECs on the ocular surface. Mimura et al.<sup>26</sup> initially detected the VEGFR-3 and VEGF-C gene expression in neovascularized rat corneas with RT-PCR. They also reported VEGF-C expression in infiltrating (not resident) "inflammatory" cells. However, these cells were not phenotyped. Very recently, Hamrah et al.<sup>28</sup> characterized VEGFR-3 on non-ECs in the normal and inflamed conjunctiva as monocytic bone marrow-derived cells. For the first time, they demonstrated the expression of VEGFR-3 in corneal DCs and its upregulation in inflammation. VEGFR-3<sup>+</sup> DCs belong to immature DCs of the monocytic lineage. They are phenotyped CD11c<sup>+</sup>CD45<sup>+</sup>CD11b<sup>+</sup>, but mostly major histocompatibility complex (MHC) class II-CD80<sup>-</sup>CD86<sup>-</sup>. During inflammation, rapid, heightened membranous expression of VEGFR-3 occurs in DCs, whereas in uninflamed tissue, the intracellular VEGFR-3 expression increases. VEGFR-3<sup>+</sup> DCs in normal corneas are VEGF-C<sup>-</sup>NRP-2<sup>-</sup> but express VEGF-C in inflammation. The expression of VEGFR-3 and VEGF-C in tissue DCs underlines a novel potential relationship between lymphangiogenesis and leukocyte trafficking in the eye. Until now, insufficient evidence identifies the expression of

VEGFR-3 in DCs residing in other tissues. Moreover, a few studies discuss the expression of VEGFR-1 and -2 in DCs (reviewed below), but they are significantly less comprehensive and have inconsistent results. Can VEGF receptors be a new emerging marker for DCs? Further exploitation is needed to solve this problem.

### Interaction between VEGF and DCs

VEGF have a complex effect on DCs. Katoh et al.<sup>22</sup> demonstrated that VEGF can suppress the apoptotic cell death of normal hematopoietic stem cells caused by gamma ray irradiation. Gabrilovich et al.<sup>29</sup> demonstrated that VEGF produced by breast and colon cancer cells dramatically inhibited the differentiation and functional maturation of DCs from CD34<sup>+</sup> precursor. Cells generated from stem cells in the presence of VEGF lack the typical morphological features of DCs and have a low level of MHC II expression and a reduced ability to take up soluble antigens. An explanation may be the following: VEGF can bind to the flt-1 receptor in CD34<sup>+</sup> hematopoietic progenitor cells (HPCs), and when HPC developed into mature DCs, it down-regulated the receptors and made the VEGF lose its binding site<sup>30</sup>. They further showed that the continuous infusion of VEGF into mice dramatically affected the number and function of DCs, and this effect became more obvious with time. The mechanism lies in the fact stating that the

differentiation of DCs from CD34<sup>+</sup> HPCs is induced by signal transduction between TNF- $\alpha$ /TNF- $\alpha$  receptors on the cell surface and the nuclear factor (NF)- $\kappa$ B/inhibitory  $\kappa$ B complex in the nucleus, whereas VEGF inhibits the activation of NF- $\kappa$ B in HPCs, which is the dominant negative effect of I $\kappa$ B subsequently preventing DC differentiation<sup>31</sup>. The antibody against VEGF could also restore the reduced DC functions. In oral squamous cell carcinoma (OSCC), Kikuchi et al.<sup>12</sup> showed that tumor-secreted VEGF may promote the tumor immunologic escape by inhibiting the differentiation of immature DC (CD1a<sup>+</sup> DC) from peripheral blood monocyte cells and increasing the levels of dysfunctional mature DC (CD83<sup>+</sup> DCs). Moreover, VEGF also could increase the expression of VEGFR-1 and -2 in CD1a<sup>+</sup> DCs, indicating that the VEGF produced and secreted by various tumor cells is a significant immunosuppressive factor (**Table 1**).

VEGF can also influence the immune response of DCs in the lung. Chapoval et al.<sup>32</sup> demonstrated that VEGF can influence the local populations, activation state, and function of DCs in the lung. An increase in both myeloid DCs (mDC) and plasmacytoid DCs (pDC) was observed in VEGF transgenic (tg) compared with wild-type (WT) mice. For the receptors, the sorted mDCs from VEGF tg lungs increased VEGFR-2 but downregulated VEGFR-1 expression. The antigen-uptake and the migration abilities of antigen-loaded DCs to local lymph nodes in VEGF tg mice were better than

**Table 1** The influence of VEGF on different immune and cancer cells

Cell type	The influence of VEGF on the cell	Reference
Dendritic cell	Inhibit the differentiation of DC in the hematopoietic process	30
	Influence the function and maturation of DC	29
	Increase the mDC and pDC in lung	32
	Enable DC to differentiate into endothelial-like cell	33
	Enable DC to get an angiogenic property	34
Macrophage	Prevent inflammation during bacterial infection	35
	Mediate the infiltration of macrophage in the lung	36
T lymphocyte	Cause thymic atrophy	37
	Costimulate IFN- $\gamma$ production, increase chemotaxis	38
	Induce epithelial activation, neutrophil chemotaxis in OAD	39
Treg	Promote the induction and maintenance of Treg in the tumor site	40
	Chemotaxis for Treg	5
Tumor cell	Increase the migration, cell mobility and invasiveness of tumor cells	41-43
	Promote cancer stemness	44

VEGF, vascular endothelial growth factor; DC, dendritic cell; mDC, myeloid DC; pDC, plasmacytoid DC; OAD, obliterative airway disease.

WT mice. In their experimental context, VEGF can regulate the expression of innate immunity effector molecules by lung myeloid DCs (mDCs) and can functionally promote the transition of mDCs from the innate response to a Th2-type inflammatory response<sup>32</sup>.

DCs may gain an angiogenesis property in the presence of VEGF. Riboldi et al.<sup>33</sup> found that human alternatively-activated DCs that matured in the presence of anti-inflammatory molecules, such as calcitriol, PGE<sub>2</sub>, or IL-10, can selectively secrete potent angiogenic cytokine VEGF isoforms: VEGF<sub>165</sub> and VEGF<sub>121</sub>. However, no VEGF production was detected in immature or classically activated (LPS, TNF- $\alpha$ , SAC, or CD40L) DCs. The former are inhibited in their ability to produce IL-12, which is a cytokine involved in the antiangiogenic process<sup>45</sup>. Evidence shows that solid tumors are infiltrated by DCs that usually lack the phenotype of classically activated DC<sup>46</sup>. Deregulated VEGF expression has been implicated in the development of solid tumors by supporting tumor angiogenesis<sup>47</sup>. These data suggest that within the tumor microenvironment, alternatively activated DCs may represent a source of angiogenic factors (by producing VEGF) and may contribute to tumor neovascularization and growth.

DCs can differentiate into endothelial-like cells (ELCs) in the presence of VEGF. Previous studies have indicated that DCs, macrophages, and ECs are closely related<sup>35</sup>. DCs derived from peripheral monocytes have the potential to differentiate along different lineages: in the presence of these angiogenic growth factors including VEGF, FGF, and IGF-1, the immature cells developed into ELCs, which are characterized by the increased expression of EC markers vWF, KDR, and Flt-4 and the disappearance of CD1a and CD83. The ELCs morphologically have the characteristics of both macrophages and ECs, and they exhibit cell structures resembling Weibel-Palade bodies, which are the storage granules of vWF<sup>34</sup>. VEGF intermediates the decrease in the expression of the DC markers CD1a and CD83 in the transition<sup>48</sup>. In conclusion, VEGF not only inhibits DC function but also stimulates the immature DCs to differentiate into ECs and contribute to neovascularization in the tumor environment.

## Relationship between VEGF/VEGFR and macrophages

### Expression of VEGF receptors in macrophages

Macrophages orchestrate vessel fusion and formation. Macrophages can promote vascular tip cells anastomosing

and bridge them together by releasing angiogenic factors<sup>49</sup>. In disease, the function of macrophages varies depending on the surrounding environment. For example, they are good stimulators for collateral vessel growth in ischemia<sup>50</sup>. In tumors, M2-polarized macrophages promote tumor vascularization by producing proangiogenic factors, such as VEGF, whereas M1-polarized macrophages have tumor-killing effect<sup>51</sup>. Many studies have reported the expression of VEGF receptors in this cell lineage. For instance, the Flt-1 gene messenger RNA is found in human peripheral blood monocytes, and the Flt-1 tyrosine kinase activity is important for the VEGF-induced cell migration of macrophages<sup>52</sup>. Sawano et al.<sup>53</sup> further demonstrated that the protein of the Flt-1 receptor is expressed on the cell surface of monocytes, and Flt-1 protein is expressed during the differentiation of monocyte-macrophage lineage, so they suggested that Flt-1 can be a novel cell surface marker for the lineage of monocyte-macrophages in humans. Similarly, Fernandez Pujol et al.<sup>48</sup> reported that VEGFR-1, 2, and 3 were detected in human CD14-positive monocytes. In addition, the expression of VEGF-C, VEGF-D, and VEGFR-3 in tumor-associated macrophages has also been reported in human cervical cancer<sup>54</sup>.

Profoundly, Zhang et al.<sup>55</sup> demonstrated that LPS treatment and gram-negative bacterial infection can enhance VEGF-C and VEGFR-3 expression in macrophages. Upon their interaction, VEGFR-3 restrained TLR4-NF- $\kappa$ B activation by regulating the PI3K-Akt signaling pathway and SOCS1 expression, and then they attenuated proinflammatory cytokine production. Notably, the ablation of the ligand-binding domain or tyrosine kinase activity of VEGFR-3 rendered mice to be more sensitive to septic shock. Aside from targeting lymphatic vessels, VEGFR-3 plays a role on macrophages to prevent infections that are complicated with lymphoedema. The significance of their results lies in the fact that VEGF-C-VEGFR-3 signaling represents a "self-control" mechanism during antibacterial innate immunity<sup>55</sup> (Table 2).

**Table 2** Expression of VEGF receptors in different immune and cancer cells

Cell type	Expression of VEGF receptors	Reference
Hematopoietic cell	VEGFR-1	30
Dendritic cell	VEGFR-3	28
Macrophage	VEGFR-1, VEGFR-3	53-55
T lymphocyte	VEGFR-1, VEGFR-2	38,56
Treg tumor cell	VEGFR-2, NRP-1	40
	VEGFR-1, VEGFR-2, NRP-1	57,58

## VEGF/VEGFR and macrophage function

Identifying the functions of the VEGF/VEGFR axis in macrophage infiltration/activation and the manner in which these tumor-educated macrophages affect tumor progression is important because infiltrating macrophages are correlated significantly and negatively with cancer patient prognosis and survival<sup>59,60</sup>. Reports show that tumor-associated macrophages play a crucial role in angiogenesis and lymphangiogenesis: on the one hand, they can directly incorporate into the endothelial layer; on the other hand, they can stimulate the division of preexistent local lymphatic ECs under inflammatory conditions<sup>61,62</sup>. However, whether the VEGF/VEGFR signaling mediated the interplay in this process is unclear. Su et al.<sup>36</sup> also found that VEGF-C plays a critical role in the macrophage infiltration in lung cancer through VEGFR-3, and this VEGFR-3-mediated macrophage infiltration may be involved in the radiosensitization of lung cancer. As mentioned above, VEGF can also stimulate the migration of macrophages, and the VEGF-C/VEGFR-3 signaling can protect against endotoxin shock during severe infection<sup>55</sup>. Furthermore, in renal clear cell carcinoma, the knockdown of VEGFR-1 impairs macrophage infiltration, angiogenesis, and growth of the tumor cell<sup>63</sup>.

Another unresolved problem is the membranous or nuclear localization of VEGF receptors. This problem is unresolved because many RTKs are detected in the cell nucleus and function as transcription cofactors to activate gene promoters, such as the EGFR family<sup>64</sup>. Evidence also shows that deleting the VEGF expression in the EC lineage can lead to the widespread degeneration of the endothelium and the sudden death of more than half of the mutant mice<sup>65</sup>. We speculate that the autocrine VEGF signaling in ECs seems to be entirely intracellular because only cell-permeable VEGF receptor antagonists, as opposed to non-permeable antagonists, can produce an inhibitory effect. This speculation is in concert with the finding that VEGF-C-activated VEGFR-3 was translocated to the nucleus of lung adenocarcinoma cells and primary lymphatic ECs<sup>36</sup>.

## Relationship between VEGF/VEGFR and T cells

T cells are master regulators of immune system function, continually walking the biological tightrope between adequate host defense and accidental host pathology. Initial studies showed that when mice were exposed to recombinant VEGF at concentrations equal to those observed in advanced-stage cancer patients, they can produce profound thymic

atrophy and a dramatic reduction in CD4/CD8 thymocytes<sup>37</sup>. The mechanism of this effect lies in the fact that VEGF did not induce thymocyte apoptosis but instead downregulated the number of the earliest observable progenitors in the thymus. These data demonstrate that at pathophysiological concentrations, VEGF interferes with the development of T cells from early HPCs, and this interference may contribute to tumor-associated immune deficiencies.

Researchers have identified that the CD45RO<sup>+</sup> memory populations of CD4<sup>+</sup> T lymphocytes express VEGFR-1 and -2 at the mRNA and protein levels. Moreover, VEGF stimulates KDR-mediated signals, costimulates the IFN- $\alpha$  production, and increases the chemotaxis within this subtype of T cells<sup>38</sup>. Gavalas et al.<sup>56</sup> demonstrated for the first time that T lymphocytes derived from ovarian cancer patients' ascites secrete VEGF and express VEGFR-2 on their surface upon their activation. In turn, this process promotes the suppression of activated T cells by the VEGF produced by tumor cells, thus leading to an immunosuppressive effect. In addition, FOXP3<sup>+</sup> regulatory T cells, which suppress an antitumor immune response, express NRP-1 and are "guided" to the tumors by VEGF, which functions as a chemoattractant<sup>6</sup>. Wada et al.<sup>40</sup> showed that VEGFR-2 is expressed in Tregs, and VEGF contributes to the induction or maintenance of Tregs in the tumor microenvironment.

VEGF regulates T cell responses. The VEGF-C/VEGFR-3 signaling can modulate innate and adaptive immune responses in the development of obliterative airway disease (OAD) in rat tracheal allografts. VEGF-C overexpression in tracheal allografts induces epithelial activation, neutrophil chemotaxis, and a shift toward a Th17-adaptive immune response. Subsequently, lymphangiogenesis is enhanced, and OAD develops. By contrast, the inhibition of VEGF-C activity with VEGFR-3-Ig inhibited lymphangiogenesis and reduced the infiltration of CD4<sup>+</sup> T cells and the development of OAD. Krebs et al.<sup>39</sup> suggested VEGFR-3-signaling as a novel strategy to regulate T cell responses in the development of obliterative bronchiolitis after lung transplantation. Reports also suggested that VEGF contributes to tumor growth through an indirect mechanism of the regulation of T cells against tumor cells<sup>66</sup>.

## VEGF can be secreted by tumor cells and further stimulate cancer stemness

Apart from interacting with different immune cells, VEGF/VEGFR signaling also plays a vital role in cancer. Generally, VEGF produced by tumor cells is accepted to act on neighboring VEGFR-expressing ECs to promote

neovascularization for continued tumor growth<sup>67</sup>. This concept has been challenged by reports demonstrating the expression of the Flt-1, Flk-1, and NRP-1 in tumor cells<sup>57</sup>. Tumor cells themselves can express VEGF receptors and respond to autocrine and paracrine VEGF signals. Later studies also established that VEGF receptors are abundantly expressed by a large percentage of solid tumors<sup>68</sup>, and the VEGF/VEGFR signaling is significantly and negatively correlated to the progression of certain types of cancer<sup>69, 70</sup>. Lichtenberger et al.<sup>58</sup> demonstrated that *in vivo* autocrine VEGF is required for epithelial tumor cell proliferation by activating Flt-1 in a cell-autonomous and angiogenesis-independent manner. Moreover, Flt-1 is expressed both intracellularly and in the cell membrane in human squamous cell carcinoma biopsies. Surprisingly, tumor development was completely inhibited in the absence of epidermal VEGF and EGFR expression, demonstrating a synergistic, tumor-promoting effect of EGFR and VEGF signaling in neoplastic cells<sup>58</sup>. The reason lies in the fact that EGFR signaling can upregulate VEGF, Flt-1, and NRP-1 in an Erk-dependent manner, which activates an autocrine proliferation loop, whereas EGFR prevents tumor cells from apoptosis. VEGF signaling has also been implicated in the ability of breast cancer to proliferate, evade apoptosis, and migrate. Studies also show that VEGFR-2 are expressed in circulating epithelial tumor cells of breast-cancer patients<sup>71</sup>. Lysophosphatidic acid (LPA) stands out as a unique lysophospholipid growth factor that regulates multiple events to promote epithelial ovarian cancer (EOC) invasion and metastasis within the ovarian tumor microenvironment<sup>72</sup>. Studies have shown that LPA induces the expression of VEGF and IL-8 to promote EOC invasion and migration<sup>73</sup>. Recently, Wang et al.<sup>41</sup> reported that LPA-induced EOC invasion is partially mediated by the VEGF/VEGFR-2 signaling axis. Consistently, Dutta et al.<sup>74</sup> showed that the NF- $\kappa$ B pathway mediates the LPA-induced VEGF/VEGFR-2 signaling and EOC invasion.

The VEGF/VEGFR axis has different biological effects on cancer cells. VEGF-A was reported to promote the migration of cancer cells<sup>42</sup>. VEGF-C was reported to enhance resistance to chemotherapy and induction of Bcl-2 in leukemia cells through the activation of Flt-4 and KDR heterodimer<sup>43</sup>. VEGF-C/VEGFR-3 axis enhances cancer cell mobility and invasion capabilities and promotes cancer cell metastasis. Using a mouse model of skin tumor, Beck et al.<sup>7</sup> identified a dual role for tumor-cell-derived VEGF in promoting cancer stemness: (I) by stimulating angiogenesis in a paracrine manner, VEGF creates a perivascular niche for cancer stem cells (CSCs); (II) by directly affecting CSCs through NRP-1

in an autocrine loop, VEGF stimulates cancer stemness and renewal. These identifications are in concert with another study that reveals that autocrine VEGF/VEGFR-2-NRP-1 signaling promotes glioma stem-like cell viability and tumor growth<sup>44</sup>. Waldner et al.<sup>75</sup> observed that chronic inflammation leads to an upregulation of VEGFR-2 on intestinal epithelial cells. VEGF receptor signaling links inflammation and tumorigenesis in colitis associated cancer.

## Conclusions

VEGF/VEGFR signaling has a complicated relationship with immune and cancer cells, which is beyond its prominent proangiogenic property. On the one hand, it can cause an aberrant hematopoiesis that inflicts the development process of DCs and mononuclear and T lymphocyte lineage. On the other hand, VEGF has a context-dependent interaction with the immune cells varying on the disease type and the cytokine milieu. Moreover, increasing evidence has identified the expression of different types of VEGF receptors in immune cells; although scientists have not arrived at a consistent conclusion, they have proposed some possible mechanisms by which VEGF plays its biological role. But several issues remain to be addressed. First, are VEGF receptors steadily expressed, or are they markers of immune cells? Second, are they only transiently expressed in a certain development stage, or are they expressed only in a small subtype of immune cells? Many undefined functions and molecular mechanisms are involved in this process. We are looking forward to future studies to answer the question: staggering or forward?

The autocrine VEGF signaling can promote tumor initiation and stimulate cancer stemness, and it is an alert on developing targeted therapies. Tumor cells themselves can express VEGF receptors, and they may enable VEGF to perform its function in a completely different manner. We can imagine that in the tumor environment, VEGF/VEGFR signaling can simultaneously influence the immune and cancer cells, leading to comprised immune response and unrestrained proliferation. As a new method for tumor cells to invade the immune surveillance, VEGF/VEGFR signaling has broadened our insights into cancer development. However, more studies are needed to explore and dampen the mechanisms by which VEGF interacts with immune and cancer cells.

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## Conflict of interest statement

No penitential conflicts of interest are disclosed.

## References

- Leung DW, Cachianes G, Kuang WJ, Goeddel DV, Ferrara N. Vascular endothelial growth factor is a secreted angiogenic mitogen. *Science*. 1989; 246: 1306-9.
- Senger DR. Vascular endothelial growth factor: much more than an angiogenesis factor. *Mol Biol Cell*. 2010; 21: 377-9.
- Ferrara N, Carver-Moore K, Chen H, Dowd M, Lu L, O'Shea KS, et al. Heterozygous embryonic lethality induced by targeted inactivation of the VEGF gene. *Nature*. 1996; 380: 439-42.
- Ziegler BL, Valtieri M, Porada GA, De Maria R, Muller R, Masella B, et al. KDR receptor: a key marker defining hematopoietic stem cells. *Science*. 1999; 285: 1553-8.
- Yaqoob U, Cao S, Shergill U, Jagavelu K, Geng Z, Yin M, et al. Neuropilin-1 stimulates tumor growth by increasing fibronectin fibril assembly in the tumor microenvironment. *Cancer Res*. 2012; 72: 4047-59.
- Hansen W, Hutzler M, Abel S, Alter C, Stockmann C, Kliche S, et al. Neuropilin 1 deficiency on CD4+Foxp3+ regulatory T cells impairs mouse melanoma growth. *J Exp Med*. 2012; 209: 2001-16.
- Beck B, Driessens G, Goossens S, Youssef KK, Kuchnio A, Caauwe A, et al. A vascular niche and a VEGF-Nrp1 loop regulate the initiation and stemness of skin tumours. *Nature*. 2011; 478: 399-403.
- Philip B, Ito K, Moreno-Sanchez R, Ralph SJ. HIF expression and the role of hypoxic microenvironments within primary tumours as protective sites driving cancer stem cell renewal and metastatic progression. *Carcinogenesis*. 2013; 34: 1699-707.
- Koch S, Claesson-Welsh L. Signal transduction by vascular endothelial growth factor receptors. *Cold Spring Harb Perspect Med*. 2012; 2: a006502.
- Roberts WG, Palade GE. Increased microvascular permeability and endothelial fenestration induced by vascular endothelial growth factor. *J Cell Sci*. 1995; 108 (Pt 6): 2369-79.
- Brogi E, Wu T, Namiki A, Isner JM. Indirect angiogenic cytokines upregulate VEGF and bFGF gene expression in vascular smooth muscle cells, whereas hypoxia upregulates VEGF expression only. *Circulation*. 1994; 90: 649-52.
- Kikuchi K, Kusama K, Sano M, Nakanishi Y, Ishige T, Ohni S, et al. Vascular endothelial growth factor and dendritic cells in human squamous cell carcinoma of the oral cavity. *Anticancer Res*. 2006; 26: 1833-48.
- Kowanetz M, Ferrara N. Vascular endothelial growth factor signaling pathways: therapeutic perspective. *Clin Cancer Res*. 2006; 12: 5018-22.
- Shibuya M, Yamaguchi S, Yamane A, Ikeda T, Tojo A, Matsushime H, et al. Nucleotide sequence and expression of a novel human receptor-type tyrosine kinase gene (flt) closely related to the fms family. *Oncogene*. 1990; 5: 519-24.
- Neufeld G, Kessler O, Herzog Y. The interaction of Neuropilin-1 and Neuropilin-2 with tyrosine-kinase receptors for VEGF. *Adv Exp Med Biol*. 2002; 515: 81-90.
- Jiang T, Zhuang J, Duan H, Luo Y, Zeng Q, Fan K, et al. CD146 is a coreceptor for VEGFR-2 in tumor angiogenesis. *Blood*. 2012; 120: 2330-9.
- Kendall RL, Thomas KA. Inhibition of vascular endothelial cell growth factor activity by an endogenously encoded soluble receptor. *Proc Natl Acad Sci U S A*. 1993; 90: 10705-9.
- Singh N, Tiem M, Watkins R, Cho YK, Wang Y, et al. Soluble vascular endothelial growth factor receptor 3 is essential for corneal alymphaticity. *Blood*. 2013; 121: 4242-9.
- Dikov MM, Ohm JE, Ray N, Tchekneva EE, Burlison J, Moghanaki D, et al. Differential roles of vascular endothelial growth factor receptors 1 and 2 in dendritic cell differentiation. *J Immunol*. 2005; 174: 215-22.
- Ginhoux F, Tacke F, Angeli V, Bogunovic M, Loubeau M, Dai XM, et al. Langerhans cells arise from monocytes in vivo. *Nat Immunol*. 2006; 7: 265-73.
- Hoehn GT, Stokland T, Amin S, Ramirez M, Hawkins AL, Griffin CA, et al. Tnk1: a novel intracellular tyrosine kinase gene isolated from human umbilical cord blood CD34+/Lin-/CD38- stem/progenitor cells. *Oncogene*. 1996; 12: 903-13.
- Katoh O, Tauchi H, Kawaiishi K, Kimura A, Satow Y. Expression of the vascular endothelial growth factor (VEGF) receptor gene, KDR, in hematopoietic cells and inhibitory effect of VEGF on apoptotic cell death caused by ionizing radiation. *Cancer Res*. 1995; 55: 5687-92.
- Kaipainen A, Korhonen J, Mustonen T, van Hinsbergh VW, Fang GH, Dumont D, et al. Expression of the fms-like tyrosine kinase 4 gene becomes restricted to lymphatic endothelium during development. *Proc Natl Acad Sci U S A*. 1995; 92: 3566-70.
- Partanen TA, Arola J, Saaristo A, Jussila L, Ora A, Miettinen M, et al. VEGF-C and VEGF-D expression in neuroendocrine cells and their receptor, VEGFR-3, in fenestrated blood vessels in human tissues. *FASEB J*. 2000; 14: 2087-96.
- Wilting J, Eichmann A, Christ B. Expression of the avian VEGF receptor homologues Quek1 and Quek2 in blood-vascular and lymphatic endothelial and non-endothelial cells during quail embryonic development. *Cell Tissue Res*. 1997; 288: 207-23.
- Mimura T, Amano S, Usui T, Kaji Y, Oshika T, Ishii Y. Expression of vascular endothelial growth factor C and vascular endothelial growth factor receptor 3 in corneal lymphangiogenesis. *Exp Eye Res*. 2001; 72: 71-8.
- Cursiefen C, Schlotzer-Schrehardt U, Kuchle M, Sorokin L, Breiteneder-Geleff S, Alitalo K, et al. Lymphatic vessels in vascularized human corneas: immunohistochemical investigation using LYVE-1 and podoplanin. *Invest Ophthalmol Vis Sci*. 2002;

- 43: 2127-35.
28. Hamrah P, Zhang Q, Dana MR. Expression of vascular endothelial growth factor receptor-3 (VEGFR-3) in the conjunctiva--a potential link between lymphangiogenesis and leukocyte trafficking on the ocular surface. *Adv Exp Med Biol.* 2002; 506: 851-60.
  29. Gabrilovich DI, Chen HL, Girgis KR, Cunningham HT, Meny GM, Nadaf S, et al. Production of vascular endothelial growth factor by human tumors inhibits the functional maturation of dendritic cells. *Nat Med.* 1996; 2: 1096-103.
  30. Oyama T, Ran S, Ishida T, Nadaf S, Kerr L, Carbone DP, et al. Vascular endothelial growth factor affects dendritic cell maturation through the inhibition of nuclear factor-kappa B activation in hemopoietic progenitor cells. *J Immunol.* 1998; 160: 1224-32.
  31. Ohm JE, Carbone DP. VEGF as a mediator of tumor-associated immunodeficiency. *Immunol Res.* 2001; 23: 263-72.
  32. Chapoval SP, Lee CG, Tang C, Keegan AD, Cohn L, Bottomly K, et al. Lung vascular endothelial growth factor expression induces local myeloid dendritic cell activation. *Clin Immunol.* 2009; 132: 371-84.
  33. Riboldi E, Musso T, Moroni E, Urbinati C, Bernasconi S, Rusnati M, et al. Cutting edge: proangiogenic properties of alternatively activated dendritic cells. *J Immunol.* 2005; 175: 2788-92.
  34. Fernandez Pujol B, Lucibello FC, Gehling UM, Lindemann K, Weidner N, Zuzarte ML, et al. Endothelial-like cells derived from human CD14 positive monocytes. *Differentiation.* 2000; 65: 287-300.
  35. Peichev M, Naiyer AJ, Pereira D, Zhu Z, Lane WJ, Williams M, et al. Expression of VEGFR-2 and AC133 by circulating human CD34(+) cells identifies a population of functional endothelial precursors. *Blood.* 2000; 95: 952-8.
  36. Su JL, Yen CJ, Chen PS, Chuang SE, Hong CC, Kuo IH, et al. The role of the VEGF-C/VEGFR-3 axis in cancer progression. *Br J Cancer.* 2007; 96: 541-5.
  37. Ohm JE, Gabrilovich DI, Sempowski GD, Kisseleva E, Parman KS, Nadaf S, et al. VEGF inhibits T-cell development and may contribute to tumor-induced immune suppression. *Blood.* 2003; 101: 4878-86.
  38. Basu A, Hoerning A, Datta D, Edelbauer M, Stack MP, Calzadilla K, et al. Cutting edge: Vascular endothelial growth factor-mediated signaling in human CD45RO+ CD4+ T cells promotes Akt and ERK activation and costimulates IFN-gamma production. *J Immunol.* 2010; 184: 545-9.
  39. Krebs R, Tikkanen JM, Ropponen JO, Jeltsch M, Jokinen JJ, Yla-Herttuala S, et al. Critical role of VEGF-C/VEGFR-3 signaling in innate and adaptive immune responses in experimental obliterative bronchiolitis. *Am J Pathol.* 2012; 181: 1607-20.
  40. Wada J, Suzuki H, Fuchino R, Yamasaki A, Nagai S, Yanai K, et al. The contribution of vascular endothelial growth factor to the induction of regulatory T-cells in malignant effusions. *Anticancer Res.* 2009; 29: 881-8.
  41. Wang FQ, Barfield E, Dutta S, Pua T, Fishman DA. VEGFR-2 silencing by small interference RNA (siRNA) suppresses LPA-induced epithelial ovarian cancer (EOC) invasion. *Gynecol Oncol.* 2009; 115: 414-23.
  42. Wang FQ, So J, Reierstad S, Fishman DA. Vascular endothelial growth factor-regulated ovarian cancer invasion and migration involves expression and activation of matrix metalloproteinases. *Int J Cancer.* 2006; 118: 879-88.
  43. Dias S, Choy M, Alitalo K, Rafii S. Vascular endothelial growth factor (VEGF)-C signaling through FLT-4 (VEGFR-3) mediates leukemic cell proliferation, survival, and resistance to chemotherapy. *Blood.* 2002; 99: 2179-84.
  44. Hamerlik P, Lathia JD, Rasmussen R, Wu Q, Bartkova J, Lee M, et al. Autocrine VEGF-VEGFR2-Neuropilin-1 signaling promotes glioma stem-like cell viability and tumor growth. *J Exp Med.* 2012; 209: 507-20.
  45. Penna G, Adorini L. 1 Alpha, 25-dihydroxyvitamin D3 inhibits differentiation, maturation, activation, and survival of dendritic cells leading to impaired alloreactive T cell activation. *J Immunol.* 2000; 164: 2405-11.
  46. Goerdts S, Orfanos CE. Other functions, other genes: alternative activation of antigen-presenting cells. *Immunity.* 1999; 10: 137-42.
  47. Ferrara N, Gerber HP, LeCouter J. The biology of VEGF and its receptors. *Nat Med.* 2003; 9: 669-76.
  48. Fernandez Pujol B, Lucibello FC, Zuzarte M, Lutjens P, Muller R, Havemann K. Dendritic cells derived from peripheral monocytes express endothelial markers and in the presence of angiogenic growth factors differentiate into endothelial-like cells. *Eur J Cell Biol.* 2001; 80: 99-110.
  49. Fantin A, Vieira JM, Gestri G, Denti L, Schwarz Q, Prykhodzhiy S, et al. Tissue macrophages act as cellular chaperones for vascular anastomosis downstream of VEGF-mediated endothelial tip cell induction. *Blood.* 2010; 116: 829-40.
  50. Takeda Y, Costa S, Delamarre E, Roncal C, Leite de Oliveira R, Squadrito ML, et al. Macrophage skewing by Phd2 haploinsufficiency prevents ischaemia by inducing arteriogenesis. *Nature.* 2011; 479: 122-6.
  51. Wynn TA, Chawla A, Pollard JW. Macrophage biology in development, homeostasis and disease. *Nature.* 2013; 496: 445-55.
  52. Barleon B, Sozzani S, Zhou D, Weich HA, Mantovani A, Marme D. Migration of human monocytes in response to vascular endothelial growth factor (VEGF) is mediated via the VEGF receptor flt-1. *Blood.* 1996; 87: 3336-43.
  53. Sawano A, Iwai S, Sakurai Y, Ito M, Shitara K, Nakahata T, et al. Flt-1, vascular endothelial growth factor receptor 1, is a novel cell surface marker for the lineage of monocyte-macrophages in humans. *Blood.* 2001; 97: 785-91.
  54. Schoppmann SF, Birner P, Stockl J, Kalt R, Ullrich R, Caucig C, et al. Tumor-associated macrophages express lymphatic endothelial growth factors and are related to peritumoral lymphangiogenesis. *Am J Pathol.* 2002; 161: 947-56.
  55. Zhang Y, Lu Y, Ma L, Cao X, Xiao J, Chen J, et al. Activation of vascular endothelial growth factor receptor-3 in macrophages restrains TLR4-NF-kappaB signaling and protects against endotoxin shock. *Immunity.* 2014; 40: 501-14.
  56. Gavalas NG, Tsiatas M, Tsitsilonis O, Politi E, Ioannou K, Ziogas AC, et al. VEGF directly suppresses activation of T cells from ascites



- secondary to ovarian cancer via VEGF receptor type 2. *Br J Cancer*. 2012; 107: 1869-75.
57. Chung GG, Yoon HH, Zerkowski MP, Ghosh S, Thomas L, Harigopal M, et al. Vascular endothelial growth factor, FLT-1, and FLK-1 analysis in a pancreatic cancer tissue microarray. *Cancer*. 2006; 106: 1677-84.
  58. Lichtenberger BM, Tan PK, Niederleithner H, Ferrara N, Petzelbauer P, Sibilina M. Autocrine VEGF signaling synergizes with EGFR in tumor cells to promote epithelial cancer development. *Cell*. 2010; 140: 268-79.
  59. Ogawa E, Takenaka K, Yanagihara K, Kurozumi M, Manabe T, Wada H, et al. Clinical significance of VEGF-C status in tumour cells and stromal macrophages in non-small cell lung cancer patients. *Br J Cancer*. 2004; 91: 498-503.
  60. Schoppmann SF, Fenzl A, Nagy K, Unger S, Bayer G, Geleff S, et al. VEGF-C expressing tumor-associated macrophages in lymph node positive breast cancer: impact on lymphangiogenesis and survival. *Surgery*. 2006; 139: 839-46.
  61. Kerjaschki D. The crucial role of macrophages in lymphangiogenesis. *J Clin Invest*. 2005; 115: 2316-9.
  62. Maruyama K, Ii M, Cursiefen C, Jackson DG, Keino H, Tomita M, et al. Inflammation-induced lymphangiogenesis in the cornea arises from CD11b-positive macrophages. *J Clin Invest*. 2005; 115: 2363-72.
  63. Li C, Liu B, Dai Z, Tao Y. Knockdown of VEGF receptor-1 (VEGFR-1) impairs macrophage infiltration, angiogenesis and growth of clear cell renal cell carcinoma (CRCC). *Cancer Biol Ther*. 2011; 12: 872-80.
  64. Lo HW, Hung MC. Nuclear EGFR signalling network in cancers: linking EGFR pathway to cell cycle progression, nitric oxide pathway and patient survival. *Br J Cancer*. 2006; 94: 184-8.
  65. Lee S, Chen TT, Barber CL, Jordan MC, Murdock J, Desai S, et al. Autocrine VEGF signaling is required for vascular homeostasis. *Cell*. 2007; 130: 691-703.
  66. Zindl CL, Chaplin DD. Immunology. Tumor immune evasion. *Science*. 2010; 328: 697-8.
  67. Bergers G, Benjamin LE. Tumorigenesis and the angiogenic switch. *Nat Rev Cancer*. 2003; 3: 401-10.
  68. Toi M, Taniguchi T, Yamamoto Y, Kurisaki T, Suzuki H, Tominaga T. Clinical significance of the determination of angiogenic factors. *Eur J Cancer*. 1996; 32A: 2513-9.
  69. Jüttner S, Wissmann C, Jöns T, Vieth M, Hertel J, Gretschel S, et al. Vascular endothelial growth factor-D and its receptor VEGFR-3: two novel independent prognostic markers in gastric adenocarcinoma. *J Clin Oncol*. 2006; 24: 228-40.
  70. Su JL, Yang PC, Shih JY, Yang CY, Wei LH, Hsieh CY, et al. The VEGF-C/Flt-4 axis promotes invasion and metastasis of cancer cells. *Cancer Cell*. 2006; 9: 209-23.
  71. Pizon M, Zimon DS, Pachmann U, Pachmann K. Insulin-like growth factor receptor I (IGF-IR) and vascular endothelial growth factor receptor 2 (VEGFR-2) are expressed on the circulating epithelial tumor cells of breast cancer patients. *PLoS One*. 2013; 8: e56836.
  72. Meng Y, Kang S, So J, Reierstad S, Fishman DA. Translocation of Fas by LPA prevents ovarian cancer cells from anti-Fas-induced apoptosis. *Gynecol Oncol*. 2005; 96: 462-9.
  73. Hu YL, Tee MK, Goetzl EJ, Auersperg N, Mills GB, Ferrara N, et al. Lysophosphatidic acid induction of vascular endothelial growth factor expression in human ovarian cancer cells. *J Natl Cancer Inst*. 2001; 93: 762-8.
  74. Dutta S, Wang FQ, Wu HS, Mukherjee TJ, Fishman DA. The NF-kappaB pathway mediates lysophosphatidic acid (LPA)-induced VEGF signaling and cell invasion in epithelial ovarian cancer (EOC). *Gynecol Oncol*. 2011; 123: 129-37.
  75. Waldner MJ, Wirtz S, Jefremow A, Warntjen M, Neufert C, Atreya R, et al. VEGF receptor signaling links inflammation and tumorigenesis in colitis-associated cancer. *J Exp Med*. 2010; 207: 2855-68.
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