The Role of Vascular Endothelial Growth Factor Genetic Variability in Cancer

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Abstract

Angiogenesis is a hallmark of tumor pathogenesis. Vascular endothelial growth factor (VEGF) is a critical regulator of angiogenesis and its inhibition has become a successful approach to antitumor therapy across tumor types. The VEGF gene is highly polymorphic with multiple common single nucleotide polymorphisms (SNPs) in the promoter, 5′ untranslated region and 3′ untranslated region. There is evidence that these SNPs in the regulatory regions can affect VEGF expression. In vitro and in vivo data show that genetic variability affects the activity and expression of VEGF. Case-control and cohort studies suggest that genetic variability may affect risk and outcome of a variety of disease states that are tightly regulated by angiogenesis. Recently, genetic variability in VEGF has been studied as a potential predictive biomarker for bevacizumab. The VEGF-1154 AA and -2578 AA genotypes predicted an improved median overall survival, whereas the VEGF-634 CC and -1498 TT genotypes predicted protection from grade 3-4 hypertension in the pivotal trial, E2100. If validated, these finding could help direct which subgroup of patients should receive bevacizumab. (Clin Cancer Res 2009;15(17):5297–302)

Background

Angiogenesis is important in multiple pathological conditions, including malignancy, and is a basic requirement for sustained tumor growth (1–3). This is largely mediated via activation and/or upregulation of vascular endothelial growth factor (VEGF)-A. VEGF-A is produced by the tumor but also produced by platelets and skeletal muscle in large quantities (4). VEGF-A production can be induced by hypoxia, changes in pH, and a variety of other mediators and cytokines. The common pathway is often through variable activation of the VEGF-A promoter (5). One well-established means is from the binding of hypoxia inducible factor (HIF) 1α and 1β binding to transcription factor binding sites on the promoter of VEGF-A in periods of hypoxia (6). VEGF-A subsequently binds its cognate receptors (the VEGF receptors), which activate multiple downstream pathways, and ultimately results in endothelial cell survival, mitogenesis, migration, differentiation, vascular permeability, and mobilization of endothelial progenitor cells from the bone marrow (Fig. 1; ref. 7).

Inhibition of VEGF-A and its receptors (e.g., VEGFR-2) is a successful strategy for inhibition of angiogenesis and as a result has become a standard class of drug for multiple malignancies (8). Within anti-VEGF therapeutics, there is heterogeneity in outcome both with respect to efficacy and toxicity. Because the target for these drugs (angiogenesis) is a host-mediated process in contradistinction to conventional chemotherapy (in which mutated tumor cells are the target), germline genetic variability may be an important contributor to this heterogeneity. VEGF-A is a highly polymorphic gene, and this genetic variability affects function and/or expression and alters risk and prognosis of multiple diseases that are controlled by angiogenesis. Genetic variability may also serve as a predictive biomarker for anti-VEGF therapies.

VEGF (and VEGF pathway) inhibition effective across disease processes. There are multiple ways to inhibit the VEGF-A pathway including inhibition of VEGF-A and its receptor, VEGFR-2 (8). The small molecule tyrosine kinase inhibitor sunitinib is approved by the U.S. Food and Drug Administration (FDA) for renal cell cancer and GIST and sorafenib is approved by the FDA for renal cell cancer and hepatocellular cancer. Bevacizumab is a humanized monoclonal antibody that binds to VEGF-A and is FDA approved for renal cell cancer, colon cancer, lung cancer, and breast cancer. Its activity in breast cancer is based on the findings of E2100 (9) and the AVADO (10) trials. These findings led to the FDA-accelerated approval for bevacizumab in combination with paclitaxel as initial chemotherapy for HER2-negative metastatic disease.

Global limitations to the nonselective implementation of anti-VEGF therapies. Although some patients gain significant benefit with the addition of an anti-VEGF therapeutic, others do not. As an example, in E2100, despite demonstrating a clear
improvement in overall response rate (ORR) and median progression free survival (PFS), the addition of bevacizumab did not significantly improve median overall survival (OS; ref. 9). The addition of bevacizumab also increased grade 3–4 toxicity including: cerebrovascular ischemia, headaches, proteinuria, and hypertension. In the context of heterogeneous benefit and real toxicity, the monetary cost further heightens the need for effective implementation of these agents. Thus, investigators have invested much effort into finding a signature that would successfully predict the most benefit but least toxicity for an individual patient and allow for the selection of those who absolutely should not receive therapy.

Early setbacks in the identification of biomarkers for selective implementation. Early efforts to identify the optimal subgroup of patients to receive bevacizumab largely included the traditional approach to biomarker discovery; clinical and tumor-related variables. Within E2100, a standard subgroup analysis was done on the basis of conventional clinical subgroups and showed an improved PFS for essentially all subgroups (9). Serum vascular cell adhesion molecule and urine VEGF-A did not correlate with outcome in E2100 (11). Tumor microvessel density, tumor VEGF and thrombospondin-2 expression, and mutations in k-ras and p53 did not predict benefit from bevacizumab in colorectal cancer (12, 13). Thus, disappointingly, traditional, tumor-specific and clinical variables were not successful biomarkers.

Clinical-Translational Advances

Rational approach to biomarker discovery: Will germline variability be the key?

There is clearly therapeutic heterogeneity among patients treated with anti-VEGF therapies. The most common established etiologies for therapeutic heterogeneity are: pharmacokinetic, somatic, or germline variability. It seems that the clinically employed doses of bevacizumab are well above that necessary for the elimination of circulating VEGF (14). Additionally, as bevacizumab is a humanized monoclonal antibody, it does not need to be metabolized in order to be activated or cleared in a classical fashion (15). Thus, pharmacokinetic variation likely plays a minor and/or no role in the variable efficacy and toxicity of bevacizumab. The evaluation of somatic variation is widely accepted as a potential biomarker for many therapeutic drugs in oncology. The use of anti-angiogenic therapy is unique, however, in that it does not target the tumor (as most conventional cytotoxic chemotherapies do). Instead, it targets
the host endothelium, which is controlled by host genetic variation rather than somatic changes (8, 16). Given the pathophysiology of angiogenesis, does germline genetic variability hold the most promise for biomarker discovery?

The polymorphic appeal of VEGF: It’s all about the regulatory domains

VEGF has multiple well-established and common polymorphisms. These single nucleotide polymorphisms (SNP) lie within the promoter, 5′UTR, and 3′UTR. There are no established, common nonsynonymous SNPs within the VEGF gene. We resequenced a region of VEGF consisting of 4,017 base pairs upstream of the VEGF start codon in 48 Caucasians and 48 African Americans. This region was chosen because it contained all known regulatory factors and SNPs in proximity to the VEGF gene. Analysis of resequencing data revealed 20 previously reported SNPs, insertions, and/or deletions, one novel SNP, and one novel deletion. Haplowiew 4.0 was used to determine the haplotypes of the VEGF promoter using all SNPs and rare variants. In the combined population, 74% of the genetic variation was explained by six major haplotypes. When stratified by race, five haplotypes explained 81.2% of genetic variation in Caucasians compared with seven haplotypes that explained 73.5% of genetic variation in African Americans. Using the elucidated haplotype structure, tagSNPs were then chosen for each haplotype. Five designated tagSNPs (Table 1) accounted for the six most common haplotypes in a population comprised mostly of Caucasian and African American subjects. There is substantial linkage disequilibrium within the VEGF promoter.

Consequences of VEGF variation: The functional side of the story

In vitro work showed that the -2578CC and -1154GG genotypes had increased VEGF secretion in human peripheral blood mononuclear cells compared with carriage of a rare allele (17, 18). VEGF expression in human breast cancer specimens assessed by immunohistochemistry revealed a trend toward lower expression with the VEGF-2578 AA (P = 0.12) and VEGF-1154 AA (P = 0.08) genotypes (19). VEGF serum levels were significantly higher in healthy subjects with the between the -634 CC wild-type genotype (20), and increased VEGF expression was also seen in MCF7 cell lines with this genotype (21). There is a high degree of linkage disequilibrium between the -2578C/A, -1154G/A, and -634 G/C alleles, and an analysis of haplotype versus expression was done via luciferase activity levels in transfected glioma cells. These studies revealed that haplotypes containing the -1154A, -2578A, and the -634G alleles had lower expression in normoxic and/or hypoxic conditions (22). A possible mechanism for variable function is the association of the -2578 C/A with the presence of an 18-bp insertion-deletion polymorphism at -2549 position (23). The -634 G/C likely affects expression at the post-transcriptional level by altering the activity of the internal ribosomal entry site B, thereby enhancing initiation of translation at the AUG start codon and regulating production of the large VEGF isoform, which is translated at an alternative CLIG codon (24). In total, these findings suggest a decreased VEGF production and/or expression for those who carry the variant promoter-5′UTR alleles. An evaluation of effect of 3′UTR SNPs on plasma VEGF levels showed that the +702 C/T and +1612 G/A SNPs did not impact plasma levels, whereas those with the +936T allele had significantly lower VEGF levels (25, 26). One potential mechanism is the loss of an AP-4 binding site with the presence of the +936T allele (26). To better understand the functional role of common haplotypes on promoter activity, however, our group has cloned the common tagSNPs (Table 1) into four common breast cancer cell lines and are evaluating the role of variability in a variety of conditions.

Consequences of VEGF variation: An inconsistent theme of clinical importance

There is a multitude of case-control studies that have tested for a correlation between a given disease process regulated by angiogenesis and VEGF genotype. These studies include those that evaluate cancer risk and prognosis (25, 27–36), retinopathy (20, 37–42), nephropathy (43–46), pre-eclampsia (47, 48), and vasculopathy (49–51), among many others. Many of these studies show an increased association of disease and/or inferior outcomes in the subgroup with genotype that would predict a higher VEGF expression. There are also many conflicting results (in which no association is detected or even the opposite association is observed). These genres of studies are fraught with problems. First, gene-gene and gene-environment interactions are clearly overlooked by many. Important genetic and clinical variables are rarely included in the multivariate analyses. Second, SNP (and gene) selection are inconsistent and often rarely functionally based (i.e., haplotype approach). Third, multiple SNPs are often assessed without proper corrections for multiple testing, thus resulting in high false discovery rates. Finally, the phenotypes are often not clearly defined, and confounding variables are not imputed. These inconsistencies make it difficult to determine the true clinical relevance of VEGF genetic variability. Although the body of literature as a whole would suggest there is an association with many of these angiogenesis-driven conditions, the degree of risk (after correcting for proper covariates) and the confidence in these associations is less than clear.

SNPs as biomarkers for anti-VEGF therapy

Ovarian cancer. A recent study evaluated the role of multiple genetic polymorphisms with efficacy parameters in a phase II trial of patients with platinum-refractory ovarian cancer (52). In the parent phase II trial, 70 women were treated with bevacizumab and daily oral cyclophosphamide. In a companion correlative study, efficacy parameters were compared with genotype (53 patients) for 33 SNPs in 30 different genes. This study found that patients who carried an A allele for the IL-8 -251 T/A gene polymorphism had a significantly lower response rate than those homozygous for TT (P = 0.006). Patients carrying a C allele of the

| Table 1. tagSNPs for common VEGF promoter haplotypes |
|-------------------------------|----------------|-------------|
| Haplotype (Frequency %)       | Location       | tagSNPs (Allele) |
| 1 (20.8)                      | VEGF -3818 G/T | T           |
| 2 (20.3)                      | VEGF -1154 G/A | A           |
| 3 (10.9)                      | VEGF -7 C/T    | T           |
| 4 (9.7)                       | SNPs of haplotypes 1, 2, 3, 5, 6 All wild type |         |
| 5 (7.8)                       | VEGF -2305 G/T | T           |
| 6 (4.6)                       | VEGF -1210 C/A | A           |
CXCR2 785 C/T polymorphism had a superior median PFS compared with those homozygous for TT \((P = 0.026)\). Patients with the VEGF 936 CT genotype had a superior median PFS compared with the homozygous wild-type and homozygous variant genotypes. Unfortunately, only two VEGF SNPs were studied, and the association with the VEGF 936 C/T did not show an allele-dose affect. Additionally, corrections were not made for multiple comparisons leading to the very real possibility of false discovery. It is not clear if these represent true predictive markers for bevacizumab or simply prognostic markers because there was no placebo group with which to compare.

Breast cancer. Our group evaluated the role of VEGF SNPs as a potential biomarker within the pivotal trial E2100. Because only tumor samples were available and germline DNA was not collected, we first evaluated whether the variability seen in selected polymorphic sites was the same in the tumor as it was in germline. Thus we genotyped paraffin-embedded human breast cancer samples and compared it to germline DNA (16). We studied the primary breast tumor \((n = 17)\), a histologically involved lymph node (LN) \((n = 17)\), and a histologically normal LN \((n = 19)\), which served as germline DNA. We assessed three SNPs including: VEGF 936C/T, eNOS -786T/C, and eNOS 894G/T. When evaluating all polymorphisms there was 100% concordance between samples that involved malignancy compared with germline DNA [95% confidence interval (CI), 0.88–1.00]. Thus we felt confident that by genotyping from tumor in E2100 the majority of variation would largely reflect germline variability. Paraffin-embedded tumors were available from 363 eligible cases in E2100 for genotyping with a median follow-up of 43 months. A total of 180 cases were from the experimental arm, and 183 from the control arm. Clinical covariates in these subgroups (with available DNA) were similar to the remainder of patients in each arm.

The VEGF -2578AA and VEGF -1154AA genotypes predicted a superior median OS for patients in the combination arm (19). These genotypes did not predict an improved median OS for patients in the control arm thus supporting a predictive.
related toxicity, hypertension, is biologically linked to outcome or alternatively, from prolonged anti-VEGF blockade (even be best served by either never being exposed to anti-VEGF therapy rebound angiogenesis after brief exposure to anti-VEGF therapy baseline VEGF expression have a sustained benefit to anti-VEGF continuation of anti-VEGF therapy (59). Perhaps those with low this finding could be due to rebound angiogenesis after discon- sponse rate. This would imply that the biologic effect is impor- tunity and expression of VEGF. Case-control and cohort studies suggest that variability may affect risk and outcome of a variety of disease states that are tightly regulated by angiogenesis. In general, increased VEGF production seems to correlate best with increases in risk and worse disease outcome, although many studies have been negative or contradictory. Early data suggest VEGF SNPs might serve as biomarkers that can successfully predict subgroups of patients that will experience the optimal outcome and others who will be at higher risk of drug-induced toxicity. From the existing data, patients with VEGF genotypes that predict low VEGF production and/or expression gain the most substantial benefit with VEGF inhibition. Currently, we suggest that future studies implementing a candidate approach for risk and/or prognosis discovery of VEGF should include a functional assessment (implementing haplotypes and/or tagSNPs) with clearly defined phenotypes. The biomarker data for anti-VEGF therapy discussed here should not be regarded as standard of care for clinical practice and should be validated in independent data sets.

Conclusion

Angiogenesis plays a critical role in the pathogenesis of can- cer. VEGF is one of the major growth factors that regulates angiogenesis and is a highly polymorphic gene. This genetic variability may be biologically significant and account for heterogeneity in disease risk and outcome. There are no nonsynonym- ymous polymorphisms to account for structural changes in protein product, but, in vitro and in vivo data show that genetic variability in the promoter, 5′UTR, and 3′ UTR affect the activity and expression of VEGF. Case-control and cohort studies suggest that variability may affect risk and outcome of a variety of disease states that are tightly regulated by angiogenesis. In general, increased VEGF production seems to correlate best with increases in risk and worse disease outcome, although many studies have been negative or contradictory. Early data suggest VEGF SNPs might serve as biomarkers that can successfully predict subgroups of patients that will experience the optimal outcome and others who will be at higher risk of drug-induced toxicity. From the existing data, patients with VEGF genotypes that predict low VEGF production and/or expression gain the most substantial benefit with VEGF inhibition. Currently, we suggest that future studies implementing a candidate approach for risk and/or prognosis discovery of VEGF should include a functional assessment (implementing haplotypes and/or tagSNPs) with clearly defined phenotypes. The biomarker data for anti-VEGF therapy discussed here should not be regarded as standard of care for clinical practice and should be validated in independent data sets.

Disclosure of Potential Conflicts of Interest

B.P. Schneider: commercial research grant, Genentech; speaker’s bureau, GSK; consultant, BMS, Genome Health, and Genentech. K.D. Miller: commercial research grants, Genentech, Roche, and Synday, GSK, Entremed, and Pfizer; speaker’s bureau, Genentech and Roche; consultant, Genentech, Roche, and Entremed.

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