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Anti-VEGF Therapy in Cancer: A Double-Edged Sword

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Abstract

Vascular endothelial growth factor (VEGF) is a mitogen that plays a crucial role in angiogenesis and lymphangiogenesis. It is involved in tumor survival through inducing tumor angiogenesis and by increasing chemoresistance through autocrine signaling. Because of its importance in tumor formation and survival, several medications have been developed to inhibit VEGF and reduce blood vessel formation in cancer. Although these medications have proven to be effective for late-stage and metastatic cancers, they have been shown to cause side effects such as hypertension, artery clots, complications in wound healing, and, more rarely, gastrointestinal perforation and fistulas. Current research in using anti-VEGF medication as a part of cancer treatments is focusing on elucidating the mechanisms of tumor resistance to VEGF medication, developing predictive biomarkers that assess whether a patient will respond to VEGF therapy and creating novel treatments and techniques that increase the efficacy of antiangiogenic medication. This chapter aims to review the role of VEGF in tumor angiogenesis and metastasis, the structure and function of VEGF and its receptors, and VEGF's role in cancer are discussed. Furthermore, tumor therapies targeting VEGF along with their side effects are presented and, finally, new directions in anti-VEGF therapy are considered along with the challenges.

Keywords: VEGF, angiogenesis, side effect, medication

1. Introduction

Oxygen and nutrients are critical to the functioning and survival of cells in the body. This need is met through the creation of an extensive vascular system, which is maintained through the process of angiogenesis, and the creation of blood vessels from the existing vasculature [1]. In the angiogenesis process, endothelial cells initially respond to changes in the local environment and migrate toward the growing tumor. The endothelial cells then migrate

together forming tubular structures that are ultimately encapsulated by recruiting periendothelial support cells to establish a vascular network that facilitates tumor growth and metastasis. Angiogenesis is subject to a complex regulatory system of both pro- and antiangiogenic factors after a tissue is fashioned [1–3] and deregulating angiogenesis—a classic trademark of cancer—leads to an aberrant microenvironment and promotion of tumor progression. Angiogenesis is initiated by the binding actions of vascular endothelial growth factor (VEGF) and fibroblast growth factors (FGF1/2) [4].

VEGF is an essential proangiogenic factor whose production is itself extensively regulated by a plethora of growth factors, cytokines, and other extracellular molecules produced in response to the various metabolic and mechanical conditions present in the cell's environment [2–6]. VEGF plays a pivotal role in tumor angiogenesis. The overexpression of VEGF is one of the central factors that leads to the onset and progression of cancer. In order to sustain their growth beyond any current size, tumors require an increased supply of blood, and this is achieved through the expression and secretion of VEGF, which stimulates the induction of new blood vessels around the tumor. Furthermore, the cancer cells, through the action of this subfamily of growth factors, invade other organs and areas of the body (metastasis) [4]. Consequently, VEGF and the resulting tumor angiogenesis present an attractive therapeutic target in the treatment of cancer. Inhibitors of VEGF/angiogenesis have been garnering interest and studied for their therapeutic application in most solid tumors [7, 8]. Moreover, a series of preclinical studies have revealed that anti-VEGF compounds increase the efficacy of ensuing antitumor treatment, although the mechanism of this effect is unclear [9].

2. Structure and function of VEGF and VEGFRs

VEGF is a dimeric glycoprotein secreted by cells that is able to induce permeability of blood vessels and promotes angiogenesis. The VEGF family contains seven members, all part of the platelet-derived growth factor (PDGF) supergene family: VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, VEGF-F, and PlGF [10, 11]. All members contain a core region comprised of eight cysteine residues forming a cysteine knot motif. These residues are involved in both inter- and intramolecular disulfide bonds at one end of a central four-stranded β -sheet within each monomer, which dimerizes in antiparallel fashion [11]. VEGFs A–D and PlGF are all produced in humans, whereas VEGF-E is produced in the Orf virus, has a 25% amino acid homology to mammalian VEGF, and lacks a heparin-binding domain [12]. VEGF-F is produced in snake venom and varies its structure and function by species, helping to produce a variety of venom [13].

The VEGF-A gene contains eight different exons that create six different isoforms through alternative splicing. These isoforms have lengths (in amino acids) of 121, 145, 165, 183, 189, and 206 that are produced by the alternate splicing of a single gene containing eight exons, and they all contain exons 1–5 and 8. All forms of VEGF-A except for VEGF-A₁₂₁ can bind to heparin [11]. VEGF₁₆₅ is the one most commonly secreted by tumor cells and acts most strongly

on endothelial cells to lead them to form new capillaries. VEGF-B exists as two isoforms of lengths 167 and 186 amino acids and has been shown to act as a cell survival factor while exhibiting little proangiogenic effect [14]. VEGF-C and VEGF-D are released proteolytically from their respective precursor proteins and play important roles in regulating lymphangiogenesis [10, 11]. PlGF upregulates angiogenesis through binding to VEGFR-1 (thereby freeing VEGF-A to bind to VEGFR-2) and exists in four isoforms of amino acid lengths 131, 152, 203, and 224 [15]. It has also been shown to induce a specific phosphorylation and activation of c-Jun N-terminal kinase (JNK) and p38 [16].

VEGF signaling pathway plays a major role in angiogenesis. VEGF receptors (VEGFRs) are type V receptor tyrosine kinases (RTKs) activated upon ligand-mediated dimerization [17]. Two high-affinity VEGF receptors, VEGFR-1 (Flt-1) and VEGFR-2 (Flk-1/KDR), have been identified in endothelial cells. Flk-1 (R2) has been shown to play a major role in tumor angiogenesis. In all, there are three types of receptors, with VEGFR-3 only binding to VEGFs -C and -D. Each of the three types of receptor (VEGFR-1, -2, and -3) is composed of seven immunoglobulin-like domains in the extracellular region, a transmembrane region, and a tyrosine kinase sequence with a kinase insert domain [11].

Signaling of VEGF is initiated via binding to its receptors, which are tyrosine kinases that are able to transphosphorylate tyrosine residues of SH2 domain-containing signaling molecules, thus activating kinase-dependent transcription factors known as STAT proteins, to modulate cell responses induced by VEGF. VEGFR-1 and -2 are both involved in endothelial cell function and angiogenesis [18], while VEGFR-3, to which only VEGFs -C and -D can bind, plays a critical role in lymphangiogenesis and primarily involved in normal embryonic development [11]. VEGF-1 has also been shown to be required in inducing the migration of monocytes and macrophages [19]. Neuropilins-1 and -2 are important coreceptors for VEGF signaling and increase the affinity of VEGF-A₁₆₅ for its receptors [5]. Refer to **Figure 1** for a summary of how each VEGF pairs with each VEGF receptor.

VEGF is heavily involved in promoting angiogenesis and research suggests that it also plays a role in regulating intussusceptive angiogenesis as well. In sprouting angiogenesis, hypoxia induces parenchymal cells to release VEGF-A into the extracellular matrix (ECM). VEGF-A then causes tip cells to produce the Delta-like-4 (Dll4) ligand, which is a membrane-bound ligand that serves to activate the Notch receptor, a highly conserved transmembrane receptor that regulates cell proliferation, cell fate, and cell death in metazoans on neighboring cells through cell to cell contact [20]. Dll4 then inhibits migratory behavior through activating the Notch receptor on neighboring stalk cells. Tip cell filopodia, actin-rich protrusions on the cell membrane that serve as a mechanism for a cell to explore its environment, sense a gradient in VEGF-A and align their sprouting to this gradient. The tip cell then anchors itself onto the substratum while actin microfilaments in the filopodia contract, pulling the tip cell toward the VEGF-A source while stalk cells proliferate. When the tip cells from different sprouts meet, they fuse to become a functional capillary through which blood can flow [1]. The function of VEGF in sprouting angiogenesis is less well understood, although it is suspected that VEGF cooperating with angiopoietin-1 (Ang-1) plays a role in stimulating the process [21, 22].

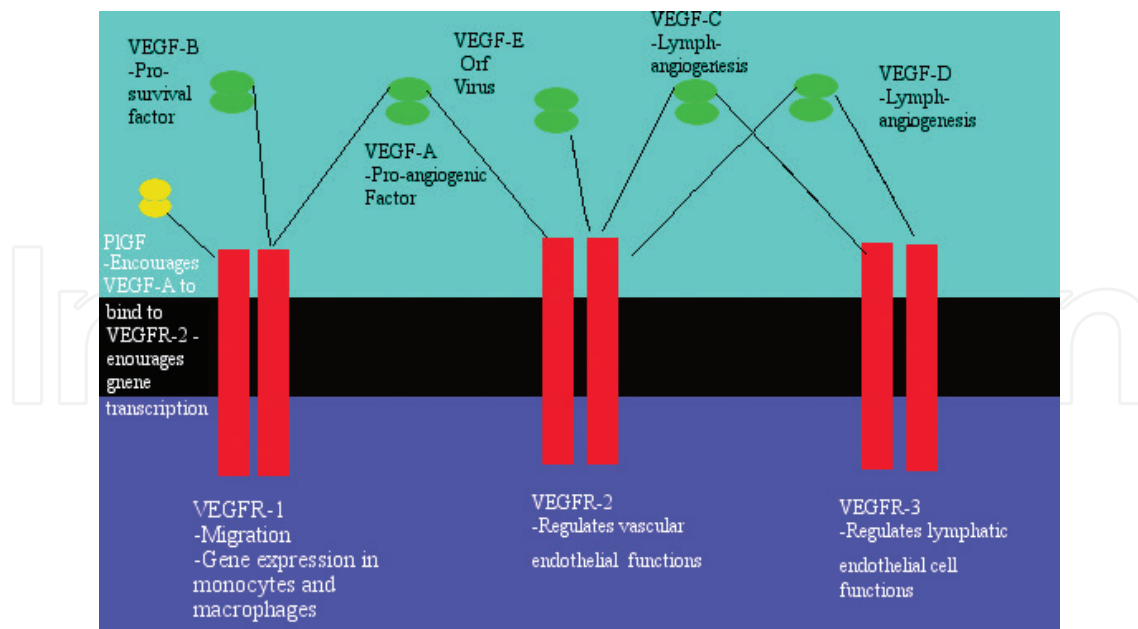


Figure 1. A summary of the functions of each form of VEGF and PlGF and each VEGF receptor. VEGFR-1 regulates cell migration and gene expression in monocytes and macrophages; VEGFR-2 regulates vascular endothelial functions; and VEGFR-3 regulates lymphatic endothelial functions. VEGF-A is a proangiogenic factor; VEGF-B is a prosurvival factor; VEGF-C and -D regulate lymphangiogenesis; VEGF-E is found in the Orf virus; and PlGF encourages VEGF-A to bind to VEGFR-2, thereby stimulating angiogenesis, and encourages the transcription of JNK and p38.

3. The role of VEGF in cancer

Tumors need oxygen to survive. At first, they are able to obtain enough oxygen by coopting the surrounding vasculature, altering its morphology, physiology, and response to therapy in the process. However, when a tumor becomes too large to be sufficiently supplied by existing vasculature, an “angiogenic switch” is turned on, and the tumor begins the process of tumor angiogenesis, thereby creating its own vasculature for an oxygen supply [23–26].

The angiogenic switch is triggered by hypoxia occurring when the tumor becomes too large for oxygen to diffuse from existing vasculature to tumor cells [27]. Hypoxia induces the production of VEGF in tumor cells through hypoxia-inducible factor-1 α (HIF-1 α) [25, 27], a master transcriptional factor that regulates a group of downstream genes including VEGF that promote angiogenesis and metastasis, while inhibitors of angiogenesis such as angiostatin and interferon are downregulated [23]. A suite of other proangiogenic genes (such as Ang-1 and -2) and regulatory mechanisms (such as micro-RNAs) are also regulated by the hypoxia-induced HIF pathway [25]. Tumor cells then release VEGF into the surrounding extracellular space, which binds to VEGF R of surrounding or nearby endothelial cells, promoting local angiogenesis and forming tumor-associated microvessels in order to delivering oxygen-carrying blood to the tumor.

Compared with normal vasculature, tumor vessels are highly irregular and inefficient at delivering nutrients. They branch irregularly, follow a tortuous path, are far larger than their normal counterparts, are unusually permeable to large molecules, have a high interstitial

pressure, and are inefficient at carrying blood [26, 28]; these abnormalities of the tumor vasculature result in poor delivery of nutrients, causing certain areas of the tumor to be chronically hypoxic, stabilizing the HIF/VEGF signaling pathway described above and therefore resulting in even more tumor vasculature [25]. The overproduction of VEGF-A results in an abundance of tip cells from the Dll4 signaling pathway, which in part causes the malformed vasculature associated with tumors through excessive branching of these tip cells [25, 29].

Tumor blood vessel can be divided into six general classes: (1) mother vessels, which are enlarged, tortuous, thin-walled, lacking in pericytes, and highly permeable; (2) capillaries, which are similar to normal capillaries; (3) glomeruloid microvascular proliferations, which are tangles of vessels situated within a mixture of disordered pericytes; (4) vascular malformations, which are large vessels with an irregular coat of smooth muscle cells; (5) feeder arteries; and (6) draining vessels, which are enlarged, serpentine smooth muscle cell-coated vessels that supply and drain the blood vessels within the tumor [30]. The irregular pericyte and smooth muscle cell formations on these vessels, which in normal vasculature serve to enhance tight junctions and decrease leakiness, serve to decrease vessel efficacy in tumor angiogenesis [31].

Oncogenes play a prominent role in triggering the angiogenic switch. Expression of the H-Ras oncogene in the immortalized rat intestinal epithelial cell line IEC-18 led to the upregulation of VEGF and a significant increase of *in vivo* vascularization [32]. Ras signaling also results in the stabilization of the resulting mRNAs and possible enhancement of their transcription [33]. The p53 suppressor gene normally serves to downregulate VEGF while upregulating thrombospondin-1, an antiangiogenic factor; mutations in these genes serve to increase the activity of VEGF [34, 35]. p53 acts as a foil to C-Myc, a gene that triggers the expression of VEGF while downregulating thrombospondin-1. In tumors, mutations in p53 serve to increase the activity of c-Myc, thereby increasing the activity of VEGF [34].

Compared to VEGF-A, VEGF-B plays an insignificant role in angiogenesis. Rather, it acts as a potent survival factor, inhibiting the production of several proapoptotic factors such as BH-3-related proteins. The prosurvival effects of VEGF-B are mediated by both VEGFR-1 and the coreceptor NP-1 [36, 37]. More recent research suggests that VEGF-B may trigger tumor angiogenesis through a VEGF-A-independent pathway and that it may even be a prognostic marker for cancer metastasis [38].

VEGF-C and VEGF-D are both heavily involved in lymphangiogenesis. In tumors, these two forms of VEGF are overexpressed and activate VEGFR-3 by means of a paracrine signaling loop, thereby encouraging lymphatic growth within the tumor [39, 40]. Lymphatic vessels created through tumor angiogenesis tend to be larger than normal, enhancing the delivery of tumor cells to the lymph nodes, from which metastasis can occur (while VEGFR-3 activation causes lymphatic vessels to sprout, it should be noted that VEGFR-2 causes the vessels to become dilated) [41]. For example, metastasis of breast cancer occurs primarily through the lymphatic system, and VEGF-C has been shown to enhance tumor metastasis in this disease [42]. Because both lymphatic vessels and blood vessels provide nutrients and a metastatic pathway for a tumor, vascular density (lymphatic vessels density or blood vessel density) within the tumor may be a prognostic factor of metastatic potential [41, 43].

4. Anti-VEGF medications

Because of tumor dependence on VEGF for growth and survival, much work has been put into developing VEGF inhibitors for use in the clinic. Most of these inhibitors fall under two broad categories that differ in structure and mechanism of action: small-molecule inhibitors (SMIs) and monoclonal antibodies (mAbs). **Table 1** contains a list of the anti-VEGF medications mentioned in this chapter, their types, FDA approval dates, the forms of cancer they are approved to treat, and some common side effects associated with their use.

Some SMIs targeting VEGF signaling pathway are able to pass through the cellular membrane and interact with the cytoplasmic domain of receptor tyrosine kinases (RTKs) [44, 45]. Most act as competitive inhibitors with ATP. As the ATP binding site is common to all RTKs, specificity in the SMI is created by engineering the part of the molecule not similar to ATP [44]. Small molecule tyrosine kinase inhibitors (SMTKIs) can be divided into three broad categories: those that hydrogen bond with the ATP binding site of the enzyme's active conformation (type I), those that hydrogen bond with the hydrophobic pocket directly next to the ATP binding site in the enzyme's inactive conformation (type II), and those that bond covalently and irreversibly with specific cysteine residues on the kinase (type III) [44].

Sunitinib is a type I SMTKI [44] that is able to inhibit RTKs containing a split-kinase domain, such as VEGFRs -1, -2, and -3; PDGFRs -A and -B; cKIT; FLT3; CSF-1R; and RET [46]. The inhibition of the RTKs blocks signal transduction, thereby preventing tumor growth and angiogenesis among other processes. Sunitinib is administered orally in a recommended dose of 50 mg once daily for 4 weeks followed by a 2-week rest [47]. The medicine is currently FDA approved for use in progressive well-differentiated pancreatic neuroendocrine tumors in patients with unresectable, locally advanced, or metastatic disease; metastatic renal cell carcinoma; and gastrointestinal stromal tumors after intolerance to imatinib mesylate [46].

Sorafenib is a type II SMTKI [45]. Sorafenib inhibits VEGFR-2, VEGFR-3, PDGFR- β , and Kit; therefore, it operates through a dual mechanism of action, inhibiting both tumor growth and angiogenesis [48]. Sorafenib is administered in a recommended dose of 400 mg twice daily around mealtimes [47]. The medicine is FDA approved for use in recurrent or metastatic progressive differentiated thyroid carcinoma, advanced renal cell carcinoma, and unresectable hepatocellular carcinoma [49].

Vandetanib is a type III SMTKI [44]. It inhibits VEGFR-2, EGFR, and RET, blocking several signal transduction pathways that control tumor growth and angiogenesis [50]. Vandetanib was approved in 2011 for use against unresectable, locally advanced, or metastatic medullary thyroid cancer. The recommended daily dose of the medicine is 300 mg per day, administered orally [51].

In contrast to small molecule inhibitors, monoclonal antibodies (mAbs) cannot translocate through the plasma membrane to interact with the cytoplasmic domains of RTKs; they are, however, more specific in action than SMIs [45]. mAbs used in antiangiogenic therapies can be divided into two broad categories: those that bind to VEGF and inhibit VEGF's ability to bind with its receptors, and those that bind to VEGFRs and inhibit ligand-receptor interaction and activate immune responses.

Medicine	Type of medication	FDA approval date	Types of cancers approved for to date	Common Grade 3-4 side effects
Apatinib [114]	SMI	N/A	N/A	N/A
Bevacizumab [125]	mAb	26-Feb-04	Metastatic colorectal cancer, nonsmall cell lung cancer, glioblastoma, metastatic renal cell carcinoma, cervical cancer (in combination with chemotherapy), platinum-resistant recurrent epithelial ovarian, fallopian tube, or primary peritoneal cancer in combination with chemotherapy FDA approval for the use of bevacizumab in metastatic HER2-negative breast cancer was revoked on 18 Nov. 2011 on account of potentially life-threatening side effects and the few benefits associated with its use	Sensory neuropathy, hypertension, fatigue, neutropenia, vomiting, diarrhea
Cabozantinib [116]	SMI	25-Apr-16	Renal cell carcinoma in patients who have received prior antiangiogenic therapy	Abdominal pain, pleural effusion, diarrhea, and nausea
Pazopanib [126]	SMI	Oct-09	Advanced renal cell carcinoma, advanced soft tissue sarcoma	Diarrhea, hypertension, and proteinuria
Ramucirumab [96]	mAb	21-Apr-14	Gastric/gastroesophageal junction adenocarcinoma (with and without paclitaxel), with docetaxel for platinum-resistant metastatic nonsmall cell lung cancer, with FOLFIRI for metastatic colorectal cancer	Hypertension, hyponatremia, neutropenia, pneumonia
Sorafenib [50]	SMI	20-Dec-05	Advanced renal cell carcinoma, unresectable hepatocellular carcinoma, progressive differentiated thyroid carcinoma	Diarrhea, hand-foot syndrome
Sunitinib [46]	SMI	26-Jan-06	Gastrointestinal stromal tumor, advanced kidney cancer, pancreatic neuroendocrine tumors	Hypertension, diarrhea, nausea, vomiting
Vandetanib [52]	SMI	6-Apr-11	Medullary thyroid cancer in patients with unresectable, locally advanced, or metastatic disease	Diarrhea/colitis, hypertension and hypertensive crisis, QT prolongation, fatigue, and rash
Zif-Aflibercept [127]	VEGF-Trap (hybrid of VEGFR-1 binding domain and VEGFR-2 domain 3)	3-Aug-12	Metastatic colorectal cancer that is resistant to an oxaliplatin-containing regimen	Neutropenia, diarrhea, hypertension, leukopenia, stomatitis, fatigue, proteinuria, and asthenia
33C3 [57]	mAb	N/A	N/A	N/A

Table 1. FDA approvals for antiangiogenic drugs.

Perhaps the most well-known mAb targeting VEGF is bevacizumab, first approved in the EU in January 2005 [52]. The medicine targets all forms of VEGF-A, thereby inhibiting its ability to activate angiogenesis [53]. 2C3 is another mAb that binds to VEGF, preventing it from interacting with VEGFR-2, but not VEGFR-1; it blocks the growth of blood vessels in tumors and inhibits increases in vascular permeability [54]. IMC1121B (ramucirumab) binds to the ligand binding site of VEGFR-2 [55]. 33C3 is an antibody that binds to Ig domains 4-7 of VEGFR-2 and therefore, on account of binding to VEGFR-2 as opposed to a VEGF molecule, has the potential to act independently of VEGF concentration [56].

A type of VEGF inhibitor that defies simple classification into one of these two categories was developed recently as VEGF-trap, otherwise known as aflibercept. Aflibercept is a fusion protein consisting of the VEGFR-1 and VEGFR-2 binding domains and the Fc region of the IgG1 antibody [57, 58]. The protein binds to VEGF-A, VEGF-B, and PlGF, inhibiting activation of VEGFR-1 and VEGFR-2 and thereby inhibiting angiogenesis [57, 58]. Aflibercept was approved as Zaltrap on 3 August 2012 for use in combination with a FOLFIRI (folinic acid, fluorouracil, and irinotecan) chemotherapy regimen in adults with colorectal cancer [59], and has been shown to provide significant benefits in OS and PFS [60].

5. Side effects of anti-VEGF medications

No metabolically active tissue in the human body is more than a few hundred micrometers away from a capillary vesicle. The extensive nature of the vascular system in humans is produced and maintained through angiogenesis; changes in metabolic activity lead to changes in demand for oxygen which in turn regulate angiogenesis [1]. Therefore, angiogenesis inhibitors are bound to have adverse side effects. These side effects are generally less severe than those encountered from chemotherapy, although they can still be life-threatening [61]. Common side effects include hypertension, arterial thromboembolic events (ATEs), cardiotoxicity, and problems with bleeding, gastrointestinal perforation, and wound healing. See **Table 2** for a description of grades of adverse events.

Perhaps the most well documented side effect of angiogenesis inhibitors is hypertension. VEGF has been shown to decrease blood pressure; for example, in a phase I clinical trial (the VIVA trial, a double-blind placebo-controlled study), recombinant VEGF was shown to decrease systolic blood pressure by as much as 22% [62]. This decrease in blood pressure is caused by the generation of blood capillaries, which increases the total cross-sectional surface area available for blood to flow and thereby reduces blood pressure, and VEGF-induced vasodilation, which occurs when VEGF induces the production of nitric oxide and PGI₂ as part of its signal transduction pathway [63]. VEGF inhibitors therefore cause hypertension by inhibiting the production of nitric oxide and PGI₂, leading to vasoconstriction and an increase in blood pressure [64, 65]. Hypertension caused from VEGF inhibition can be managed using standard therapies, such as angiotensin-converting enzyme inhibitors, beta blockers, calcium channel blockers, diuretics, and angiotensin II receptor blockers [66].

VEGF-dependent interactions between the glomeruli and endothelial cells are also inhibited through anti-VEGF therapies (such as bevacizumab, sunitinib, and sorafenib [67]), leading

to proteinuria, a common side effect in anti-VEGF treatment [65, 68]. Inhibition of the VEGF signaling pathway leads to the suppression of nephrin, which in turn leads to a decrease in maintenance of the glomerular slit diaphragm [68]. Luckily, most instances of proteinuria are mild, presenting as only grades I and II, although more severe proteinuria has been reported in a share of cases [67, 68].

Treatment with VEGF inhibitors is also associated with an increased risk of arterial thromboembolic events (ATEs) [65, 69, 70]. This increased risk is caused by a reduction in the regenerative capacity of endothelial cells and can diminish antiapoptotic factors while encouraging procoagulant changes in the blood vessels [70]. The rate of venous thromboembolic events does not seem to be affected by VEGF inhibition, at least when comparing bevacizumab with chemotherapy and chemotherapy alone [69]. It is recommended that patients on anti-VEGF therapy who develop an ATE be taken off the therapy immediately [65]. The use of aspirin during therapy has been shown to increase the likelihood of grade 3 and 4 bleeding events, although no significant difference has been found between aspirin users in control and bevacizumab-treated groups [69]. Patients with a history of ATEs and older patients are at greater risk in developing a thromboembolic event when using VEGF inhibitors such as bevacizumab [69].

Cardiotoxicity is also a common side effect of VEGF inhibition, and has been observed in patients on bevacizumab, sunitinib, and sorafenib. The exact mechanisms of this toxicity are often unclear, and they may either have to do with inhibition of VEGF, inhibition of other signaling pathways concomitantly with VEGF, or both. Cardiomyopathy has been observed in sunitinib monotherapy in a phase I/II trial in which 11% of all participants (8/75) had a cardiovascular event [71]. Another study found evidence that sunitinib induces cardiotoxicity through the inhibition of the AMPK signal transduction pathway [72]. Moreover, bevacizumab given after acute myelogenous leukemia chemotherapy resulted in an increase in cardiovascular toxicity, although the mechanisms for this toxicity remain unknown [73]. Sorafenib has also been demonstrated to cause cardiotoxicity in mice due to myocyte necrosis [74].

Grade	Description	Example with gastric fistula
I	Mild with few or no symptoms; no interventions required	Asymptomatic; clinical or diagnostic observations only; intervention not indicated
II	Moderate, with minimal intervention needed; some limitation of activities	Symptomatic; altered GI function
III	Severe but not life threatening; hospitalization required; limitation of patient's ability to care for him or herself	Severely altered GI function; bowel rest tube feeding; TPN or hospitalization indicated
IV	Life threatening; urgent intervention required	Life-threatening consequences; urgent operative intervention indicated
V	Death related to adverse event	death

Table 2. Explanation of grades of adverse events [76].

Some side effects are caused by the fact that VEGF impairment impairs wound healing on account of its antiangiogenic properties [65]. Gastrointestinal perforation has been known to occur in patients on anti-VEGF therapy. In the AVOREN trial, three patients receiving bevacizumab in combination with IFN- α experienced grade 4 gastrointestinal perforations, while one patient experienced grade 3 gastrointestinal perforations [66]. Sorafenib and sunitinib have also been shown to cause gastrointestinal perforations [65]. These perforations are caused by tumors, often metastatic, and the healing of these perforations is impaired because angiogenesis is itself prevented. Moreover, a significantly increased risk of hemorrhagic events of all grades is observed in patients on sorafenib, sunitinib, pazopanib, bevacizumab, and aflibercept [65, 75].

6. New directions

Although much progress has been made in angiogenesis inhibition therapy, research in the field is still ongoing. The following section reviews (i) how does tumor resistance against anti-VEGF mediation grow? (ii) what are the challenges in assessing biomarkers for the efficacy of antiangiogenic therapies in specific cases? and (iii) how can treatment methodologies and new treatments improve overall survival (OS)?

6.1. How does tumor resistance against anti-VEGF medication grow?

Tumor resistance to antiangiogenic therapies can be classified into two broad categories: intrinsic and acquired [65, 77]. Acquired antiangiogenic resistance, in contrast to normal methods of acquired drug resistance in which mutational alteration of the drug target prevent the drug from working, consists of tumors initiating alternative methods to cope with hypoxia [77]. There are at least four distinct mechanisms through which this acquired resistance operates: (1) through the activation and upregulation of alternative proangiogenic pathways; (2) through the recruitment of bone marrow-derived proangiogenic cells including increased pericyte coverage of tumor vasculature; (3) through increased tumor aggressiveness resulting in metastasis to provide vasculature through widespread vessel cooption as opposed to tumor angiogenesis; and (4) through the adoption of alternative angiogenic mechanisms [77].

6.1.1. Proangiogenic pathways not involving VEGF

Several proangiogenic molecular pathways are upregulated when the VEGF/VEGFR pathway is inhibited. This often results in a return of tumor vascularization after a period of refractoriness [65, 78]. Some examples of alternative proangiogenic pathways include fibroblast growth factors (FGFs), platelet-derived growth factors (PDGFs), tumor necrosis factor- α (TNF- α), placenta growth factor (PlGF), angiogenin, stromal-derived factor 1 α (SDF-1 α), and interleukins-1 α and -1 β (IL-1 α , IL-1 β) [79–81]. Several of these molecules are regulated through HIF-1 expression, which in turn is controlled by hypoxia [79]. Therefore, through inducing the regression of tumor vasculature, anti-VEGF therapies can induce the expression of other proangiogenic pathways that reduce their own efficacy.

Several therapies are being developed that target both VEGF and alternative angiogenic pathways at the same time. For example, blockage of both VEGF and bFGF with brivanib resulted in prolonged tumor stasis following previous angiogenic inhibition in mouse neuroendocrine tumors [82]. Another study reported that inhibiting SDF-1 α after irradiating mice with implanted human U251 GBM tumors prevented the revascularization of irradiated tumors more effectively than inhibiting VEGF, thereby suggesting that SDF-1 α may be involved in acquired resistance to anti-VEGF therapies as well [81]. It has also been suggested that synergism between FGF-2 and PDGF-bb could induce angiogenesis, even if they are only present at low concentrations within the cytoplasm [79]. Taken together, these results suggest that inhibition of alternative angiogenic pathways in addition to inhibiting VEGF could increase the efficacy of antiangiogenic therapy.

6.1.2. BMDCs

Bone marrow-derived cells (BMDCs) such as pericytes and macrophages play important roles in both normal and pathological angiogenesis [1, 83, 84]. Tumor-associated macrophages (TAMs) are attracted to hypoxic regions of tumors through the upregulation of chemoattractants caused by hypoxia. Following extravasation into the tumor region, monocytes migrate into hypoxic areas of the tumor, following a chemoattractant gradient [85]. Monocyte chemoattractant protein-1 (MCP-1) has been shown to be an important chemoattractant in this process [86]. Once in the hypoxic region of the tumor, macrophages will promote tumor progression and metastasis through their trophic role (breaking down the ECM and encouraging tumor cell motility) and through excreting compound such as mutagenic oxygen, nitrogen radicals, and several proangiogenic factors such as VEGF and angiopoietin-2 [83, 87].

The process through which pericytes contribute to cancer progression and metastasis is poorly understood. Normally, pericytes are associated with newly formed blood vessels, creating a single, often discontinuous, layer around the endothelial cell tube that serves to support a mature vessel. However, in tumor angiogenesis, pericyte coverage can be greater than, or less than normal tissue vasculature depending on the tumor type; for example, glioblastoma exhibits lower than normal pericytes coverage, while islet carcinomas exhibit higher than normal coverage. This aberrant pericyte coverage can result in tumor growth and metastasis [88]. Current pericytes-targeted cancer therapies aim to reach a balance between pro- and anti-angiogenic factors, encouraging vascular normalization [89].

6.1.3. Increased tumor aggressiveness

In most cancers, the appearance of a proinvasive phenotype following antiangiogenic therapy is in question; however, in glioblastoma it is relatively undisputed [78], and there is evidence suggesting its occurrence in pancreatic cancer [90]. The proinvasive phenotype allows tumors from these cancers to circumvent the need for a blood supply, obviating the necessity of tumor angiogenesis. The mechanisms underlying this increased tumor aggressiveness are not fully known. HIFs have been widely accepted as playing a role in increased tumor aggressiveness and metastasis [90]. Moreover, increased collagen signaling in the presence of VEGF inhibition, including activation of discoidin domain receptor 1, protein tyrosine kinase 2, and pseudopo-

dium-enriched atypical kinase 1, has been shown to increase tumor progression and aggressiveness in murine models of pancreatic ductal adenocarcinoma [91]. In glioblastoma, VEGF inhibition creates hypoxic conditions in the tumor, which in turn causes increased expression of c-Met, an HGF receptor tyrosine kinase, through HIF-1 α ; this increase in c-Met expression correlates with increased invasion and poorer survival [80]. When both VEGF and c-Met are blocked, the increased tumor invasiveness and aggressiveness associated with anti-VEGF medications is suppressed in murine GBM and PNET, suggesting new routes for research in antiangiogenic therapies [80, 92].

6.1.4. *Alternative angiogenic mechanisms*

Another method through which tumors can circumvent angiogenic inhibition is through alternative angiogenic mechanisms such as intussusceptive angiogenesis, glomeruloid angiogenesis, vasculogenic mimicry, looping angiogenesis, and vessel cooption [78, 83]. These forms of angiogenesis occur through other signaling pathways that do not involve VEGF, and are upregulated when VEGF signaling is inhibited. One such alternative angiogenic pathway is vasculogenic mimicry (VM), which may be encouraged when anti-VEGF medications provide selective pressure for stem-like cancer cells that participate in the process. This phenomenon highlights the need for novel therapeutic methods that target the signaling pathways that control VM in addition to VEGF [93]. Similar combination therapies could be used to increase the efficacy of antiangiogenic medication in general by restricting the tumor's ability to acquire resistance to antiangiogenic therapies.

6.2. Challenges in biomarkers

Unfortunately, no validated biomarkers are currently available for determining which patients will respond best to antiangiogenic therapy [93, 94]. An array of biomarkers has been studied in hope to find effective biomarkers, including systemic measurements, gene analysis, circulating biomarkers, tissue markers, and imaging parameters [95]. Various challenges stand in the way of establishing effective biomarkers, such as the inability to perform repeated biopsies (inhibiting researchers' ability to assess dynamic biomarkers), the unpredictability of response and toxicity, the expensive and complex nature of human trials, the unpredictability of toxicity and response to therapies, and the specificity of each biomarker to disease [95].

The degree of hypertension experienced by a patient has been proposed as a potential systemic biomarker of tumor response, although more research is needed to establish the validity of this measurement [95, 96, 97]. Because hypertension is dependent on the VEGF-signaling pathway, it is possible that patients who do not develop hypertension are not responding to VEGF treatment at the current dose. In fact, the degree of hypertension correlates with survival, with patients who develop more severe hypertension experiencing better cancer remission than patients who develop no hypertension [97]. Interestingly, VEGF polymorphisms may play a role in determining the degree of hypertension, of anti-VEGF medications, which certain polymorphisms being more susceptible to inhibition than others. For example, VEGF-634 SNP (single nucleotide polymorphism) G/G is correlated with higher hypertension in sunitinib-treated patients with mRCC than either VEGF-634 C/G or VEGF-634 C/C [98].

Several circulating biomarkers have been proposed that circulate in the bloodstream of patients. For example, high levels of soluble VEGF-R1 (sVEGFR1) correlate with decreased efficacy of bevacizumab in GBM, rectal carcinoma, breast cancer, hepatocellular carcinoma, and metastatic colorectal carcinoma. This correlation is probably caused by sVEGFR1 acting as an endogenous VEGF-trap, so adding a VEGF-specific monoclonal antibody does little to further inhibit VEGF signaling pathways [94]. Another potential circulating biomarker is SDF1 α , as levels of this chemokine correlate with evasion of anti-VEGF therapies, although further study is needed to assess this potential biomarker [94]. Pretreatment levels of circulating VEGF-A has been shown to be prognostic in metastatic colorectal, lung, and renal cell cancers, but it is not predictive for bevacizumab treatment [99]. Moreover, a phase II study presented evidence that plasma concentrations of VEGF-A and IL-8 are prognostic for OS in MRCC, with high levels being unfavorable, while low plasma levels or sVEGFR-3 are may be predictive for a positive outcome in patients with mRCC receiving sunitinib [100]. Another phase II trial found that serum levels of Ang-2 and MMP-2, along with tumor levels of HIF-1 α , are potential baseline efficacy biomarkers for sunitinib in advanced RCC [101]. Finally, low circulating endothelial cell levels (<65 CEC/4 mL blood) have been found to correlate with longer median PFS and OS in patients with colorectal cancer receiving bevacizumab [102].

Imaging techniques provide potential of imaging parameters as biomarkers as well. For example, MRI- and CT-based tissue vascular measured such as blood flow, blood volume, and permeability have been shown to occur after bevacizumab administration, although more research is needed to assess the efficacy of these measures as biomarkers [95]. Moreover, pretreatment ADC histogram analysis has been shown to be a possible predictive biomarker for bevacizumab treatment in recurrent glioblastoma [103]. Imaging studies can also be used to augment other biomarker studies, and can be used in cooperation with systems pharmacology to create multilevel computational models that predict the efficacy of treatment in patients, as well as suggesting dosing schedules that could be more efficacious than current practices [104]. Another potential biomarker could be patient genotype. As discussed above, some VEGF SNPs may be more receptive to treatment than others. However, some genes show little or no correlation with the efficacy of antiangiogenic therapies. For example, the mutation status of KRAS, a common oncogene, does not correlate with VEGF therapy efficacy [105]. More research must be done to establish which genes can and cannot be considered biomarkers.

6.3. Treatment methodologies and new treatments

According to the normalization hypothesis, during the course of antiangiogenic therapy, there is a dose-dependent window of time during which aberrant tumor vasculature is normalized. In this state, it is easier to deliver cytotoxic drugs from conventional chemotherapy to the tumor in a treatment schedule that takes advantage of the window presented by antiangiogenic agents given in low doses [106]. However, there are two important considerations to take into account when scheduling chemotherapy with antiangiogenic agents: first, the dose of antiangiogenic agents affects the normalization window during which chemotherapy can be delivered effectively. Second, the size of the chemotherapeutic agents matters, as vascular normalization causes the pores in aberrant tumor vessels to shrink, limiting the ability of large molecules to

pass through to the tumor [94]. Vascular normalization has also been shown to improve the outcome of immunotherapy, making delivery of immune cells to the tumor easier, and can even decrease the intravasation of cancer cells, limiting the possibility of metastasis [94].

Most of the time, resistance to chemotherapy occurs through heritable changes in the tumor genotype. However, because resistance to VEGF inhibitors does not occur through natural selection, as discussed above, it is possible that rechallenging after disease progression following an intervening interval of time during which VEGF therapy is suspended may allow for a return of efficacy in antiangiogenic VEGF inhibitors. For example, patients with metastatic renal cell carcinoma (mRCC) who experience disease progression after initial response to sunitinib can eventually respond to the drug upon rechallenge after an intervening period on alternative therapies, such as sorafenib (patients with more than 6 months off sunitinib experienced greater PFS than patients with less than 6 months off sunitinib, although in each case the second PFS was shorter than the original) [99]. Moreover, in a randomized phase III trial, patients with unresectable metastatic colorectal cancer progressing up to 3 months after discontinuing bevacizumab plus chemotherapy experienced moderate survival benefits when bevacizumab plus chemotherapy was given as a second line treatment as compared to chemotherapy alone [107].

More research must be done to assess the efficacy of antiangiogenic agents in the adjuvant and neoadjuvant settings. In the neoadjuvant setting, VEGF inhibition may cause tumor regression, converting an unresectable tumor to a resectable one [82], with 12 of 30 patients in one single-arm phase II study who received oxaliplatin plus bevacizumab having initial nonsynchronous unresectable CLM become resectable [108]. However, antiangiogenic neoadjuvant treatment in mouse models of metastatic disease has been shown not to correlate with postsurgical survival [109]. In the adjuvant setting, it is possible that antiangiogenic therapies may prevent relapse by preventing the reestablishment of tumor vasculature. However, bevacizumab has delivered poor results in OS when used in combination with chemotherapy for adjuvant colorectal cancer, although PFS is improved [78].

Some work is also being put into developing novel VEGF and VEGFR inhibitors. For example, ramucirumab, a monoclonal antibody that inhibits VEGFR-2, was given FDA approval in 2014 for use as a single agent in the treatment of patients with gastroesophageal carcinoma; it has since been given approval for use in combination with paclitaxel, docetaxel, and FOLFIRI [110]. Ramucirumab is the first biological treatment to show moderate survival benefits as a single agent after gastroesophageal adenocarcinoma progression following first-line chemotherapy in a phase 3 trial (ramucirumab vs. placebo, OS = 5.2 months vs. 3.8 months, respectively) [111]. The drug has also shown moderate OS benefits when used in combination with docetaxel for second-line treatment of stage IV NNSCLS compared with docetaxel alone after progression on platinum-based chemotherapy (10.5 months vs. 9.1 months, ramucirumab plus docetaxel vs. docetaxel alone, respectively) [112]. Apatinib is another novel VEGFR-2 inhibitor, a small molecule not yet given FDA approval (although it is approved for use in China in treating metastatic gastric or gastroesophageal adenocarcinoma after second-line chemotherapy) [113]. The drug thus far has shown only moderate survival benefits of 1.8 months, and several trials are ongoing to assess its efficacy in different settings [114].

Yet another new small molecule VEGF inhibitor, cabozantinib (Cabometyx, Exelixis, Inc.) was given FDA approval on 25 April, 2016, for the treatment of renal cell carcinoma in patients who have received prior antiangiogenic therapy. Approval was given based on improved progression-free survival (7.4 months vs. 3.8 months in the cabozantinib and everolimus arms, respectively), improved overall survival (21.4 months vs. 16.5 months) and improved confirmed response rate (17 vs. 3%). The drug exhibits the standard side effects associated with VEGF inhibition; 40% of patients who received cabozantinib experienced a serious adverse event such as abdominal pain, pleural effusion, diarrhea, and nausea [115].

Another potential target for anticancer therapy has also been found in lymphangiogenesis. Several studies are examining the potential of inhibiting the VEGFR-3/VEGF-C/VEGF-D signaling axis in preventing lymph-node-mediated metastasis and disease progression. Inhibition of VEGF-C/-D with soluble VEGFR-3 has been shown to reduce tumor metastasis in mouse models, as has blocking VEGF-C/-D proteolysis or blocking VEGF-C from binding with the Nrp-2 receptor [116]. Moreover, foretinib, a multiple kinase inhibitor currently undergoing clinical trials, has shown promise in inhibiting the activity of VEGFR-3 and lymphangiogenesis and could potentially be used to inhibit lymph-node-mediated metastasis [117]. Corosolic acid has also been shown to induce apoptosis in CT-26 colon carcinoma in a mouse model, in addition to inhibiting lymphangiogenesis, but more study is needed before this substance becomes useable as a cancer therapy [118].

PlGF inhibition is another potential novel therapeutic approach in the fight against cancer, and preclinical studies have shown that inhibiting PlGF using genetic inhibition or anti-PlGF antibodies slows tumor growth and metastasis, and can even induce tumor regression in preexisting medulloblastoma [119]. However, the efficacy of inhibiting PlGF in tumors has come under question, with some preclinical studies showing that inhibiting PlGF does not significantly inhibit tumor growth [120, 121]. More research is needed to assess whether PlGF inhibition could be an efficacious cancer therapy.

7. Conclusion

Ever since its discovery, VEGF has been at the center of attention in new and developing cancer therapies. Since its early successes, however, antiangiogenic therapy has often presented only modest improvements in overall survival and progression-free survival [122]. Researchers have not given up hope that this therapeutic technique contains promise in the arsenal against cancer. Therefore, much recent research has been done in pushing the frontier of antiangiogenic therapies, trying to improve patient outcome.

Because the biology of VEGF and its receptors is well understood, current research focuses on why some tumors become resistant to antiangiogenic therapies and others are intrinsically resistant, how to circumvent this intrinsic or acquired resistance, and how to best utilize these expensive therapies by developing predictive biomarkers for treatment outcome. More research is also being done to develop novel VEGF inhibition techniques, and in characterizing the rare yet serious toxicities associated with these drugs.

As they stand now, antiangiogenic therapies face a set of limitations that severally impacts their efficacy. Tumors can acquire resistance to the drugs (if they do not already have intrinsic resistance) and demonstrate an increase in aggressiveness. Moreover, antiangiogenic therapies may cause a decrease in chemotherapy perfusion, lowering the efficacy of chemotherapies given in combination with antiangiogenic medicine [123]. These difficulties suggest that, at least when given alone, antiangiogenic therapies may face severe limitations in survival benefits. Therefore, future research should focus on more than simply inhibiting VEGF on a continuous schedule. Rather, it should focus on increasing the efficacy of chemotherapy through utilizing antiangiogenic therapy to induce vascular normalization, allowing for more efficient delivery of chemotherapeutic agents [123, 124]. Moreover, research should also find ways to decrease resistance to these therapies through inhibiting proangiogenic factors that are upregulated in response to the inhibition of VEGF and through developing predictive biomarkers for the efficacy of these expensive treatments [123, 124].

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