Vascular Endothelial Growth Factor Genotypes, Haplotypes, Gender, and the Risk of Non–Small Cell Lung Cancer

Rihong Zhai,[1] Geoffrey Liu,[1,3] Wei Zhou,[1] Li Su,[1] Rebecca Suk Heist,[1,2] Thomas J. Lynch,[2] John C. Wain,[4] Kofi Asomaning,[1] Xihong Lin,[5] and David C. Christiani[1,6]

Abstract

Purpose: The vascular endothelial growth factor (VEGF) is a major mediator of angiogenesis involving tumor growth and metastasis. Polymorphisms in the *VEGF* gene may regulate VEGF production. In this large case-control study, we investigated whether functional polymorphisms (*-460C/T*, *+405C/G*, *+936C/T*) in the *VEGF* gene are associated with the risk of non–small cell lung cancer (NSCLC).

Experimental Design: *VEGF* genotypes and haplotypes were determined in 1,900 Caucasian patients with NSCLC and 1,458 healthy controls. The results were analyzed using logistic regression models, adjusting for age, gender, smoking status, pack-years of smoking, and years since smoking cessation (for ex-smokers). The false-positive report probability was estimated for the observed odds ratios (OR).

Results: There were no overall associations between individual *VEGF* genotypes and the risk of NSCLC. Stratified analysis suggested that the combined *+405CC+CG* genotype was significantly associated with increased risk of lung adenocarcinoma in males (adjusted OR, 1.40; 95% confidence interval, 1.03-1.87). In haplotype analysis, haplotypes were globally associated with differences between cases and controls in males ($P = 0.03$). Specifically, the *-460T/+405G/+936C* haplotype was significantly ($P = 0.02$) associated with decreased risk of adenocarcinoma in males when compared with the most common *CGC* haplotype (adjusted OR, 0.76; 95% confidence interval, 0.50-0.98). None of the *VEGF* genotypes and haplotypes studied significantly influenced the susceptibility to NSCLC in females.

Conclusions: Polymorphisms of *-460C/T*, *+405C/G*, and *+936C/T* in the *VEGF* gene do not play a major role in NSCLC risk. However, we could not exclude a minor role for the *+405CC+CG* genotypes and the *460T/+405G/+936C* haplotype in lung adenocarcinogenesis in male Caucasians.

Angiogenesis is a process by which endothelial cells divide and migrate to form new blood vessels (1). Angiogenesis not only supplies metabolic demands but also provides potential routes for tumor dissemination and metastasis (2). Increased angiogenesis has been reported to be associated with tumor progression and metastasis in a number of human solid tumors, including non–small cell lung cancer (NSCLC; refs. 3, 4).

Vascular endothelial growth factor (VEGF) is one of the most potent mediators of angiogenesis and vascular permeability (5). Expression of *VEGF* mRNA and proteins in tumors of NSCLCs has been associated with higher microvessel counts, tumor size, and poorer prognosis (6–8). In NSCLC, *VEGF* expression in adenocarcinomas was significantly higher than that in squamous cell carcinoma (7). Androgen could stimulate angiogenesis via promoting VEGF production in both normal and tumor tissues (9), suggesting that *VEGF* expression may be regulated by hormonal status. Clinically, higher serum VEGF levels in patients with NSCLC have been associated with higher NSCLC staging and shorter survival (10–12). Therapeutic strategies, such as the use of anti-VEGF antibody bevacizummab, have shown favorable antitumor results in NSCLC (13).

The *VEGF* gene is located on chromosome 6p21.3. Polymorphisms in the *VEGF* gene have been associated with differential *VEGF* expression and protein production. For example, the *+405CG* and *CC* genotypes have been associated with higher vascular density in tumors of NSCLC (14). However, the exact function is controversial as some studies have shown that the *+405C* allele is correlated with lower VEGF protein production (15, 16). The *+936T* allele has been related to lower VEGF

Authors' Affiliations: Departments of [1]Environmental Health, [2]Hematology-Oncology, and [3]Medicine and Medical Biophysics, Princess Margaret Hospital/Ontario Cancer Institute, University of Toronto, Toronto, Ontario, Canada; [4]Surgery and [5]Biostatistics, Harvard School of Public Health, Boston, Massachusetts; and [6]Medicine, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts

Received 7/5/07; revised 10/17/07; accepted 10/25/07.

Grant support: NIH grants CA92824, CA74386, CA90578, ES/CA 06409, and CA119650 (D. Christiani). The Flight Attendants Medical Research Institute (R. Zhai and W. Zhou), and the Kevin Jackson Memorial Fund and Alan Brown Chair of Molecular Genomics (G. Liu).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Requests for reprints: David C. Christiani, Department of Environmental Health, Harvard School of Public Health, 665 Huntington Avenue, Boston, MA 02115. Phone: 617-432-1641; Fax: 627-432-6981; E-mail: dchris@hsph.harvard.edu.

doi:10.1158/1078-0432.CCR-07-1655

plasma levels (17), as has the *-460T* allele (15). These three *VEGF* polymorphisms have been associated with increased risks for several types of tumors (18–21), including lung cancer in Asian populations (22). Nevertheless, the association of these polymorphisms with NSCLC risks in Caucasian populations has not been evaluated. Based on the pathologic significance of VEGF in NSCLC and the potential biological effects of *VEGF* polymorphisms on VEGF production, we hypothesized that functional single nucleotide polymorphisms of the *VEGF* gene would be associated with differential risk of NSCLC. Furthermore, we hypothesized that the association might be modified by gender or cell type. In addition, we estimated the false-positive report probability (FPRP) by incorporating the prior probability that these specific single nucleotide polymorphisms are associated with NSCLC risk (23).

Materials and Methods

Study population. This study was approved by the Human Subjects Committees of Massachusetts General Hospital and Harvard School of Public Health, Boston, MA. The study population was derived from a large ongoing molecular epidemiologic study that began in 1992 and now has >1,900 NSCLC patients recruited at the Massachusetts General Hospital. Details of this case-control population have been described previously (24). Briefly, all histologically confirmed, newly diagnosed patients with NSCLC at the Massachusetts General Hospital were recruited between December 1992 and February 2006. Before 1997, only early stage (stage I and II) patients were recruited. After 1997, all stages of NSCLC cases were recruited in this study. Controls were recruited at the Massachusetts General Hospital from healthy friends and non–blood-related family members (usually spouses) of several groups of hospital patients: (*a*) patients with cancer, whether related or not related to a case; or (*b*) patients with a cardiothoracic condition undergoing surgery. No matching was done. Importantly, none of the controls were themselves patients. Potential controls that carried a previous diagnosis of any cancer (other than non–melanoma skin cancer) were excluded from participation. More than 85% of the eligible cases and >90% of the controls participated in this study and provided blood samples. A research nurse administered questionnaires on demographic information and a detailed smoking history of each participant. To reduce potential variation in allele frequency by ethnicity, only Caucasians were considered in the analysis.

Genotyping. DNA was extracted from peripheral blood samples using the Purgene DNA Isolation Kit (Gentra Systems). *VEGF -460C/T* (rs833061), *+405C/G* (rs2010963), and *+936C/T* (rs3025039) genotypes were determined using the 5′ nuclease assay (TaqMan) and ABI Prism 7900HT Sequence Detection System (Applied Biosystems). The primers, probes, and reaction conditions are available upon request. Genotyping was done by laboratory personnel blinded to case-control status. For quality control, a random 5% of the samples were repeated to assess the reproducibility of results. Two authors independently reviewed all genotyping results.

Statistical analysis. We analyzed all Caucasians with complete information on age, gender, smoking status (never smoking, ex-smoking, and current smoking), pack-years of smoking, and years since smoking cessation (for ex-smokers). Hardy-Weinberg disequilibrium of each polymorphism in controls was tested using the χ^2 test. Detection of linkage disequilibrium between the three polymorphisms was based on Lewontin's D' in controls. Haplotype frequencies and individual haplotypes were generated using the SAS HAPPY program (25, 26).

Demographic and clinical information between cases and controls was compared using χ^2 tests for categorical variables and the Student's *t* test or the nonparametric Kruskal-Wallis test for continuous variables, where appropriate. Logistic regression models were used to analyze the associations of all genotypes and haplotypes with NSCLC risks, adjusting for potential confounding factors such as age, gender, smoking status, pack-years of smoking, and years since smoking cessation (if ex-smoker). In addition to the overall association analysis, we did a stratified analysis by various factors including gender, histology, and smoking status to further explore the association between *VEGF* polymorphisms and the risk NSCLC in each stratum because previous studies suggested that *VEGF* expression or production might relate to gender or cell types (7, 9). All reported *P* values were based on two-sided tests. $P < 0.05$ were considered statistically significant. All analyses were done using SAS software version 9.1 (SAS Institute).

We estimated the FPRP for statistically significant observations using the methods described by Wacholder et al. (23). We calculated the FPRP for prior probability ranging from 50% to 0.1%. We considered that a prior probability of 50% may be appropriate when there is very strong biological and epidemiologic evidence that the association is real, and that a prior probability of 0.1% may be appropriate when both biological and epidemiologic data are inadequate.

Results

Population characteristics. Demographic data stratified by case and gender are presented in Table 1. Both male and female cases were generally older and smoked more. The frequency of nonsmokers was higher among controls, whereas the frequency of ever smokers was higher among cases. The number of years that a participant had quit smoking was higher among controls. The distribution of the smoking variables in our controls was similar to that of the general Massachusetts population over age 45 (27). The proportion of non-, ex-, and current smokers were 36%, 45%, and 20% in our controls and 36%, 47, and 17% in the general Massachusetts population over age 45, respectively. Adenocarcinoma, squamous cell carcinoma, and others (large cell carcinoma, carcinoid, mixed cell type, and uncertain cell type) represented 57%, 23%, and 20% of NSCLC cases, respectively.

Association between VEGF genotypes and NSCLC risk. All of the *VEGF* polymorphisms in the control and case populations were consistent with Hardy-Weinberg equilibrium ($P > 0.05$, χ^2 goodness-of-fit). Genotype frequencies of the *-460C/T*, *+405C/G*, and *+936C/T* polymorphisms in controls were in close agreement with those previously published for healthy Caucasian individuals (15–17, 26). There was no statistical difference in genotype distributions between cases and controls, overall or for different genders (Table 2).

There were no overall crude or adjusted associations between individual *VEGF* polymorphisms and the risk of NSCLC (Table 3). In the subgroup analysis, the combined *+405CC+CG* genotype was significantly associated with higher risk of NSCLC in males, specifically for adenocarcinomas [adjusted odds ratio (OR), 1.39; 95% confidence interval (95% CI), 1.07-1.81; Table 4]. No subset associations were found in never smokers, ex-smokers, current smokers, or in three different strata according to pack-years of smoking ($P > 0.05$ for all comparisons).

Association between VEGF haplotypes and NSCLC risk. Haplotype analyses were conducted to evaluate the combined effect of the three polymorphisms on NSCLC risk. Consistent with observations from early studies (22, 26), the *-460C/T* and *+405C/G* polymorphisms in our study were in strong linkage disequilibrium (Lewontin's $D' = 0.94$), whereas linkage between the *-460C/T* and *+936C/T* polymorphism was weaker

Table 1. Demographic characteristics among NSCLC cases and controls

Characteristics	Overall			Male			Female		
	Cases (*n* = 1,900)	Controls (*n* = 1,458)	*P*	Cases (*n* = 981)	Controls (*n* = 638)	*P*	Cases (*n* = 919)	Controls (*n* = 820)	*P*
Age (mean ± SD)	65 ± 10.8	58 ± 12.2	<0.01	66.0 ± 10.2	60.0 ± 12.9	<0.01	64.1 ± 11.0	57.3 ± 11.5	<0.01
Gender, *n* (%)									
Female	919 (48.8%)	820 (56.2%)	<0.01						
Male	981 (51.6%)	638 (43.8%)							
Smoking, *n* (%)									
Never	177 (9.3%)	517 (35.5%)	<0.01	69 (7.0%)	195 (30.6%)	<0.01	108 (11.8%)	322 (39.3)	<0.01
Ex-smoker	989 (52.1%)	656 (45.0%)		553 (56.4%)	339 (53.1)		436 (47.4%)	317 (38.7%)	
Current smoker	734 (38.6%)	285 (19.6%)		359 (36.6%)	104 (16.3)		375 (40.8%)	181 (20.1%)	
Years since quit (median)*,†	12 (1-59)	18 (1-65)	<0.01	14 (1-59)	20 (1-65)	<0.01	12 (1-55)	17 (1-59)	<0.01
Pack-years†	50 (0.1-231)	25 (0.1-218)	<0.01	58 (0.2-231)	29 (0.1-210)	<0.01	44 (0.02-210)	21 (0.03-218)	<0.01
Tumor stage (%)									
I and II	48.6%			49.0%			48.0%		
III and IV	51.3%			51.0%			52.0%		
Cell type (%)									
Adenocarcinoma	56.6%			50.8%			62.8%		
Squamous cell carcinoma	23.3%			29.0%			17.2%		
Others	20.1%			20.2%			20.0%		

NOTE: Continuous variables tested with the Student's *t* test; categorical variables tested using the χ^2 test.
*Ex-smokers only.
†Median (range), tested by nonparametric Wilcoxon's rank sum test.

(Lewontin's $D' = 0.26$). There were five haplotypes with frequencies >5% among both cases and controls. The most common haplotype was the *460C/405G/936C* (*CGC*) haplotype, with frequencies of 40% in cases and 41% in controls. Haplotype frequency distributions in controls were similar to those reported in other healthy Caucasian populations (26). The overall distribution of different haplotypes were similar between cases and controls ($\chi = 9.66$, $P = 0.22$). However, in male subjects, haplotype frequencies were significantly different between cases and controls (Table 2). Moreover, in multivariate analyses, haplotypes were globally associated with NSCLC risk in males ($P = 0.03$). When the male subjects were further stratified by histologic cell type, the *TGC* haplotype was significantly associated with decreased risk of adenocarcinoma compared with the *CGC* haplotype (adjusted OR, 0.76; 95% CI, 0.50-0.98; $P = 0.04$; Table 4). No associations were found

Table 2. *VEGF* genotype and haplotype frequencies among NSCLC cases and controls

Characteristics	Overall			Male			Female		
	Cases (*n* = 1,900)	Controls (*n* = 1,458)	*P*	Cases (*n* = 981)	Controls (*n* = 638)	*P*	Cases (*n* = 919)	Controls (*n* = 820)	*P*
VEGF -460C/T, n (%)									
TT	539 (28.4%)	422 (28.9%)	0.87	287 (29.3%)	179 (28.1%)	0.79	252 (27.4%)	243 (29.6%)	0.59
CT	922 (48.5%)	694 (47.6%)		473 (48.2%)	307 (48.1%)		449 (48.9%)	387 (47.2%)	
CC	439 (23.1%)	342 (23.5%)		221 (22.5%)	152 (23.8)		218 (23.7%)	190 (23.2)	
VEGF+405C/G, n (%)									
GG	805 (42.4%)	650 (44.6%)	0.22	397 (40.5%)	279 (43.7%)	0.14	408 (44.4%)	371 (45.2%)	0.89
CG	848 (44.6%)	644 (44.1)		454 (46.3%)	294 (46.1%)		394 (42.9%)	350 (42.7%)	
CC	247 (13.0%)	164 (11.3%)		130 (13.3%)	65 (10.2%)		117 (12.7%)	99 (12.1%)	
VEGF +936C/T, n (%)									
CC	1,441 (75.8%)	1,125 (77.2%)	0.67	754 (76.9%)	496 (77.7%)	0.87	687 (74.7%)	629 (76.7%)	0.63
CT	424 (22.3%)	308 (21.2%)		209 (21.3%)	132 (20.7%)		215 (23.4%)	176 (21.5%)	
TT	35 (1.8%)	25 (1.7%)		18 (1.8%)	10 (1.6%)		17 (1.9%)	15 (1.8%)	
Haplotypes*									
CGC	40.0%	40.8%	0.22	40.0%	40.3%	0.0041	40.1%	41.7%	0.40
TCC	28.1%	29.1%		27.6%	31.4%		28.2%	26.8%	
TGC	19.0%	16.6%		19.8%	15.6%		18.6%	17.3%	
CGT	6.5%	6.0%		7.0%	6.1%		6.1%	5.6%	
TCT	4.5%	5.6%		4.7%	4.7%		4.6%	6.5%	

*The combined haplotype frequencies (in the order of *-460C/T*, *+405C/G*, *+936C/T*) <5% were not included in the analysis.

Table 3. Adjusted ORs of VEGF genotypes and haplotypes for NSCLC risks

	All cases (*n* = 1,900) vs. controls (*n* = 1,458)		Male cases (*n* = 981) vs. controls (*n* = 638)		Female cases (*n* = 919) vs. controls (*n* = 820)	
	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
Genotypes						
VEGF -460C/T						
TT	1.0		1.0		1.0	
CT+CC	1.16 (0.96-1.40)	0.12	1.07 (0.81-1.40)	0.65	1.27 (0.98-1.65)	0.07
VEGF -405C/G						
GG	1.0		1.0		1.0	
CG+CC	1.14 (0.96-1.36)	0.13	1.27 (0.99-1.63)	0.06	1.04 (0.82-1.32)	0.73
VEGF +936C/T						
CC	1.0		1.0		1.0	
CT+TT	1.05 (0.87-1.27)	0.59	1.13 (0.87-1.50)	0.37	0.99 (0.27-1.29)	0.95
Haplotypes*						
Global test	0.54		0.03		0.68	
CGC	1.0		1.0		1.0	
TCC	1.02 (0.88-1.17)	0.84	1.15 (0.94-1.42)	0.10	0.91 (0.74-1.11)	0.34
TGC	0.88 (0.75-1.04)	0.14	0.81 (0.63-1.02)	0.08	0.93 (0.74-1.17)	0.53
CGT	0.98 (0.74-1.31)	0.89	0.90 (0.59-1.36)	0.61	1.04 (0.69-1.55)	0.87
TCT	1.14 (0.84-1.55)	0.39	1.15 (0.72-1.85)	0.55	1.04 (0.69-1.57)	0.85

NOTE: Adjusted for age, gender, smoking status, pack-years of smoking, and years since smoking cessation (if ex-smoker).
*The combined haplotype frequencies (in the order of -460C/T, +405C/G, +936C/T) <5% were not included in the analysis.

between any haplotype and NSCLC risk, overall or stratified among female subjects.

Table 5 shows the FPRP for the two statistically significant associations we observed. In general, at a FPRP cut point of 0.5, the observed OR for an association is likely to reflect a true association with a prior probability of >10%. However, an association with prior probability of 1% or lower is likely to be a false-positive finding. Because our statistically significant findings were restricted to subgroups, a more stringent cut point at 0.2 for FPRP may be appropriate. Given the available epidemiologic data and the known functional significance of *VEGF* polymorphisms, a prior probability of at least 25% may be appropriate. Thus, the observed ORs may not reflect false-positive associations.

Discussion

Our results suggest that polymorphisms of *-460C/T*, *+405C/G*, and *+936C/T* in the *VEGF* gene do not play a major role in NSCLC carcinogenesis. Nevertheless, we could not exclude the

Table 4. Adjusted ORs of *VEGF* polymorphisms according to gender and NSCLC histology

	Male				Female			
	Adenocarcinoma (706 cases/638 controls)		Squamous cell carcinoma (285 cases/638 controls)		Adenocarcinoma (770 cases/820 controls)		Squamous cell carcinoma (158 cases/820 controls)	
	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
Genotypes								
VEGF -460C/T								
TT	1.0		1.0		1.0		1.0	
CT+CC	1.14 (0.85-1.52)	0.38	0.92 (0.61-1.39)	0.69	1.29 (0.99-1.69)	0.06	1.21 (0.73-2.01)	0.47
VEGF+405C/G								
GG	1.0		1.0		1.0		1.0	
CG+CC	1.39 (1.07-1.81)	0.01	0.98 (0.67-1.42)	0.90	1.07 (0.84-1.36)	0.58	0.75 (0.47-1.77)	0.20
VEGF+936C/T								
CC	1.0		1.0		1.0		1.0	
CT+TT	1.11 (0.84-1.48)	0.46	1.39 (0.92-2.09)	0.11	0.94 (0.75-1.27)	0.85	1.23 (0.76-2.00)	0.40
Haplotypes*								
Global test		0.02		0.23		0.54		0.67
CGC	1.0		1.0		1.0		1.0	
TCC	1.18 (0.95-1.47)	0.14	1.09 (0.80-1.47)	0.23	0.95 (0.75-1.21)	0.67	1.09 (0.80-1.47)	0.59
TGC	0.76 (0.50-0.98)	0.04	0.95 (0.67-1.35)	0.76	0.95 (0.72-1.16)	0.47	0.95 (0.67-1.35)	0.76
CGT	0.87 (0.56-1.35)	0.53	1.13 (0.60-2.10)	0.71	1.36 (0.86-2.16)	0.19	1.13 (0.60-2.10)	0.71
TCT	1.14 (0.69-1.90)	0.61	1.32 (0.68-2.58)	0.55	0.91 (0.56-1.46)	0.68	1.32 (0.68-2.58)	0.42

NOTE: Adjusted for age, gender, smoking status, pack-years of smoking, and years since smoking cessation (if ex-smoker).
*The combined haplotype frequencies <5% were not included in the analysis.

Table 5. False-positive report probability

Polymorphism	Stratum	OR (95% CI)	Power	Reported *P* value	Prior probability*			
					0.5	0.25	0.1	0.001
+405CG+CC	Adenocarcinoma in male	1.39 (1.07-1.81)	0.714 †	0.01	0.020	0.057	0.115	0.668
TGC	Adenocarcinoma in male	0.76 (0.50-0.98)	0.737 ‡	0.04	0.045	0.123	0.296	0.822

*FPRP for the observed ORs.
†Estimation of the statistical power to detect an OR of 1.5 with a level equal to the observed *P* value.
‡Estimation of the statistical power to detect an OR of 0.7 with a level equal to the observed *P* value.

possibility that the combined*+405CC+CG* genotype and *460T/+405G/+936C* haplotype (*TGC*) play minor roles in adenocarcinoma in males.

Our study has a number of strengths. First, the polymorphisms investigated in this study are thought to affect *VEGF* expression and protein production, and several epidemiologic studies have reported associations between these polymorphisms and cancer risk. The combination of these evidences suggested a likely high prior probability. Second, the large sample size gave high statistical power and therefore was less susceptible to fluctuating results. Third, we used the FPRP to interpret the results. Estimates of the FPRP can be used to decide whether a statistically significant finding is noteworthy based on prior probability. However, prior probability varies among individuals, and investigators who use different prior probabilities may reach different conclusions. We tested a range of prior probabilities which allowed us to identify how sensitive our findings were to changing prior probability.

Our results indicated that the *+405CC/CG* genotype may influence adenocarcinoma susceptibility, particularly in males. Previous studies have reported that *+405C/G* polymorphisms were associated with *VEGF* expression, production, and disease development. Individuals carrying the *+405CC* and *CG* genotypes were linked with higher *VEGF* expression and vascular density in the tumor of NSCLC (14). Sfar et al. (19) reported that the combined *+405CC/CG* genotype was associated with increased risk of prostate cancer. In Asian populations, the combined *+405CC/CG* genotype was associated with increased risk of small cell lung cancer (22). The *+405CC* genotype was also reported to be associated with higher serum VEGF levels, increased risks of retinopathy and myocardial infarction in diabetes (28–30), and higher tumor aggressiveness in breast cancer (18). However, attributing functional causality to these findings should be limited, as there is debate on the exact function of the *405G/C* polymorphism and some studies have shown that the *+405C* allele is associated with lower VEGF production and *VEGF* promoter activity (15, 16). In addition, some studies do not show a role of the *+405G/C* polymorphism in VEGF production or disease risk (26, 28, 30).

In the present study, neither the *VEGF -460C/T* nor *+936C/T* polymorphisms significantly influenced susceptibility to NSCLC. However, the haplotype *TGC* containing the *-460T* allele and the *+936C* allele showed a decreased effect on the risk of adenocarcinoma in males. This finding suggested that the effects of *+405C/G* polymorphisms on adenocarcinoma in males may be at least in part due to the fact that *+405C/G* was in linkage disequilibrium with *-460C/T* and *+936C/T*. Neither the *-460C/T* nor the *+936C/T* polymorphism alone was sufficient to influence the susceptibility to NSCLC, but a set of three polymorphisms (haplotype) might have a more powerful effect on NSCLC susceptibility due to a combined effect on gene function. Another possible reason may be that the effect of haplotype *TGC* on NSCLC risk was owing to linkage disequilibrium with other functional variants in the *VEGF* gene (HapMap database).[7] Thus, additional studies covering more functional single nucleotide polymorphisms in the *VEGF* gene will be needed to confirm our findings.

There was a striking difference in the effects of *VEGF* genotype and haplotype on NSCLC risk between female and male subjects. This gender difference in relation to *VEGF* polymorphism association in NSCLC was unlikely to be due to the differences in smoking status between males and females, because after adjusting for smoking exposure levels, the combined *+VEGF 405CC/CG* genotype and haplotype *TGC* remained significantly associated with NSCLC risk in male subjects for adenocarcinoma. Although a number of epidemiologic studies have proved that the incidence, risk, histology, and pathogenesis of lung cancer differed between women and men, the mechanisms driving these differences are largely unknown (31, 32). Genetic factors have been proposed to account for gender differences in lung cancer risks. For example, there was a higher frequency of tumor suppressor gene *p53* mutations among women with NSCLC than among men with NSCLC; the proto-oncogene *K-ras* gene mutations have been found to be more common in female patients with lung cancer who were smokers than among male smokers with lung cancer (33). Androgen is probably another factor determining gender difference in response to VEGF. Both *in vitro* and *in vivo* studies have proved that androgen could up-regulate *VEGF* expression (34, 35), whereas androgen ablation inhibited *VEGF* expression (36). On the contrary, estrogen reduced *VEGF* expression (37, 38).

The finding that *VEGF* polymorphisms tended to have a stronger association with adenocarcinoma risk than with squamous cell carcinoma risk has not been previously reported. However, several lines of compelling evidence may explain our observations. First, in the lungs of patients with NSCLC, *VEGF* expression and VEGF protein levels in adenocarcinoma were significantly higher than that in squamous cell carcinoma (4, 7, 39). Second, higher *VEGF* expression in tumors was associated with higher microvessel count, advanced tumor stages, and shorter survival (7). Lastly, the microvessel counts in adenocarcinoma were significantly higher than that in the squamous cell carcinoma (40).

[7] http://www.hapmap.org

One of the limitations of this study was the inability to directly address how the *VEGF* haplotypes was involved in NSCLC development. Further studies are needed to investigate the functions of haplotype *TGC* on NSCLC and to address why *VEGF* genotype and haplotype associations with NSCLC risks were stronger in males than in females. Additional research is required to elucidate the mechanisms behind gender differences of genetic associations in NSCLC.

In summary, our results did not support a major independent role for any of the polymorphisms investigated in this study in NSCLC. However, this study could not exclude the possibility that the *+405CG+CC* genotype and the *TGC* haplotype had minor roles in NSCLC carcinogenesis. These results need to be validated by other independent studies, and further studies are necessary to investigate the gene-environment interactions between *VEGF* polymorphisms and NSCLC risk.

Acknowledgments

We thank the following staff members of the Lung Cancer Susceptibility Group: Barbara Bean, Jessica Shin, Andrea Solomon, Andrea Shafer, Thomas Van Geel, Lucy Ann Principe, Salvatore Mucci, Richard Rivera-Massa, David P. Miller, and the generous support of the physicians and surgeons of the Massachusetts General Hospital Cancer Center.

References

1. Voelkel NF, Vandivier RW, Tuder RM. Vascular endothelial growth factor in the lung. Am J Physiol Lung Cell Mol Physiol 2006;290:L209–21.
2. D'Amico TA. Angiogenesis in non-small cell lung cancer. Semin Thorac Cardiovasc Surg 2004;16:13–8.
3. Macchiarini P, Fontanini G, Hardin MJ, Squartini F, Angeletti CA. Relation of neovascularisation to metastasis of non-small-cell lung cancer. Lancet 1992;340:145–6.
4. Yuan A, Yang PC, Yu CJ, et al. Tumor angiogenesis correlates with histologic type and metastasis in non-small-cell lung cancer. Am J Respir Crit Care Med 1995;152:2157–62.
5. Carmeliet P. VEGF as a key mediator of angiogenesis in cancer. Oncology 2005;69 Suppl 3:4–10.
6. Fontanini G, Boldrini L, Chine S, et al. Expression of vascular endothelial growth factor mRNA in non-small-cell lung carcinomas. Br J Cancer 1999;79:363–9.
7. Yuan A, Yu CJ, Chen WJ, et al. Correlation of total *VEGF* mRNA and protein expression with histologic type, tumor angiogenesis, patient survival and timing of relapse in non-small cell lung cancer. Int J Cancer 2000;89:475–83.
8. Han H, Silverman JF, Santucci TS, et al. Vascular endothelial growth factor expression in stage I non-small cell lung cancer correlates with neoangiogenesis and a poor prognosis. Ann Surg Oncol 2001;8:72–9.
9. Joseph IB, Nelson JB, Denmeade SR, Isaacs JT. Androgens regulate vascular endothelial growth factor content in normal and malignant prostatic tissue. Clin Cancer Res 1997;3:2507–11.
10. Kaya A, Ciledag A, Gulbay BE, et al. The prognostic significance of vascular endothelial growth factor levels in sera of non-small cell lung cancer patients. Respir Med 2004;98:632–6.
11. Dudek AZ, Mahaseth H. Circulating angiogenic cytokines in patients with advanced non-small cell lung cancer: correlation with treatment response and survival. Cancer Invest 2005;23:193–200.
12. Shimanuki Y, Takahashi K, Cui R, et al. Role of serum vascular endothelial growth factor in the prediction of angiogenesis and prognosis for non-small cell lung cancer. Lung 2005;183:29–42.
13. Yano S, Matsumori Y, Ikuta K, Ogino H, Doljinsuren T, Sone S. Current status and perspective of angiogenesis and antivascular therapeutic strategy: non-small cell lung cancer. Int J Clin Oncol 2006;11:73–81.
14. Koukourakis MI, Papazoglou D, Giatromanolaki A, et al. *VEGF* gene sequence variation defines *VEGF* gene expression status and angiogenic activity in non-small cell lung cancer. Lung Cancer 2004;46:293–8.
15. Watson CJ, Webb NJ, Bottomley MJ, Brenchley PE. Identification of polymorphisms within the vascular endothelial growth factor (*VEGF*) gene: correlation with variation in VEGF protein production. Cytokine 2000;12:1232–5.
16. Stevens A, Soden J, Brenchley PE, et al. Haplotype analysis of the polymorphic human vascular endothelial growth factor gene promoter. Cancer Res 2003;63:812–6.
17. Renner W, Kotschan S, Hoffmann C, Obermayer-Pietsch B, Pilger E. A common *936 C/T* mutation in the gene for vascular endothelial growth factor is associated with vascular endothelial growth factor plasma levels. J Vasc Res 2000;37:443–8.
18. Jin Q, Hemminki K, Enquist K, et al. Vascular endothelial growth factor polymorphisms in relation to breast cancer development and prognosis. Clin Cancer Res 2005;11:3647–53.
19. Sfar S, Hassen E, Saad H, Mosbah F, Chouchane L. Association of *VEGF* genetic polymorphisms with prostate carcinoma risk and clinical outcome. Cytokine 2006;35:21–8.
20. Krippl P, Langsenlehner U, Renner W, et al. A common *936 C/T* gene polymorphism of vascular endothelial growth factor is associated with decreased breast cancer risk. Int J Cancer 2003;106:468–71.
21. Kataoka N, Cai Q, Wen W, et al. Population-based case-control study of *VEGF* gene polymorphisms and breast cancer risk among Chinese women. Cancer Epidemiol Biomarkers Prev 2006;15:1148–52.
22. Lee SJ, Lee SY, Jeon HS, et al. Vascular endothelial growth factor gene polymorphisms and risk of primary lung cancer. Cancer Epidemiol Biomarkers Prev 2005;14:571–5.
23. Wacholder S, Chanock S, Garcia-Closas M, El Ghormli L, Rothman N. Assessing the probability that a positive report is false: an approach for molecular epidemiology studies. J Natl Cancer Inst 2004;96:434–42.
24. Su L, Zhou W, Asomaning K, et al. Genotypes and haplotypes of matrix metalloproteinase 1, 3 and 12 genes and the risk of lung cancer. Carcinogenesis 2006;27:1024–9.
25. Kraft P, Cox DG, Paynter RA, et al. Accounting for haplotype uncertainty in matched association studies: a comparison of simple and flexible techniques. Genet Epidemiol 2005;28:261–72.
26. Zhai R, Gong MN, Zhou W, et al. Genotypes and haplotypes of *VEGF* gene are associated with higher ARDS mortality and lower VEGF plasma levels. Thorax 2007;62:718–22.
27. Zhou W, Liu G, Park S, et al. Gene-smoking interaction associations for the *ERCC1* polymorphisms in the risk of lung cancer. Cancer Epidemiol Biomarkers Prev 2005;14:491–6.
28. Awata T, Inoue K, Kurihara S, et al. A common polymorphism in the 5′-untranslated region of the *VEGF* gene is associated with diabetic retinopathy in type 2 diabetes. Diabetes 2002;51:1635–9.
29. Awata T, Kurihara S, Takata N, et al. Functional *VEGF C-634G* polymorphism is associated with development of diabetic macular edema and correlated with macular retinal thickness in type 2 diabetes. Biochem Biophys Res Commun 2005;333:679–85.
30. Petrovic D, Verhovec R, Globocnik M, Osredkar J, Peterlin B. Association of vascular endothelial growth factor gene polymorphism with myocardial infarction in patients with type 2 diabetes. Cardiology 2007;107:291–5.
31. Twombly R. New studies fan controversy over gender risk in lung cancer. J Natl Cancer Inst 2004;96:898–900.
32. Caracta CF. Gender differences in pulmonary disease. Mt Sinai J Med 2003;70:215–24.
33. Olak J, Colson Y. Gender differences in lung cancer: have we really come a long way, baby? J Thorac Cardiovasc Surg 2004;128:346–51.
34. Sordello S, Bertrand N, Plouët J. Vascular endothelial growth factor is up-regulated *in vitro* and *in vivo* by androgens. Biochem Biophys Res Commun 1998;251:287–90.
35. Stewart RJ, Panigrahy D, Flynn E, Folkman J. Vascular endothelial growth factor expression and tumor angiogenesis are regulated by androgens in hormone responsive human prostate carcinoma: evidence for androgen dependent destabilization of vascular endothelial growth factor transcripts. J Urol 2001;165:688–93.
36. Sibug RM, Helmerhorst FM, Tijssen AM, de Kloet ER, de Koning J. Estrogen reduces vascular endothelial growth factor (164) expression in the mouse nucleus paraventricularis of the hypothalamus. Neurosci Lett 2002;333:199–202.
37. Niklaus AL, Babischkin JS, Aberdeen GW, Pepe GJ, Albrecht ED. Expression of vascular endothelial growth/permeability factor by endometrial glandular epithelial and stromal cells in baboons during the menstrual cycle and after ovariectomy. Endocrinology 2002;143:4007–17.
38. Joseph IB, Isaacs JT. Potentiation of the antiangiogenic ability of linomide by androgen ablation involves down-regulation of vascular endothelial growth factor in human androgen-responsive prostatic cancers. Cancer Res 1997;57:1054–7.
39. Arinaga M, Noguchi T, Takeno S, Chujo M, Miura T, Uchida Y. Clinical significance of vascular endothelial growth factor C and vascular endothelial growth factor receptor 3 in patients with non small cell lung carcinoma. Cancer 2003;97:457–64.
40. Juczewska M, Chyczewski L, Telego M, et al. Studies on angiogenesis intensity in lung cancer in aspect of its correlation with histological type of tumor and clinical stage. Rocz Akad Med Bialymst 1997;42:254–70.

Clinical Cancer Research

Vascular Endothelial Growth Factor Genotypes, Haplotypes, Gender, and the Risk of Non–Small Cell Lung Cancer

Rihong Zhai, Geoffrey Liu, Wei Zhou, et al.

Clin Cancer Res 2008;14:612-617.