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Effect of vascular endothelial growth factor polymorphisms on survival in advanced-stage non-small-cell lung cancer

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Polymorphisms have been identified in the vascular endothelial growth factor (*VEGF*) gene that may affect VEGF production. We hypothesized that such polymorphisms may correlate with survival outcomes among advanced-stage non-small-cell lung cancer (NSCLC) patients. We evaluated the association between VEGF polymorphisms and overall survival among patients with advanced NSCLC who were treated with at least one cytotoxic regimen at Kyoto University Hospital between 2003 and 2008. We investigated the following VEGF polymorphisms: –460T > C (rs833061), +405G > C (rs2010963), +936C > T (rs3025039), –1154G > A (rs1570360), and –2578C > A (rs699947). Analyses of genotype associations with survival outcomes were performed using Cox proportional models, Kaplan–Meier methods, and the log-rank test. There were 126 patients and 80 deaths. On a Cox regression analysis of a current and former smoker (hazards ratio [HR], 1.422; 95% confidence interval [CI], 1.111–1.859; *P* = 0.0046), poor performance status (PS) (HR, 2.524; 95% CI, 1.483–3.827; *P* = 0.0019), the VEGF –460CC genotype (HR, 1.719; 95% CI, 1.166–2.390; *P* = 0.0084), VEGF –1154AA and AG genotypes (HR, 1.482; 95% CI, 1.144–1.897; *P* = 0.0034), and VEGF –2578AA genotype (HR, 1.797; 95% CI, 1.219–2.495; *P* = 0.0047) had a significant prognostic effect on survival based on univariate analysis. Based on multivariate analysis of a current and former smoker (HR, 1.407; 95% CI, 1.095–1.840; *P* = 0.0070), poor PS (HR, 2.249; 95% CI, 1.309–3.468; *P* = 0.0058), and the VEGF –1154AA and AG genotypes (HR, 1.419; 95% CI, 1.033–1.901; *P* = 0.0316) were significant independent prognostic factors for survival. In this study, polymorphisms in VEGF may affect survival in advanced NSCLC. (*Cancer Sci* 2009; 100: 1917–1922)

Lung cancer is a major cause of cancer-related mortality worldwide and is expected to remain a major health problem for the foreseeable future. Chemotherapy is the cornerstone of management of the disease. However, its therapeutic impact on patient survival has been modest. Recent discoveries have provided greater understanding of the molecular basis of the disease, yielding the success of the monoclonal antibody to VEGF, bavacizumab, in treatment with platinum-based chemotherapy of NSCLC.[1]

Senger *et al*. described a protein able to induce vascular leakage and named it vascular permeability factor (VPF).[2] Ferrara and Henzel[3] reported the purification to homogeneity and NH2-terminal amino acid sequencing of an endothelial cell-specific mitogen named VEGF. Cloning and expression of VEGF[4] and VPF[5] revealed that their activities are embodied by the same molecule. The human *VEGF* gene is organized into eight exons, separated by seven introns. The human *VEGF* gene is localized in chromosome (6) p21.3.[6] Alternative exon splicing of a single *VEGF* gene results in the generation of at least five different molecular species, having 121, 145, 165, 189, and 206 amino acids respectively, following signal sequence cleavage (VEGF121, VEGF145, VEGF165, VEGF189, VEGF206). VEGF 165 (VEGF 165; commonly called VEGF-A or VEGF) is a critical angiogenic factor.[7] Many tumor cell lines secrete VEGF *in vitro*, suggesting that this diffusible molecule is a mediator of tumor angiogenesis.[8] VEGF levels prior to chemotherapy may be associated with poor outcome in NSCLC as well as in small-cell lung carcinomas.[9,10] Since inhibition of VEGF has been correlated with suppression of tumor growth and angiogenesis,[11,12] a recombinant humanized monoclonal antibody to VEGF, bavacizumab, has been available for clinical use. Recently, bevacizmab has shown a survival benefit when compared to chemotherapy alone in first-line treatment with platinum doublet therapy against advanced NSCLC in a phase III trial, Eastern Cooperative Group trial 4599.[1,13,14] However, pulmonary hemorrhage is a serious problem with bevacizumab therapy. Thus, molecular markers may be necessary to achieve optimal treatment for each individual patient, as is the case with other molecular target drugs like epidermal growth factor receptor thyrosine kinase inhibitors.[13,14]

Polymorphisms have been identified in the *VEGF* gene that may have functional activity. Among those polymorphisms, we chose five SNPs in which the variant alleles are not so rare. The –460T > C polymorphism is located in the promoter region and the variant C allele may have increased VEGF promoter activity.[15] The variant C allele of the +405G > C polymorphism has been associated with lower VEGF levels.[15,16] The variant allele of the + –936C > T polymorphism, located in the 3′-untranslated region, has been associated with lower VEGF plasma levels.[17] And the variant alleles of the –1154G > A and –2578C > A polymorphisms are located in the promoter region. The variant alleles of the –1154G > A have been associated with lower VEGF plasma levels and the variant alleles of the –2578C > A have been associated with higher VEGF plasma levels.[16,18]

Zhai *et al*. investigated whether functional polymorphisms of –460T > C, +405G > C, and +936C > T in the *VEGF* gene are associated with the risk of NSCLC. They could not indicate apparent association between polymorphisms of –460T > C, +405G > C, and +936C > T in the *VEGF* gene and NSCLC risk. However, they suggested a minor role for the +405CC + CG genotypes and the 460T/+405G/+936C haplotype in lung adenocarcinogenesis in male Caucasians.[19]

Heist *et al*. evaluated the relationship between the VEGF polymorphisms +936C > T, –460T > C, and +405G > C and among patients with early stage NSCLC treated with surgical resection. They showed that carrying the variant C allele of the +405G > C

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polymorphism was associated with significantly improved survival among early-stage NSCLC patients treated with surgical resection and there was a trend toward improved survival among patients carrying the variant T allele of the +936C > T polymorphism.[20] However, it is unknown whether polymorphisms in VEGF may affect survival in advanced-stage NSCLC.

We hypothesized that polymorphisms in VEGF may be associated with survival outcomes in advanced NSCLC, the same way they are in early stage NSCLC.[20] We analyzed the association between these five VEGF SNPs and survival outcomes in advanced NSCLC.

Materials and Methods

Study population. A total of 126 patients with advanced NSCLC who were treated with at least one cytotoxic chemotherapy regimen between April 2003 and April 2008 at Kyoto University Hospital were enrolled in this study. Standard Response Evaluation Criteria in Solid Tumors were used for response evaluation. Written informed consent pertaining to the utilization of clinical materials was obtained from all patients. The study was approved by the Ethics Committee of the Kyoto University Graduate School and Faculty of Medicine.

Genotyping. Blood samples were collected from all study participants at the time of recruitment. DNA was extracted from peripheral blood samples using QIAamp DNA extraction kit (Qiagen, Tokyo, Japan). The VEGF polymorphisms –460T > C (rs833061), +405G > C (rs2010963), +936C > T (rs3025039), –1154G > A (rs1570360), and –2578C > A (rs699947) were genotyped by the 5′-nuclease assay (TaqMan) using the ABI prism 7300 Sequence Detecting System (Applied Biosystems, Foster City, CA, USA).

Statistical analysis. The univariate relationship between each independent clinicopathologic variable and VEGF genotype was examined using Fisher's exact tests.

In order to evaluate risk factors associated with prognosis, a Cox proportional hazards regression model with a step-down procedure was used. Proportional hazards assumptions were checked and satisfied; only those variables with statistically significant results in univariate analysis were included in a multivariate analysis. The criterion for removing a variable was the likelihood ratio statistic, which was based on the maximum partial likelihood estimate (default *P*-value of 0.05 for removal from the model). Survival time was calculated from the date of diagnosis. Survival curves were determined using the Kaplan–Meier method. The log-rank test allowed us to evaluate the differences between survival curves. All preceding statistical analyses were performed by using JMP 6 software (SAS Institute, Cary, NC, USA).

Results

Patients' characteristics. Table 1 shows the characteristics of the 126 advanced NSCLC patients. Patient selection was according to will to participate in this SNP study, so 126 patients treated in 5-years seems like a small subset of patients. All patients were Japanese, including 84 (66.7%) males and 42 (33.3%) females, with a median age of 67 years (range, 29–83 years). The pathologic diagnoses are listed in Table 1 and the number of dominant adenocarcinomas was 92 (73%). Twenty-seven (21.5%) patients were non-smokers and 99 (78.5%) patients were former or current smokers. The Eastern Cooperative Oncology Group (ECOG) performance status (PS) was 0–1 for 121 patients and 2–3 for five patients. Eighty-seven (69%) patients had been treated with platinum doublets, 19 (15.1%) patients with cytotoxic agent monotherapy, 14 (11.1%) patients with chemo-radiotherapy, and 10 patients (4.8%) with Epidermal growth factor receptor tyrosine kinase inhibitors (EGFR) TKIs as the first-line regimen.

Table 1. Patient characteristics (*n* = 126)

Characteristics	No. of patients	%
Age, years		
Median	67	
Range	29–83	
Gender		
Male	84	66.7
Female	42	33.3
Stage		
IIIB	47	37.3
IV	72	57.1
Recurrence after surgery	7	5.6
Histologic type		
Adenocarcinoma	92	73.0
Squamous	14	11.1
Large	2	1.6
NSCLC NOS	18	14.3
First-line treatment		
Chemo-radiotherapy	14	11.1
Platinum doublets	87	69.0
Cytotoxic agent monotherapy	19	15.1
EGFR TKIs	10	4.8
Smoking history		
Never	27	21.5
Former	58	46.0
Current	41	32.5
Pack-years median, range		
Median	30	
Range	0–145.5	
ECOG PS		
0–1 (%)	121	96.0
≥ 2 (%)	5	4.0
VEGF –460T > C		
TT	61	48.4
CT	54	42.9
CC	11	8.7
VEGF +405G > C		
GG	40	31.7
CG	59	46.8
CC	27	21.5
VEGF +936C > T		
CC	85	67.5
CT	35	27.8
TT	6	4.7
VEGF –1154G > A		
GG	93	73.8
AG	25	19.8
AA	8	6.4
VEGF –2578C > A		
CC	60	47.6
AC	55	43.7
AA	11	8.7

ECOG PS, Eastern Cooperative Oncology Group performance status; EGFR TKI, Epidermal growth factor receptor tyrosine kinase inhibitors; NSCLC NOS, not otherwise specified; VEGF, vascular endothelial growth factor.

Vascular endothelial growth factor (VEGF) genotypes and clinicopathological association. The associations between clinicopathologic factors and VEGF genotypes are shown in Table 2. Twelve (40%) of 30 PD cases were –1154AA and AG genotype, whereas 75 (78.1%) of 96 disease-controlled cases were GG genotype ($P = 0.049$ by χ^2-test). Fourteen (41.2%) of 34 non-adenocarcinoma cases were –1154AA and AG genotype, whereas 73 (79.3%) of 92 adenocarcinoma cases were –1154GG genotype ($P = 0.002$ by Fisher's exact test). No correlation existed between

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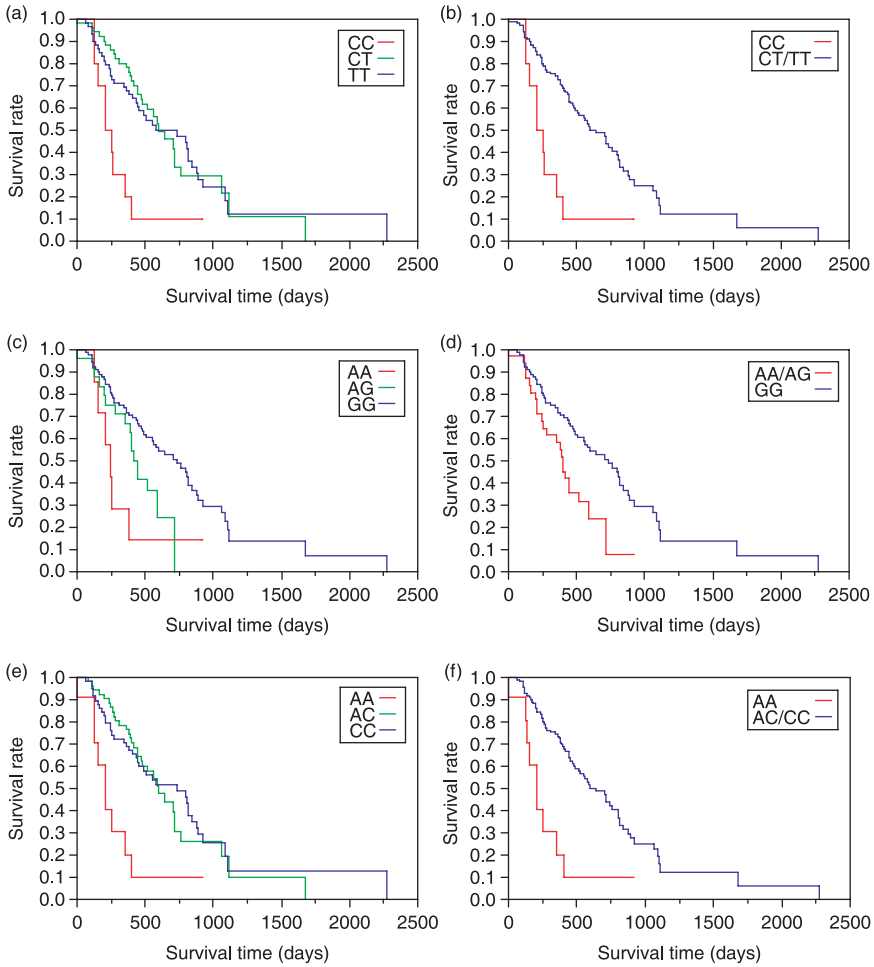

Fig. 1. Kaplan–Meier analysis of survival of 126 non-small-cell lung cancer patients according to vascular endothelial growth factor (VEGF) genotypes. The differences between groups were evaluated with the log-rank test. (a) VEGF –460CC *vs* CT *vs* TT. (b) VEGF –460CC *vs* CT/TT. (c) VEGF –1154AA *vs* AG *vs* GG. (d) VEGF –1154AA/AG *vs* GG. (e) VEGF –2578AA *vs* AC *vs* CC. (f) VEGF –2578AA *vs* AC/CC.

Table 2. Association between VEGF –1154G > A genotype and clinicopathologic factors

Characteristics	*VEGF –1154G > A			*P*-values
	AA	AG	GG	
Response to first-line therapy				
PR/SD	4	17	75	
PD	4	8	18	0.049
Pleural effusions				
Yes	3	5	37	
No	5	20	56	NS
Gender				
M	5	21	58	
F	3	4	35	NS
Smoking status				
Never	2	8	31	
Former/Current	6	17	62	NS
Histology				
Adenocarcinoma	5	14	73	
Others	3	11	20	0.002

*1154AA + AG *vs* GG. PD, progressive disease; PR, partial response; SD, stable disease.

other genotypes and clinicopathologic factors, including the response to first-line therapy, retention of effusions, gender, smoking history, and histologic type. There were also no correlations between serum Lactate dehydrogenase (LDH), platelet count, and VEGF genotypes (data not shown).

Vascular endothelial growth factor (VEGF) genotypes and survival. The median survival time of patients with the –460CC genotype was significantly shorter than those with the –460CT and TT genotypes ($P < 0.01$ by log-rank test; Fig. 1b). The same was true of patients with the –1154AA and AG genotypes compared to patients with the –1154GG genotype ($P < 0.01$ by log-rank test; Fig. 1d), the –2578AA genotype compared to the –2578AC and CC genotypes ($P < 0.01$ by log-rank test; Fig. 1f), the –1154AA or AG genotype compared to the –1154GG genotype ($P < 0.01$ by log-rank test; Fig. 1c), the –460CC genotype compared to the –460CT or TT genotype ($P < 0.01$ by log-rank test; Fig. 1a), and of patients with the –2578AA genotype compared to patients with the –2578AC and CC genotypes ($P < 0.01$ by log-rank test; Fig. 1e). A Cox regression analysis was performed on the 126 patients to determine the correlation between patient prognosis and clinicopathologic factors in whom information about age (<70 years *vs* ≥70 years), gender (female *vs* male), smoking history (smoker *vs* never smoker), PS (0–1 *vs* 2–3), disease stage (others *vs* IIIB), VEGF –460T > C (CC genotype *vs* CT + TT genotype), VEGF +405G > C (GG genotype *vs* CC + CG genotype), VEGF +936C > T (CT + TT genotype *vs* CC genotype), VEGF –1154G > A (AA + AG genotype *vs* GG genotype), VEGF –2578C > A (AA genotype *vs* AC + CC genotype) was available (Table 3). Among these factors, a current and former smoker (hazards ratio [HR], 1.422; 95% confidence interval [CI], 1.111–1.895; $P = 0.0046$), poor PS (HR, 2.524; 95% CI, 1.483–3.827; $P = 0.0019$), VEGF –460CC genotype (HR, 1.719; 95% CI, 1.166–2.390; $P = 0.0084$), VEGF –1154AA and AG genotypes (HR, 1.482; 95% CI, 1.144–1.897; $P = 0.0034$), and the VEGF –2578AA genotype (HR, 1.797; 95% CI, 1.219–2.495; $P = 0.0047$)

Table 3. Univariate analysis for overall survival

Variables	Crude hazard ratio	95% CI	*P*-values
Age (<70/≥70)	1.060	0.832–1.338	0.6329
Gender (Male/female)	1.194	0.944–1.533	0.1420
Disease stage (Others/IIIB)	1.156	0.911–1.492	0.2376
Smoking history (Smoker/never smoker)	1.422	1.111–1.859	0.0046*
PS (0–1/2–3)	2.524	1.483–3.827	0.0019*
First-line chemo-radiotherapy/others	1.301	0.913–2.016	0.1555
First-line EGFR TKIs/others	1.399	0.853–2.828	0.2070
VEGF –460T > C (CC/CT + TT)	1.719	1.166–2.390	0.0084*
VEGF +405G > C (GG/CC + CG)	1.199	0.940–1.511	0.1411
VEGF +936C > T (CT + TT/CC)	1.042	0.812–1.318	0.7388
VEGF –1154G > A (AA + AG/GG)	1.482	1.144–1.897	0.0034*
VEGF –2578C > A (AA/AC + CC)	1.797	1.219–2.495	0.0047*

CI, confidence interval; EGFR TKI, Epidermal growth factor receptor tyrosine kinase inhibitors; PS, performance status;VEGF, vascular endothelial growth factor.

Table 4. Multivariate analysis for overall survival

Variables	Hazard ratio	95% CI	*P*-values
Smoking history (Smoker/never smoker)	1.407	1.095–1.840	0.0070*
PS (0–1/2–3)	2.249	1.309–3.468	0.0058*
VEGF –460T > C (CC/CT + TT)	1.890	0.333–18.738	0.6212
VEGF –1154G > A (AA + AG/GG)	1.419	1.033–1.901	0.0316*
VEGF –2578C > A (AA/AC + CC)	2.547	0.413–24.185	0.4435

CI, confidence interval; PS, performance status; VEGF, vascular endothelial growth factor.

Table 5. Log-rank test ($P = 0.0025$): Median survival time and 95% confidence interval (CI) for Figure 1a

–460T > C	No. of patients	Died	Censored	Median survival	95% CI
CC	11	9	2	257	[125, 351]
CT	54	31	23	600	[468, 715]
TT	61	40	21	737	[438, 876]

Table 6. Log-rank test ($P = 0.0017$): Median survival time and 95% confidence interval (CI) for Figure 1b

–460T > C	No. of patients	Died	Censored	Median survival	95% CI
CC	11	9	2	257	[125, 351]
CT/TT	115	71	44	643	[342, 801]

had a significant prognostic effect on survival based on univariate analysis. Based on multivariate analysis of a current and former smoker (HR, 1.407; 95% CI, 1.095–1.840; $P = 0.0070$), poor PS (HR, 2.249; 95% CI, 1.309–3.468; $P = 0.0058$), and the VEGF –1154AA and AG genotypes (HR, 1.419; 95% CI, 1.033–1.901; $P = 0.0316$) were significant independent prognostic factors for survival (Table 4).

Precise data of median survival time of each VEGF genotypes according to Figure 1 is shown in Tables 5–10.

Discussion

The present study found that VEGF polymorphisms were associated with advanced NSCLC prognosis. Patients with the –460CC genotype (HR, 1.719; 95% CI, 1.166–2.390; $P = 0.0084$), the –1154AA and AG genotypes (HR, 1.482; 95% CI, 1.144–1.897; $P = 0.0034$), or the –2578AA genotype (HR, 1.797; 95% CI, 1.219–2.495; $P = 0.0047$) showed decreased overall survival. In addition, polymorphisms in the *VEGF* gene promoter region were associated with disease control rate with first-line chemotherapy and histology. The association between VEGF polymorphisms and overall prognosis was evident even after adjusting for clinical characteristics, indicating that the –1154 genotype may be an independent prognostic factor for advanced NSCLC. Although

Table 7. Log-rank test ($P = 0.0028$): Median survival time and 95% confidence interval (CI) for Figure 1c

–1154G > A	No. of patients	Died	Censored	Median survival	95% CI
AA	8	6	2	241	[125, 383]
AG	25	18	7	441	[278, 588]
GG	93	56	37	737	[511, 812]

Table 8. Log-rank test ($P = 0.0017$): Median survival time and 95% confidence interval (CI) for Figure 1d

–1154G > A	No. of patients	Died	Censored	Median survival	95% CI
AA/AG	33	24	9	400	[241, 520]
GG	93	56	37	737	[511, 812]

Table 9. Log-rank test ($P = 0.0007$): Median survival time and 95% confidence interval (CI) for Figure 1e

–2578 C > A	No. of patients	Died	Censored	Median survival	95% CI
AA	11	9	2	212	[125, 351]
AC	55	321	23	588	[447, 715]
CC	60	39	21	737	[438, 876]

Table 10. Log-rank test ($P = 0.0006$): Median survival time and 95% confidence interval (CI) for Figure 1f

–2578C > A	No. of patients	Died	Censored	Median survival	95% CI
AA	11	9	2	212	[125, 351]
AC/CC	115	68	47	643	[488, 801]

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more functional studies on the genotype effect are necessary, these findings may be useful for clinical therapeutic trials targeting the VEGF pathway.

The −460CC genotype was associated with poor prognosis in advanced NCSLC. Many case control studies concerning −460T > C were conducted in various kinds of carcinomas. Although the VEGF −460T > C genotype is a suitable genetic marker for oral[21] and prostate cancers,[22] the results in NSCLC are inconclusive.[23,24] The variant C allele correlates with higher VEGF production than normal.[15] The normal T allele may be associated with decreased VEGF promoter activity,[15] and the CC genotype may produce higher VEGF levels. Summers *et al*. reported the association between VEGF −460T > C polymorphism and progression to chronic kidney disease; however, there is no report which shows the significant association between −460T > C genotype and prognosis in malignant diseases including NSCLC.[20,25]

The VEGF –2578AA genotype was also associated with poor prognosis in advanced NCSLC. Many case control studies concerning –2578C > A were also conducted in various kinds of carcinomas, but as with –2578C > A −460T > C, results have not been clear.[26–28] The variant A allele correlates with higher VEGF production than normal.[16,18] A significant association between the A allele and colonic involvement in Crohn's disease patients is reported,[27] and the –2578AC genotype is associated with high VEGF expression in resected NSCLC patients.[29] However, there are no reports concerning the prognostic value of the VEGF2578 genotype.

The variant A allele of VEGF –1154G > A was an independent poor prognostic factor in advanced NSCLC patients (HR, 1.419; 95% CI, 1.033–1.901; $P = 0.0316$). Many case control studies concerning VEGF –1154G > A were also conducted in various kinds of carcinomas, with the same ambiguous outcomes noted for the two SNPs discussed above.[27,28,30] A significant association between the A allele and low serum VEGF levels in Crohn's disease patients has been reported,[27] and the –1154AA genotype is associated with low VEGF expression in resected NSCLC patients.[29] However, there are no reports concerning the prognostic value of the VEGF –1154G > A genotype.

Surprisingly, those who were estimated to show low VEGF expression appeared to have poorer outcomes than those who were estimated to show high VEGF expression. This finding was unexpected and was in contrast to previous observations, which showed a survival improvement for patients who were estimated to show low VEGF expression.[29] A clear explanation for the discrepancy in low VEGF results is not currently evident. However, a number of hypotheses to explain this result have been explored. Because of the possibility of a poor drug delivery with the low VEGF cases, because the ability of normal VEGF production may represent an important event associated with advanced settings, and because of the association of another prognostic factor such as EGFR somatic mutation, it could be useful to perform EGFR mutational analyses as well as VEGF expression level in this patient population. Unfortunately, we were unable to assess the impact of *EGFR* gene mutations on study therapies and there were too few samples to analyze the VEGF expression.

Dudek *et al*. first reported that high levels of serum VEGF at the time of pretreatment may predict worse survival in NSCLC patients.[9] However, Chakra *et al*. reported recently that the prognostic information given by a high circulating VEGF serum level is not an independent determinant of survival because of its close relationship with main prognostic variables such as PS, stage of the disease, and nodal status.[31] The variant A allele is associated with lower VEGF production,[16,18] and the loss of normal VEGF production may be associated with a worse outcome among treated advanced NSCLC patients.

Heist *et al*. reported improved survival in early stage NSCLC patients carrying the +405G > C, +936C > T allele in the *VEGF* gene. However in our study, there is no correlation to survival. A number of hypotheses to explain our results have been explored, including the effects of differences in stage, PS, mean age, and especially the treatment status of patients included in our study. In other words, the difference depends on the patients' background; for instance, there were early stage NSCLC patients who received radical operations in Heist's study and there were advanced-stage NSCLC patients who were treated with chemotherapy in our study.

This is the first report to our knowledge correlating VEGF polymorphisms and survival outcome in advanced-stage NSCLC. The limitations of our study include a small sample size and the heterogeneity of treatment regimens. These account for the small number of SNPs and the low value of HR, but they can be alleviated by conducting a larger study. The potential clinical implications of our findings are manifold. The first implication is that the ability to produce higher VEGF may be associated with a worse prognosis. The second is that the loss of ability to produce normal VEGF may also correlate with a worse prognosis. In this era of molecular-targeting therapy, VEGF polymorphisms might be used to predict who will benefit from bevacizumab in the advanced setting. Patients with lower VEGF production might tolerate a higher drug density than those with normal VEGF production.

In summary, polymorphisms in VEGF may be associated with survival in advanced stage NSCLC patients who received chemotherapy. Our findings should spur additional investigation of polymorphisms in VEGF as well as functional studies of VEGF in advanced NSCLC patients with chemotherapy.

Disclosure

The authors declare that they have no competing financial interests.

Abbreviations

NSCLC	Non-small-cell lung cancer
ECOG	Eastern Cooperative Oncology Group
PS	Performance status
VEGF	Vascular endothelial growth factor
SNP	Single nucleotide polymorphism
PR	Partial response
SD	Stable disease
PD	Progressive disease

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