Vascular Endothelial Growth Factor Polymorphisms: Role in Response and Toxicity of Tyrosine Kinase Inhibitors

Susan A. J. Vaziri · Jenny Kim ·
Mahrukh K. Ganapathi · Ram Ganapathi

Abstract Angiogenesis is central to the growth of normal tissues and tumors. Inhibiting this pathway has been a strategy for drug development for tumors not responsive to most agents used in chemotherapy. Notably, signaling mediated by vascular endothelial growth factor (VEGF) is a key target because aberrant signaling via this pathway is frequently associated with neoangiogenesis in tumors. The drug-discovery effort to blunt VEGF signaling has led to the approval of bevacizumab and several receptor tyrosine kinase inhibitors (TKIs) that have shown efficacy in the clinical management of breast, colorectal, lung, and kidney cancer. Understanding the genetic variability in VEGF and VEGF receptor has led to identifying genotypic variations (single nucleotide polymorphisms [SNPs]) associated with treatment outcome and toxicity. Notably, identification of SNPs in VEGF associated with angiogenesis inhibitor treatment-induced hypertension and outcome provides exciting opportunities for personalized medicine to improve outcome and reduced toxicity with these novel TKIs.

Keywords Angiogenesis · Tyrosine kinase inhibitors · Hypertension · VEGF polymorphism · Outcome · Toxicity

S. A. J. Vaziri · J. Kim · M. K. Ganapathi · R. Ganapathi (✉)
Taussig Cancer Institute - R40, Cleveland Clinic Foundation,
9500 Euclid Avenue,
Cleveland, OH 44195, USA
e-mail: ganapar@ccf.org

S. A. J. Vaziri
e-mail: vaziris@ccf.org

J. Kim
e-mail: kimj4@ccf.org

M. K. Ganapathi
e-mail: ganapam@ccf.org

Introduction

Because angiogenesis is central to the growth of normal tissues and tumors, inhibiting this pathway has been a strategy for drug development [1]. Notably, signaling mediated by vascular endothelial growth factor (VEGF) has been a target because aberrant signaling via this pathway is frequently associated with neoangiogenesis in tumors. The forerunner to this drug-discovery effort to blunt VEGF signaling is the antibody, bevacizumab, which is approved for use in the treatment of breast, colorectal, lung, and kidney cancer [1]. Although the mechanism of action of bevacizumab involves binding to VEGF, the complexity of the VEGF pathway suggests a requirement for a more comprehensive approach to inhibit this key signaling pathway [2]. This effort has led to the development of VEGF receptor (VEGFR) tyrosine kinase inhibitors (TKIs). These small molecule inhibitors that are essentially multi-kinase inhibitors have rapidly entered the clinical arena because of their efficacy in treating cancers, particularly renal cancer that is generally not responsive to therapy effective in several other tumor types [2].

Among the tumors in which TKIs are effective, most studies have focused on renal cell carcinoma (RCC) possibly because of the remarkable effects of sorafenib and sunitinib in the clinical management of this disease. The TKIs (also referred to as multi-kinase inhibitors) inhibit several receptor tyrosine kinases including but not restricted to VEGFR-1, -2, and -3, platelet-derived growth factor receptor (PDGFR)-α, PDGFR-β, and c-Kit [2]. The success of these TKIs in the treatment of RCC is further exemplified by the recent approval of pazopanib, which is also an inhibitor of several receptor tyrosine kinases (R5). Although it is not entirely clear, the efficacy of these TKIs

in inhibiting several receptor tyrosine kinases (RTKs) in the angiogenesis signaling pathway could be responsible for both their efficacy and toxicity.

Several excellent reviews on the clinical efficacy and toxicity of TKIs have been published. This article focuses on the mechanistic basis for the efficacy/toxicity of TKIs with emphasis on VEGF polymorphisms. Although bevacizumab is an angiogenesis inhibitor and not a TKI, this review discusses this agent along with the TKIs because there is possible overlap in VEGF polymorphisms that are involved with regard to treatment outcome and toxicity.

VEGF Levels and TKIs

The central role for VEGF, a critical angiogenic molecule, is exemplified by its role in development, normal physiology, and in certain pathologic conditions. Because the angiogenesis inhibitors target the VEGF signaling pathway, efficacy of toxicity of TKIs and other angiogenesis inhibitors could be related to the effect on circulating VEGF levels that could impact on normal homeostasis. In the case of bevacizumab that can bind to and consequently inhibit the biologic activity of human VEGF, the general effect of treatment with this agent is decreased circulating VEGF levels. However, with the TKIs that target the RTKs (eg, sunitinib), an increase in VEGF levels and a decrease in soluble VEGFR-2 (sVEGFR-2) and sVEGFR-3 was observed in cytokine refractory patients with metastatic RCC (mRCC) [3••]. Interestingly, these changes in VEGF and sVEGFR were observed during treatment with sunitinib, and levels tended to return to near-baseline after 2 weeks off treatment, indicating that these effects were dependent on drug exposure. Further, significantly larger changes in VEGF, sVEGFR-2, and sVEGFR-3 levels were observed in patients exhibiting objective tumor response compared with those exhibiting stable disease or disease progression ($P<0.05$ for each analyte).

Elucidation of the mechanism(s) responsible for VEGF and placenta growth factor (PlGF) induction will require further study, but treatment-related increases in tumor hypoxia may be involved, perhaps via increased activity of hypoxia-inducible factor (HIF)-1α or other HIFs. However, the induction may not be completely tumor dependent because laboratory studies in nontumor-bearing mice suggest that VEGF induction following treatment with anti-VEGFR2 monoclonal antibodies is dose-dependent and normalized after discontinuation of therapy [4••]. These results suggest that observed sunitinib-induced plasma changes in VEGF signaling intermediates represent a systemic tumor-independent response to therapy and may correlate with the most efficacious antitumor doses, potentially having utility for defining the optimal biologic dose range for this drug class but not as predictive markers

of tumor response or clinical benefit. It is also likely that these changes in VEGF and VEGFR may also be relevant to drug-associated toxicities, drug resistance, and observed rapid tumor (re)growth seen after cessation of therapy.

Another clinical trial examining antitumor activity of sunitinib in patients with bevacizumab refractory mRCC showed that mean plasma VEGFA and PlGF levels significantly increased, whereas VEGFC and sVEGFR3 levels decreased with sunitinib treatment in this patient cohort with subsequent restoration to near-baseline during off-treatment periods [5]. Furthermore, lower baseline levels of sVEGFR3 and VEGFC were found to be associated with longer progression-free survival and objective response rate, suggesting that baseline levels of sVEGFR3 and VEGFC may have potential utility as biomarkers of clinical efficacy in this clinical setting. Interestingly, in a more recent study by Feldman et al. [6] evaluating sunitinib plus bevacizumab, although VEGF levels were found to be inversely correlated with response, the rise in VEGF levels was not similar to that previously reported with sunitinib monotherapy [6]. Further understanding of the biologic basis for these observations is needed, but it is of interest to note that preeclampsia is associated with increased VEGF levels and the potential link to genetic polymorphisms, toxicity, and TKI therapy will be discussed later.

Toxicity Associated with TKI Therapy

Because VEGF pathway and angiogenesis are central to growth and development as well as physiologic processes of normal organ function and maintenance, it is not completely surprising that adverse effects could be expected by inhibition of this key signaling pathway [7]. Adverse effects associated with these agents include hypertension, renal dysfunction, arterial thromboembolic events, bowel perforation, reversible posterior leukoencephalopathy syndrome, wound complications, and hemorrhage. The exact molecular mechanisms of the adverse effects of VEGF/VEGFR inhibitors are not fully understood. Although bevacizumab and TKIs have distinctive targets and mechanism(s) of action, they do share the class adverse effects as a result of VEGF or VEGFR inhibition.

Hypertension

Hypertension is the most common side effect of VEGF pathway inhibitors. VEGFR2 signaling generates nitric oxide and prostaglandin I2, which induces endothelial cell-dependent arteriole and venous vasodilatation, thereby controlling blood pressure [8, 9]. Inhibition of VEGF

pathway, therefore, could lead to vasoconstriction and, thereby, hypertension. Furthermore, direct endothelial cell apoptosis by VEGF blockage and resulting rarefaction (decreased arteriole and capillary densities) has also been hypothesized as a mechanism of hypertension induced by anti-VEGF therapy [10]. Degree of hypertension caused by these agents has been shown to be dose-dependent [11, 12]. In addition to dose and inherent differences between agents, host susceptibility can also affect the rate of hypertension. Preexisting hypertension has been seen to be a risk factor in further elevation in blood pressure with these agents. Although the etiology is unclear, of particular significance is the higher incidence for development of hypertension in patients with mRCC, compared with patients with other tumors [13].

Cardiomyopathy

Cardiomyopathy and congestive heart failure (CHF) have been reported with the use of VEGF- and VEGFR targeting agents, although lack of prospective monitoring precludes definitive causality. Ventricular dysfunction with VEGF/VEGFR inhibitors is suggested in cardiomyocyte-specific VEGF knockout murine models, which present with dilated cardiomyopathy [14]. Furthermore, cardiomyopathy has been associated with sunitinib in an early phase 1/2 trial with this agent in imatinib refractory gastrointestinal stromal tumor (GIST) patients, which incorporated careful cardiac monitoring [15••]. Twenty percent of patients showed a drop in left ventricular ejection fraction below the normal range (<50%) and 8% developed clinical CHF, which was reversible upon cessation of therapy. Refractory CHF with fatal outcomes has rarely been reported in trials of anti-angiogenic agents.

Renal Damage and Proteinuria

Proteinuria has been observed in all studies with bevacizumab treatment. However, it is rarely reported in clinical trials with VEGFR TKIs, such as sunitinib or sorafenib. With axitinib, however, 32% of patients (17 of 52) with mRCC developed grade 2 or higher proteinuria and a few patients had proteinuria more than 1 g per 24 h urine [16]. Underlying mechanisms of proteinuria and renal damage may be caused by the filtration barrier of the renal glomeruli, which is formed partly by endothelial cells. It has been suggested that VEGF/VEGFR2 interaction is important in repair of the renal system [17, 18]. Furthermore, in murine models, heterozygous deletion of *VEGF* led to loss of endothelial cell fenestration, loss of podocytes, mesangiolysis, and proteinuria. Importantly,

VEGF has also been shown to have a critical protective role in the pathogenesis of microangiopathic process [19]. Pathologic findings in the past have included proliferative glomerulonephritis, interstitial nephritis, and thrombotic microangiopathy [20–22].

Hemorrhage

The risk of bleeding is increased in patients with the use of VEGF pathway inhibitor therapy. Both mucocutaneous and tumor-related bleeding have been reported. Mild epistaxis and mucocutaneous bleeding comprise the most common presentation (20–40%) with bevacizumab therapy. They have also been described with sunitinib and other TKIs [23]. Fatal hemorrhage has been most prevalent in tumors of the lung and gastrointestinal tract. Life-threatening hemoptysis has been shown to occur with lung cancer with squamous histology (a current contraindication for bevacizumab therapy) [24]. In a phase 3 trial of advanced colorectal cancer, gastrointestinal hemorrhage was 24% in the bevacizumab plus chemotherapy group compared with 6% in the chemotherapy control group. Tumor-related bleeding has also been shown with sunitinib-treated GIST patients including central nervous system bleeding [25].

Overall, it is apparent that interfering with a VEGF signaling pathway either with a VEGF-targeted antibody or TKIs is associated with a pattern of toxicity that is also observed in patients with preeclampsia.

VEGF SNPs

Given the central role for VEGF in angiogenesis and the association of VEGF levels with certain pathologic conditions, there has been considerable effort in identifying a link to genetic variability. Although the role of VEGF levels and their impact on outcome and toxicity has been addressed, these studies for the most part are correlations without genotype data, making it difficult to assess the impact of genetic polymorphisms. The VEGF gene is quite complex, with several alternatively spliced isoforms, and the regulation of expression could differ between normal and tumor tissue. Interestingly, because all identified polymorphisms in VEGF are not in the coding region, possible mechanisms for their role in gene expression have been proposed. Table 1 outlines single nucleotide polymorphisms (SNPs), with emphasis on their role in different types of human malignancies [26–28••, 29–37]. As a footnote, we also indicate other noncancer-related pathologic states that have been associated with VEGF SNPs [38–47].

Although many transcription factors bind to the promoter regions of VEGF [48], none occur at the common

Table 1 Diseases and conditions associated with vascular endothelial growth factor single nucleotide peptides[a]

SNP[b]	rs Number[c]	Location and association with type of cancer and VEGF levels	Study
–2578C>A	699947	Promoter renal cell carcinoma; survival	Kaiwi et al. [26]
		AML; prognosis	Kim et al. [27]
		Breast cancer; overall survival	Schneider et al. [28••]
		Thyroid cancer; increased risk	Hsiao et al. [29]
		Invasive breast cancer; increased risk	Jacobs et al. [30]
–1498C>T	833061	Promoter esophageal carcinoma	Zhai et al. [31]
		Colon cancer or VEGF levels; no association	Cacev et al. [32]
		AML; prognosis	Kim et al. [27]
		Breast cancer; bevacizumab-induced hypertension	Schneider et al. [28••]
		Early-stage NSCLC; survival; no association	Heist et al. [33]
–1154G>A	1570360	Promoter renal cell carcinoma; no association	Ricketts et al. [34]
		Renal cell carcinoma; smaller tumors; lower stage	Kaiwi et al. [26]
		Colon cancer or VEGF levels; no association	Cacev et al. [32]
		Breast cancer; overall survival	Schneider et al. [28••]
		Invasive breast cancer; increased risk	Jacobs et al. [30]
–634G>C	2010963	5′-UTR; esophageal cancer; no association	Zhai et al. [31]
		Ovarian cancer; survival	Smerdel et al. [35]
		Renal cell carcinoma; no association	Kaiwi et al. [26]
		AML; prognosis	Kim et al. [27]
		Breast cancer; bevacizumab-induced hypertension	Schneider et al. [28••]
		Early-stage NSCLC; overall survival	Heist et al. [33]
–7C>T	25648	5′-UTR; bladder cancer; risk	Garcia-Closas et al. [36]
936C>T	3025039	3′-UTR; esophageal adenocarcinoma	Zhai et al. [31]
		stomach cancer; gender-dependent	Bae et al. [37]
		AML; treatment outcome	Kim et al. [27]
		In situ breast cancer; increased risk	Jacobs et al. [30]

AML acute myelogenous leukemia; *NSCLC* non-small cell lung cancer; *UTR* untranslated region; *VEGF* vascular endothelial growth factor

[a] These single nucleotide peptides (SNPs) have been associated with other diseases including diabetic retinopathy [38, 39], Kawasaki disease [40], Alzheimer's disease [41], Behçet's disease [42], pregnancy loss [43], preeclampsia [43], age-related macular degeneration [44], psoriatic arthritis [45], giant cell arteritis [46], and end-stage renal disease in males [47]

[b] Nucleotide positions are noted relative to the adenosine of the translation start site

[c] Reference sequence (rs) numbers are identified in the National Center for Biotechnology's SNP database (http://www.ncbi.nlm.nih.gov/snp). Genbank Accession No. NM_001025366

polymorphic sites associated with VEGF expression. Nevertheless, these SNPs have been reported to cause changes in VEGF expression levels [49] and have been correlated with different cancers (Table 1). However, the mechanism for these associations remains to be determined.

In terms of the role of SNPs and their impact on VEGF levels, the 936C>T polymorphism (rs3025039) occurs in the 3′ untranslated region (UTR). Studies have shown an association between this SNP, VEGF levels and cancer (Table 1) [49]. This SNP occurs at a potential AP-4 binding site [50], which may affect mRNA stability. The other SNP, –634G>C (rs2010963), occurs in the 5′-UTR and this polymorphism occurs in the internal ribosome entry site (IRES)-B domain critical for expression of one of the VEGF isoforms [51••]. Currently, it is

not apparent how these SNPs affect VEGF levels in patients with TKIs and the impact on outcome or toxicity. However, with regard to TKI-induced toxicity and VEGF SNPs, we will focus on hypertension because this is a major toxicity associated with TKI therapy.

As indicated earlier, the association of VEGF polymorphisms with outcome and toxicity will also include data with bevacizumab because there is overlap both in toxicity profile and treatment-induced hypertension. With regard to TKI treatment-induced hypertension that is not associated with VEGF polymorphisms, there is compelling evidence that this is related to potency and, particularly, the affinity of the agent for binding to VEGFR2 [2]. This proposed correlation is further strengthened by the recent finding that treatment with a specific VEGFR2 antibody in vivo leads to

a sustained and rapid increase in blood pressure [52]. Based on this observation it was inferred that VEGF acting through VEGFR2 can play a critical role in blood pressure control by promoting nitric oxide synthase expression and nitric oxide activity [52]. From these observations, it can be surmised that interfering with VEGF signaling by sequestering VEGF with bevacizumab or inhibiting VEGFR2 receptor activity with a TKI can lead to hypertension induced by these agents that interfere with angiogenesis.

VEGF SNPs and Toxicity with Angiogenesis Inhibitors or TKIs

In a pioneering study evaluating a panel of VEGF genotype polymorphisms in tumor DNA (–2578 C/A, –1498 C/T, –1154 G/A, –634 G/C, and 936 C/T), Schneider et al. [28••] reported specific VEGF genotypes that correlated with bevacizumab-induced hypertension. Specifically, the genotypes VEGF –634 CC and VEGF –1498 TT compared with alternate genotypes were found to be associated with significantly less grade 3 or 4 hypertension in patients with metastatic breast cancer that were treated with paclitaxel plus bevacizumab ($P=0.005$ and $P=0.022$, respectively). Interestingly, this correlation of VEGF genotype with hypertension was not associated with VEGF expression in the tumor. However, this study did not report the impact of circulating VEGF levels, which would have been of interest because the VEGF 936 C/T polymorphism has been linked to plasma VEGF levels [50]. More recently, Kim et al. [53] have evaluated the role of VEGF genotype polymorphisms on sunitinib-induced hypertension in patients with mRCC. In this retrospective study, patients with mRCC treated with sunitinib (50 mg, 4 weeks on/ 2 weeks off) and who had available blood pressure data were analyzed. Specifically, using genomic DNA isolated from peripheral blood lymphocytes, VEGF SNPs –634 C/G and –1498 C/T were amplified using primers designed to flanking sequences for the respective SNPs. Genotypes were subsequently assigned following sequence analysis. Among a cohort of 64 patients with 63 analyzable, 89% patients had prior nephrectomy and 63% were previously treated with cytokines and/or a TKI. In this cohort, 57% patients were on antihypertensive therapy at baseline with baseline systolic and diastolic blood pressure of 139 mm Hg (range, 93–190) and 80 mm Hg (range, 47–103), respectively. For the VEGF –634 genotype (Fig. 1), the distribution of patients was C/C (10%), C/G (33%), and G/ G (57%). Further, VEGF –634 C/C < C/G < G/G genotypes were found to be associated with increasing frequency and duration of hypertension (defined as diastolic >90 mm Hg and/or systolic >150 mm Hg) during treatment with
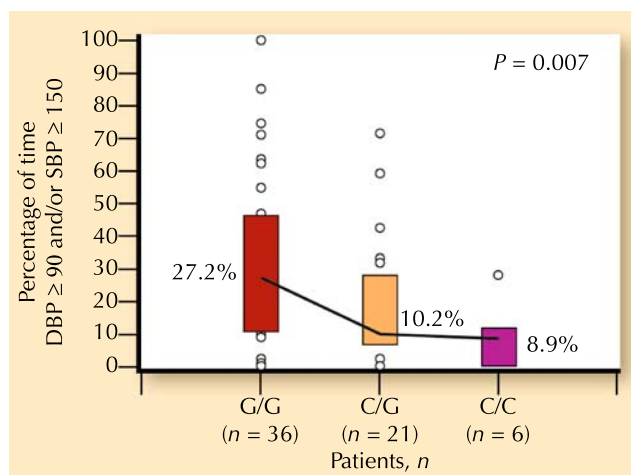

Fig. 1 Association of vascular endothelial growth factor single nucleotide polymorphism –634 genotype and duration of hypertension in metastatic renal cell carcinoma patients ($n=63$) treated with sunitinib [53]. *DBP* diastolic blood pressure; *SBP* systolic blood pressure

sunitinib ($P=0.03$ and $P=0.007$, respectively). Further, the significance of these findings persisted even after adjusting for baseline blood pressure and the use of antihypertensive medications ($P=0.05$ and $P=0.02$, respectively). In contrast, a similar correlation for the VEGF –1498 VEGF genotype was not found. It is of interest to note that, although the correlation of VEGF –634 genotype with hypertension was observed with bevacizumab [28••] and sunitinib [53], the VEGF –1498 genotype was correlated only with bevacizumab treatment. It is difficult to define a mechanistic basis, but one potential reason could be the larger patient cohort with the bevacizumab trial [28••]. A potential problem with the study by Kim et al. [53] is absence of data on plasma VEGF levels and their relationship to the VEGF SNP genotype for additional correlative data. Evaluation of other VEGF SNPs (–2578 C/ A, –2459 in/del18, 1154 G/A, and 936 C/T), as well as VEGFR-2 SNPs (889 G/A [rs2305948] and 1416 A/T [rs1870377] in mRCC patients treated with sunitinib for association with outcome and toxicity are ongoing at the Taussig Cancer Institute, Cleveland Clinic Foundation.

VEGF SNPs and Outcome with Angiogenesis Inhibitors or TKIs

In the study by Schneider et al. [28••] following treatment with paclitaxel or paclitaxel plus bevacizumab, the association of VEGF SNPs with treatment outcome was also determined. In particular VEGF –2578 A/A was found to associated with superior median overall survival in patients treated in the combination arm (plus bevacizumab) compared with alternate genotypes combined. Further, with

VEGF –1154, the A allele was correlated with a superior median overall survival and an additive effect of each active allele only for patients treated in the combination arm (plus bevacizumab). Interestingly, no significant associations with other VEGF or VEGFR-2 genotypes and efficacy were observed in this study [28••]. The only other report on VEGF genotype and treatment outcome is by Schultheis et al. [54], identifying that the VEGF 936 C/T genotype had a longer median progression-free survival compared with C/C and T/T genotype (P=0.061) in patients with recurrent/metastatic epithelial ovarian cancer treated with cyclophosphamide plus bevacizumab. A problem with this study is the absence of a treatment arm without bevacizumab for comparison. No data on association of VEGF genotype and toxicity was reported in this study. In the Kim et al. [53] study, VEGF –634 and –1498 genotypes were not found to be associated with tumor volume reduction or progression-free survival.

Future Directions

The role of VEGF/VEGFR genotype and correlations with outcome and toxicity in patients treated with angiogenesis inhibitors is still in its infancy. As this review has outlined, a substantial amount of published information exists on VEGF and/or VEGFR SNPs and the association with both nonmalignant and malignant disease conditions. Although this is helpful information, it is lacking data on the correlation between VEGF and/or VEGFR SNPS associated with prognosis/progression of a cancer type and response to the angiogenesis inhibitor treatment. Obviously, a more comprehensive analysis of both SNPs and expression of biomarkers in the VEGF signaling pathway will be important to critically understand whether association of outcome and toxicity with angiogenesis inhibitors is exclusively treatment related. Although studies have used genomic DNA from either peripheral blood mononuclear cells or tumor, our ongoing studies suggest a more than 98% concordance in paired samples. However, for correlations with expression and outcome, tumor material is important. Another unanswered question is whether the metastatic tumor that is being treated maintains the genotype and expression pattern for VEGF signaling determined with the primary tumor, so that meaningful correlative associations with outcome can be derived. Overall, these are exciting times when unique opportunities for personalized medicine with angiogenesis inhibitors are a reality with significant promise to improve treatment outcome and reduce toxicity.

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