Scientific Research Publishing

Journal of Cancer Therapy, 2018, 9, 714-728
http://www.scirp.org/journal/jct
ISSN Online: 2151-1942
ISSN Print: 2151-1934

Association of Polymorphic Variants of *VEGF* and *KDR* Genes with Development and Metastasing of Non-Small Cell Lung Cancer

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How to cite this paper: Shchayuk, A.N., Krupnova, E.V., Shapetska, M.N., Mikhalenka, A.P., Chebotareva, N.V. and Kilchevsky, A.V. (2018) Association of Polymorphic Variants of *VEGF* and *KDR* Genes with Development and Metastasing of Non-Small Cell Lung Cancer. *Journal of Cancer Therapy*, **9**, 714-728.
https://doi.org/10.4236/jct.2018.99059

Received: August 1, 2018
Accepted: September 8, 2018
Published: September 11, 2018

Abstract

Vascular endothelial growth factor (VEGF) is one of the most important and specific factors affecting angiogenesis in tumor development. VEGFR2 is a receptor encoded by the *KDR* gene. VEGF and VEGFR2 transmit a signal to intracellular tyrosine kinase cascades. Polymorphic variants of the *VEGF* and *KDR* genes significantly influence the expression levels of the endothelial growth factor and its receptor, which leads to a change in the activation of angiogenesis in oncopathological processes. In this study, the relationship between the polymorphic variants rs2010963, rs699947 and rs3025039 of the *VEGF* gene and rs1870377 and rs2071559 of the *KDR* gene was analyzed with the development of a specific histological type of non-small cell lung cancer and its clinical and morphological characteristics. It was established that the development of squamous cell carcinoma is associated with −634CC genotype of the *VEGF* gene and the genotypes containing −2578A allele of the *VEGF* gene reduce the likelihood of this cancer type development. The development of adenocarcinoma is associated with +936CC *VEGF*/1719TT *KDR* and +936CT *VEGF*/1719TT *KDR* combinations. In women with non-small cell lung cancer, −634GC genotype of the *VEGF* gene is associated with a greater degree of the primary lesion spread. Genotype −2578CC of the *VEGF* gene is associated with a higher degree of the primary tumor spread in the general group of patients and with regional metastases in women. Haplotypes −634G/−2578C/+936C are risky for the occurrence of metastases in regional lymph nodes in women.

Keywords

Non-Small Cell Lung Cancer, Angiogenesis, Vascular Endothelial Growth

Factor, *VEGF* Gene Polymorphism, *KDR* Gene Polymorphism

1. Introduction

Lung cancer is one of the most common malignant neoplasms and the leading causes of death from cancer in men. Non-small cell lung cancer (NSCLC) constitutes 85% of all lung cancers and is a heterogeneous disease. Its most common histological types are adenocarcinoma (ADC) and squamous cell carcinoma (SCC). The key process in the NSCLC development is angiogenesis. One of the most important and specific factors affecting angiogenesis in tumor development is vascular endothelial growth factor VEGF [1] [2]. The main receptor through which VEGF transmits a signal to intracellular tyrosine kinase cascades is the VEGFR2 receptor encoded by the *KDR* gene [3] [4]. High VEGF expression is associated with tumor growth and metastatic process, while the inhibited VEGF expression results in suppressed tumor growth [5]. The VEGF gene also triggers the activation of the protease cascade involved in the degradation of extracellular matrix, suppressing apoptosis, stimulates the endothelial cells survival, increases vascular permeability, inhibits the dendritic cells differentiation as well as activates tissue factors and monocytes migration [6]. The VEGF gene is located on the short arm of chromosome 6 (6p21.3) and consists of eight exons and seven introns [7], the KDR gene encoding the VEGFR2 receptor is located in the 4q11 - q12 region of chromosome 4 and contains 26 exons, 25 introns [8]. Polymorphic variants of the *VEGF* and *KDR* genes significantly influence the expression levels of the endothelial growth factor and its receptor, which leads to a change in the activation of angiogenesis in oncopathological processes. Therefore, the studies of molecular genetic disorders of these genes that will allow predicting the development and progress of this disease are of great importance.

In this research, we aim to study the relationship of the polymorphic variants rs2010963, rs699947, and rs3025039 of the *VEGF* gene and rs1870377 and rs2071559 of the *KDR* gene with the probability of development of an NSCLC specific histological type and clinical and morphological manifestations of this disease.

2. Materials and Methods

2.1. Object of Research

Peripheral blood samples of 234 patients with NSCLC, who were on treatment at Minsk City Clinical Oncology Clinic from 2004 to 2017, were used as biological material in our study. The diagnosis of lung cancer has been based on clinical picture of the disease, history data, bronchoscopy, X-ray examination and computed tomography, cytomorphology of sputum and tumor tissue biopsies. The study group consisted of patients with NSCLC stage 1 - 4 according to the TNM

Classification of Malignant Tumours (2009) who underwent surgery. The group of NSCLC included the most common histological types of NSCLC: 126 patients with squamous cell carcinoma and 108 with adenocarcinoma without primary multiple cancer (181 men and 53 women). Sociodemographic and clinical characteristics of NSCLC patients are shown in Table 1. The average patients' age

Table 1. Sociodemographic and clinical characteristics of NSCLC patients and control group.

Characteristics	Patients with NSCLC, n = 234, (%)	Control, n = 383, (%)
	Age (years):	
Median	61.9	63.8
Min/max	32/88	33/94
	Gender:	
Female	53 (22.6)	88 (23.00)
Male	181 (77.3)	295 (77.0)
	Smoking status:	
Does not smoke	68 (29.1)	166 (43.3)
Smokes	145 (61.9)	126 (32.9)
No information	21 (9.0)	91 (23.7)
	Histology:	
Squamous-cell carcinoma	126 (53.8)	
Adenocarcinoma	108 (46.2)	
	Stage:	
I	103 (44.0)	
II	45 (19.2)	
III	70 (30.0)	
IV	16 (6.8)	
	Degree of the primary lesion spread (T):	
T1	75 (32.1)	
T2	107 (45.7)	
T3	32 (13.7)	
T4	20 (8.5)	
	Regional metastases (N):	
N0	124 (53.0)	
N1	45 (19.2)	
N2	60 (25.6)	
N3	4 (1.7)	
	Distant metastases (M):	
M0	217 (92.7)	
M1	17 (7.3)	

was 61.9 ± 1.2 years (from 32 to 88). The histological type of tumor was determined based on the WHO criteria. The control group consisted of 383 people (295 men and 88 women) corresponding to the group of NSCLC patients selected by age, concomitant diseases and their nationality. The biological material was collected in compliance with the principles of voluntariness and confidentiality and the informed consent of patients. The study protocol was approved by the local Ethics Committee.

2.2. Methods of Research

Isolation of total DNA from peripheral blood was carried out using the method described by Mathew [9], followed by phenol-chloroform extraction and ethanol purification. Genotyping of polymorphic variants −634C > G (rs2010963), −2578C > A (rs699947), +936C > T (rs3025039) of the *VEGF* gene and 1719T > A (rs1870377), −906T > C (rs2071559) of the *KDR* gene was performed by polymerase chain reaction (PCR) and subsequent restriction fragment length polymorphism (PCR-RFLP), using specific primers and restriction endonucleases. Primers were synthesized by Primetech ALC (Minsk). Reagents for PCR and PCR-RFLP were provided by Thermo Fisher Scientific (Vilnius). After PCR-RFLP, DNA samples were visualized in UV light.

2.3. Statistical Processing of Results

The statistical analysis was carried out using the GraphPad InStat Version 3.05 application software and the SNPStats online program [10]. For the validity check, when comparing genotype frequencies in groups, the standard Pearson χ^2 criterion or the Fisher's exact test for small sampling were used. The relationship between genotypes, development and course of the disease was assessed according to the odds ratio (OR (95% CI)) using codominant and dominant SNPStats models.

3. Results

The association study between polymorphic variants of *VEGF* and *KDR* genes and the risk of ADC and SCC development showed (**Table 2**) that in the group of patients with SCC, −634CC genotype occurs 2.5 times more often than in the control group (OR = 2.42; 95%CI 1.03 - 5.65; p = 0.04). The distribution of −634CC genotype frequency of occurrence in patients with ADC and in the control group does not differ. It was shown that the genotypes containing −2578A allele of the *VEGF* gene reduce the risk of squamous cell carcinoma development (**Table 2**): in the group of patients with this histological type, CA and AA genotypes are less likely occurring than in the control group (OR = 0.59; 95%CI 0.37 - 0.93; p = 0.02). Such relationship was not found in patients with ADC.

In the population under study, the relationship of the studied polymorphic variants of the *KDR* gene and the development of a specific histological NSCLC

Table 2. Distribution of the *VEGF* gene genotypes' frequency in the control group and in patients with ADC and SCC.

Polymorphism	Control n (%)	ADC n (%)	OR (95% CI)	p	Control n (%)	SCC n (%)	OR (95% CI)	p
VEGF −634C > G	n = 222	n = 108			n = 315	n = 125		
GG	113 (50.9)	51 (47.2)	1.00		162 (51.4)	67 (53.6)	1.00	
GC	98 (44.1)	49 (45.4)	1.11 (0.69 - 1.78)	0.62	141 (44.8)	46 (36.8)	0.79 (0.51 - 1.22)	**0.04**
CC	11 (5.0)	8 (7.4)	1.61 (0.61 - 4.25)		12 (3.8)	12 (9.6)	**2.42 (1.03 - 5.65)**	
GC + CC	109 (49.1)	57 (52.8)	1.16 (0.73 - 1.84)	0.53	153 (48.6)	58 (46.4)	0.92 (0.61 - 1.39)	0.68
VEGF −2578C > A	n = 222	n = 108			n = 315	n = 125		
CC	54 (24.3)	26 (24.1)	1.00		70 (22.2)	41 (32.8)	1.00	
CA	110 (49.5)	63 (58.3)	1.19 (0.68 - 2.08)	0.18	168 (53.3)	52 (41.6)	**0.53 (0.32 - 0.87)**	**0.04**
AA	58 (26.1)	19 (17.6)	0.68 (0.34 - 1.37)		77 (24.4)	32 (25.6)	0.71 (0.40 - 1.25)	
CA+ AA	168 (75.7)	82 (75.9)	1.01 (0.59 - 1.73)	0.96	245 (77.8)	84 (67.2)	**0.59 (0.37 - 0.93)**	**0.02**
VEGF +936C > T	n = 222	n = 108			n = 315	n = 125		
CC	159 (71.6)	79 (73.2)	1.00		228 (72.4)	79(63.2)	1.00	
CT	55 (24.8)	27 (25.0)	0.99 (0.58 - 1.68)	0.66	77 (24.4)	37 (29.6)	1.39 (0.87 - 2.21)	0.08
TT	8 (3.6)	2 (1.8)	0.50 (0.10 - 2.43)		10 (3.2)	9 (7.2)	2.60 (1.02 - 6.62)	
CT + TT	63 (28.4)	29 (26.9)	0.93 (0.55 - 1.55)	0.77	153 (48.6)	58 (46.4)	0.92 (0.61 - 1.39)	0.68

type was not established. The frequency of occurrence of polymorphic variants of this gene in patients with ADC and SCC was close to the frequency of these variants in the control groups.

Since the signal transmission from *VEGF* into the cell is carried out through its receptor VEGFR2 encoded by the *KDR* gene, combinations of polymorphic variants of the *VEGF* and *KDR* genes were analyzed. Table 3 shows only significant combinations of these genes that affect the likelihood of the disease development both in the general group of patients with NSCLC and when divided into groups with ADC and SCC. It was revealed that the patients-carriers of +936CT/−906CC combination had a reduced risk of NSCLC development.

When considering the effects of *VEGF* and *KDR* genotype combinations on the probability of a specific histological type of tumor development, it was found that +936CC/1719TT and +936CT/1719TT combinations are associated with the risk of development in specifically with ADC, but in the carriers of the homozygous combination +936CC/1719TT this association is of higher value (p = 0.02). The combination −634GC/1719TT reduces the risk of SCC development.

Analysis of the frequency distribution of polymorphic variants of genes under study in the groups of men and women with NSCLC is presented in Table 4.

It was established (Table 4) that −634C genotype of the *VEGF* gene is risky in terms of NSCLC development in men: in the group of patients, this genotype is occurring by 2.3 times more often as compared to the control group (OR = 2.26; 95% CI 1.03 - 4.94; p = 0.05). The frequency distribution of this genotype in women with NSCLC does not differ from the corresponding control.

Table 3. Significant combinations of *VEGF* and *KDR* gene genotypes in patients with NSCLC and in the control group.

Combination of *VEGF/KDR* genotypes	n (%)		OR (95% CI)	p
	Control	NSCLC		
+936CT/−906CC	47 (18.5)	24 (10.3)	**0.51 (0.30 - 0.86)**	**0.02**
	Control	ADC		
+936CC/1719TT	75 (43.6)	62 (59.4)	**1.87 (1.14 - 3.05)**	**0.02**
+936CT/1719TT	14 (9.9)	18 (17.1)	**2.34 (1.11 - 4.92)**	**0.04**
	Control	SCC		
−634GC/1719TT	48 (23.4)	17 (13.6)	**0.41 (0.22 - 0.75)**	**0.01**

Table 4. The frequency distribution of polymorphic *VEGF* gene variants in men and women with NSCLC and in the corresponding control.

Polymorphism	Men				Women			
	Control n (%)	NSCLC n (%)	OR (95% CI)	p	Control n (%)	NSCLC n (%)	OR (95% CI)	p
VEGF −634C > G	n = 295	n = 181			n = 88	n = 52		
GG	150 (50.9)	94 (51.9)	1.00		48 (54.5)	24 (46.1)	1.00	
GC	133 (45.1)	70 (38.7)	0.84 (0.57 - 1.24)	**0.05**	35 (39.8)	25 (48.1)	1.43 (0.70 - 2.90)	0.61
CC	12 (4.1)	17 (9.4)	**2.26 (1.03 - 4.94)**		5 (5.7)	3 (5.8)	1.20 (0.26 - 5.45)	
GC+CC	145 (49.1)	87 (48.1)	0.96 (0.66 - 1.39)	0.82	40 (45.5)	28 (53.9)	1.40 (0.70 - 2.79)	0.34
VEGF −2578C > A	n = 295	n = 181			n = 88	n = 52		
CC	65 (22.0)	55 (30.4)	1.00		23 (26.1)	12 (23.1)	1.00	
CA	159 (53.9)	87 (48.1)	0.65 (0.41 - 1.01)	0.13	44 (50.0)	28 (53.9)	1.22 (0.52 - 2.84)	0.89
AA	71 (24.1)	39 (21.6)	0.65 (0.38 - 1.10)		21 (23.9)	12 (23.1)	1.10 (0.40 - 2.96)	
CA+ AA	230 (78.0)	126 (69.6)	**0.65 (0.43 - 0.99)**	**0.04**	65 (73.9)	40 (76.9)	1.18 (0.53 - 2.63)	0.69
VEGF +936C > T	n = 295	n = 181			n = 88	n = 52		
CC	217 (73.6)	125 (69.1)	1.00		59 (67.0)	33 (63.5)	1.00	
CT	70 (23.7)	48 (26.5)	1.19 (0.78 - 1.83)	0.45	24 (27.3)	16 (30.8)	1.19 (0.56 - 2.56)	0.90
TT	8 (2.7)	8 (4.4)	1.74 (0.64 - 4.74)		5 (5.7)	3 (5.8)	1.07 (0.24 - 4.78)	
CT + TT	78 (26.4)	56 (30.9)	1.25 (0.83 - 1.87)	0.29	29 (33.0)	19 (36.5)	1.17 (0.57 - 2.40)	0.67

It was shown that the genotypes containing −2578A allele of the *VEGF* gene have protective characteristics in the NSCLC development in men (OR = 0.65, 95% CI 0.43 - 0.99, p = 0.04).

At the next stage of work, the influence of molecular genetic disorders in the *VEGF* and *KDR* genes was studied for the probability of a certain histological type of NSCLC development in men and women. The frequency distribution of genotypes by the polymorphic loci of the *VEGF* gene in the control group and in the groups of patients with ADC and SCC, depending on gender, is presented in Table 5.

Table 5. Frequency distribution of polymorphic *VEGF* and *KDR* gene variants among men and women with ADC and SCC.

Polymorphism	Men							Women						
	Control n (%)	ADC n (%)	OR (95% CI)	p	SCC n (%)	OR (95% CI)	p	Control n (%)	ADC n (%)	OR (95% CI)	p	SCC n (%)	OR (95% CI)	p
VEGF −634C > G	n = 295	n = 64			n = 117			n = 88	n = 44			n = 8		
GG	150 (50.9)	33 (51.6)	1.00		61 (52.1)	1.00		48 (54.5)	18 (40.9)	1.00		6 (75.0)	1.00	
GC	133 (45.1)	25 (39.1)	0.85 (0.48 - 1.51)	0.23	45 (38.5)	0.83 (0.53 - 1.31)	0.09	35 (3.8)	24 (54.5)	1.83 (0.86 - 3.87)	0.27	1 (12.5)	0.23 (0.03 - 1.98)	0.24
CC	12 (4.1)	6 (9.4)	2.27 (0.80 - 6.49)		11 (9.4)	2.25 (0.94 - 5.38)		5 (5.7)	2 (4.5)	1.07 (0.19 - 6.00)		1 (12.5)	1.60 (0.16 - 16.10)	
GC + CC	145 (49.1)	31 (48.4)	0.97 (0.57 - 1.67)	0.92	56 (47.9)	0.95 (0.62 - 1.46)	0.81	40 (45.5)	26 (59.1)	1.73 (0.83 - 3.61)	0.14	2 (2.05)	0.40 (0.08 - 2.09)	0.25
VEGF −2578C > A	n = 295	n = 64			n = 117			n = 88	n = 44			n = 8		
CC	65 (22.0)	16 (25.0)	1.59 (0.69 - 3.67)		39 (33.3)	1.00		21 (23.9)	10 (22.7)	1.37 (0.45 - 4.12)		2 (25.0)	0.55 (0.09 - 3.30)	
CA	159 (53.9)	37 (57.8)	1.50 (0.72 - 3.11)	0.47	50 (42.7)	**0.52 (0.32 - 0.87)**	**0.05**	44 (50.0)	26 (59.1)	1.70 (0.66 - 4.35)	0.52	2 (25.0)	0.26 (0.04 - 1.54)	0.30
AA	71 (24.1)	11 (17.2)	1.00		28 (23.9)	0.66 (0.36 - 1.19)		23 (26.1)	8 (18.2)	1.00		4 (50.0)	1.00	
CA+ AA	230 (78.0)	48 (75.0)	0.85 (0.45 - 1.59)	0.73	78 (66.7)	**0.57 (0.35 - 0.91)**	**0.02**	67 (76.1)	34 (77.3)	1.07 (0.45 - 2.52)	0.88	6 (75.0)	0.94 (0.17 - 5.02)	0.94
VEGF +936C > T	n = 295	n = 64			n = 117			n = 88	n = 44			n = 8		
CC	217 (73.6)	51 (79.7)	1.00		74 (63.2)	1.00		59 (67.0)	28 (63.6)	1.00		5 (62.5)	1.00	
CT	70 (23.7)	12 (18.8)	0.73 (0.37 - 1.45)	0.56	36 (30.8)	1.51 (0.93 - 2.44)	0.08	24 (27.3)	15 (34.1)	1.32 (0.60 - 2.89)	0.51	1 (12.5)	0.49 (0.05 - 4.43)	0.20
TT	8 (2.7)	1 (1.6)	0.53 (0.07 - 4.35)	0.56	7 (6.0)	2.57 (0.90 - 7.32)		5 (5.7)	1 (2.3)	0.42 (0.05 - 3.78)		2 (25.0)	4.72 (0.72 - 30.84)	
CT + TT	78 (26.4)	13 (20.3)	0.71 (0.37 - 1.37)	0.30	43 (36.8)	**1.62 (1.02 - 2.55)**	**0.04**	29 (33.0)	16 (36.4)	1.16 (0.54 - 2.48)	0.70	3 (37.5)	1.22 (0.27 - 5.46)	0.80

The association study of polymorphic *VEGF* gene variants with the development of ADC or SCC in men and women showed that the genotypes containing −2578A allele of the *VEGF* gene contribute to the reduced SCC risk development in men (OR = 0.57, 95%CI: 0.35 - 0.91; p = 0.02) and the genotypes containing +936T allele of the *VEGF* gene trigger the SCC development in men (OR = 1.62; 95%CI 1.02 - 2.55; p = 0.04).

In women, there were no significant differences in the frequency occurrence of polymorphic *VEGF* gene variants between the control group and the patients with ADC and SCC (Table 5).

The assessed dependence of the degree of incidence of the primary NSCLC tumor on the polymorphic *VEGF* and *KDR* gene variants showed that in the carriers of −2578CC genotype of the *VEGF* gene the greater degree of incidence of the primary tumor (T2 - T4) is occurring more often (OR = 2.35; 95% CI: 1.21 - 4.58; p = 0.02) than the smaller one (T1) (Figure 1).

Analysis of the relationship between the studied polymorphisms of *VEGF* and *KDR* genes with the primary tumor spread in men and women with NSCLC showed that −634GC genotype of the *VEGF* gene is more frequently occurring in women with a greater degree of the primary lesion spread (>3 cm) (OR = 4.80, 95%CI: 1.14 - 20.09; p = 0.04) (Figure 2). There is also a tendency for the

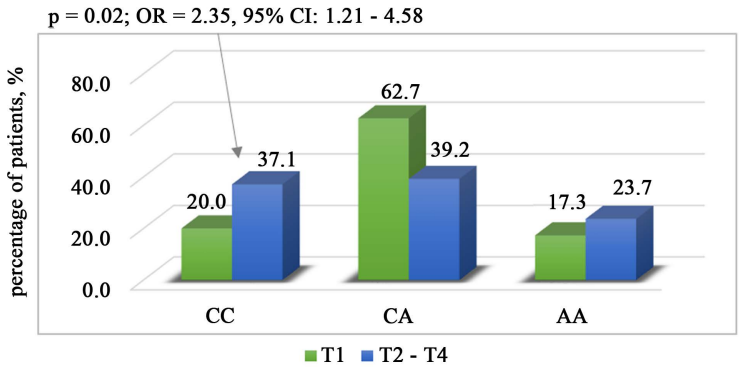

Figure 1. Genotypes' association of −2578C > A polymorphism of the *VEGF* gene in the patients with NSCLC showing the extent of the primary tumor spread.

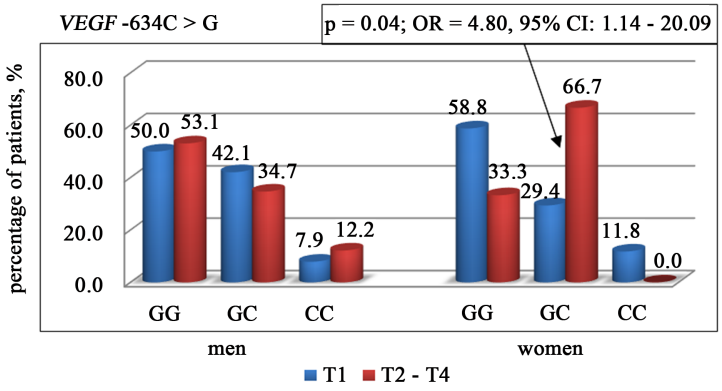

Figure 2. The relationship of polymorphic *VEGF* gene variants and a degree of the primary lesion spread in men and women with NSCLC.

increased frequency of occurrence of −634GG genotype of the *VEGF* gene in a group of patients with a tumor size less than 3 cm. In male patients, no associations of the polymorphic variants of the *VEGF* gene in −634C > G position with a tumor size were observed.

In the population under study, there were no statistically significant differences by the frequency of occurrence in men and women with NSCLC of polymorphic −2578C > A and +936C > T variants of the *VEGF* gene associated with a primary tumor size. Association study of polymorphic *KDR* gene variants with a degree of the primary lesion spread did not show statistically significant differences in men and women with NSCLC.

A comparative analysis of the distribution profile of **−2578**C > A polymorphism genotypes of the *VEGF* gene in patients with metastases (N1-N3) and without metastases (N0) showed that −2578AA genotype reduces the likelihood of regional metastasis development (OR = 0.45, 95% CI: 0.22 - 0.92; p = 0.04) (Figure 3).

When analyzing the frequency distribution of polymorphic *VEGF* gene variants depending on the gender of patients with NSCLC, it was shown that −2578CC genotype of the *VEGF* gene affects the risk of regional metastasis in women with NSCLC (OR = 10.00, 95%CI: 1.44 - 69.26; p = 0.02) (Table 6). In men, the association was not identified.

No association of polymorphic *KDR* gene variants was identified in men and women with NSCLC with the presence of regional metastases.

Association analysis of *VEGF* gene haplotypes with regional metastases was carried out both in men and women with NSCLC. The statistical significance was reached only in the group of women as shown (Table 7):
−634C/−2578C/+936C and −634G/−2578C/+936C haplotypes are risky for the metastasis occurrence in regional lymph nodes.

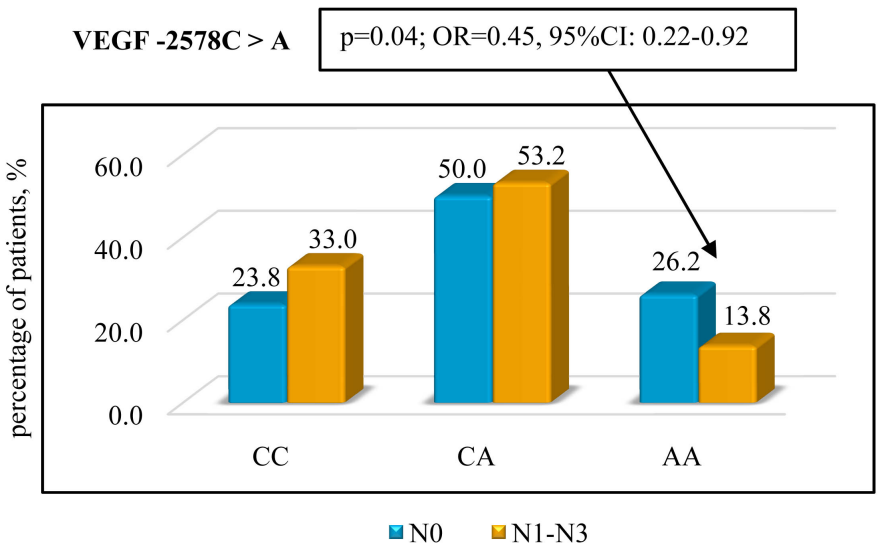

Figure 3. Association of −2578C > A polymorphism genotypes of the *VEGF* gene with regional metastases in patients with NSCLC.

Table 6. Frequency distribution of *VEGF* gene genotypes in men and women with NSCLC depending on the presence of regional metastases.

Polymorphism	Men		OR (95% CI)	p	Women		OR (95% CI)	p
	N0 n (%)	N1 - N3 n (%)			N0 n (%)	N1 - N3 n (%)		
VEGF −634C > G	n = 87	n = 79			n = 35	n = 17		
GG	45 (51.7)	43 (54.4)	1.00		16 (45.7)	8 (47.1)	1.00	
GC	33 (37.9)	30 (38)	0.95 (0.50 - 1.82)	0.82	17 (48.6)	8 (47.1)	0.94 (0.29 - 3.11)	0.99
CC	9 (10.3)	6 (7.6)	0.70 (0.23 - 2.13)		2 (5.7)	1 (5.9)	1.00 (0.08 - 12.76)	
GC+CC	42 (48.3)	36 (45.6)	0.90 (0.49 - 1.65)	0.73	19 (54.3)	9 (52.9)	0.95 (0.30 - 3.03)	0.93
VEGF −2578C > A	n = 87	n = 79			n = 35	n = 17		
CC	25 (28.7)	25 (31.6)	1.00		4 (11.4)	8 (47.1)	**10.00 (1.44 - 69.26)**	
CA	40 (46)	43 (54.4)	1.07 (0.53 - 2.17)	0.18	21 (60)	7 (41.2)	1.67 (0.29 - 9.52)	**0.02**
AA	22 (25.3)	11 (13.9)	0.50 (0.20 - 1.24)		10 (28.6)	2 (11.8)	1.00	
CA+ AA	62 (71.3)	54 (68.3)	0.87 (0.45 - 1.69)	0.68	31 (88.6)	9 (52.9)	**0.14 (0.035 - 0.60)**	**0.01**
VEGF +936C > T	n = 87	n = 79			n = 35	n = 17		
CC	60 (69)	55 (69.6)	1.00		22 (62.9)	11 (64.7)	1.00	
CT	23 (26.4)	22 (27.9)	1.04 (0.52 - 2.08)	0.77	12 (34.3)	4 (23.5)	0.67 (0.17 - 2.55)	0.39
TT	4 (4.6)	2 (2.5)	0.55 (0.10 - 3.10)		1 (2.9)	2 (11.8)	4.00 (0.33 - 49.08)	
CT + TT	27 (31)	24 (30.4)	0.97 (0.50 - 1.88)	0.93	13 (37.1)	6 (35.3)	0.92 (0.28 - 3.09)	0.90

Table 7. Association of *VEGF* gene haplotypes with regional metastases in women with NSCLC.

VEGF −634C > G	*VEGF* −2578C > A	*VEGF* +936C > T	Frequency	OR (95% CI)	p
G	A	C	0.4448	1.00	-
C	C	C	0.1966	**5.60 (1.05 - 29.83)**	**0.05**
G	C	C	0.1341	**23.27 (2.68 - 202.19)**	**0.01**
G	C	T	0.08	7.17 (0.92 - 56.11)	0.07
C	C	T	0.09	0.00 (-)	-
G	C	T	0.04	0.00 (-)	-
C	A	C	0.01	0.00 (-)	-

4. Discussion

At present, much attention is paid to the study of molecular-biologic markers characterizing the development and progression of malignant neoplasms. The studied *VEGF* and *KDR* gene polymorphisms play an important role in the oncological disease pathogenesis. Some studies show that −634C > G, −2578C > A, and +936C > T polymorphisms of the *VEGF* gene and -906T > C of the *KDR* gene are associated with the increased corresponding protein concentration in blood and correlate with an increased risk of the oncological diseases' development and progression [11] [12] [13].

Association of −634CC genotype of the *VEGF* gene with the probability of SCC development in the general group of patients and in men can be explained by the fact that −634C > G polymorphism of the *VEGF* gene is located in the 5'-untranslated region containing the binding elements of the MZF1 transcription factor [14]. These sequences can be involved in regulation of the studied gene transcription, which is directly related to the expression of the *VEGF* gene. The genotype −634CC is associated with a higher serum concentration of *VEGF* as compared to CG and GG genotypes [15] [16] and with a higher vascular density in NSCLC cases [17].

Genotypes containing −2578A allele of the *VEGF* gene also show protective characteristics in relation to the SCC development in the general group of patients and in men. Single nucleotide substitution C > A of the *VEGF* gene in the position of −2578 bp is localized in the promoter region. The gene polymorphism in this region can weaken the *VEGF* expression affecting the tumor development. According to literary sources, −2578C allele correlates with a higher *VEGF* expression as compared to the A allele [18] [19].

Polymorphism +936C > T of the *VEGF* gene is localized in the 3'-untranslated gene region and affects the circulating *VEGF* concentrations in plasma [20] directly triggering angiogenesis [21], which can explain the risky significance in our population of +936T allele of the *VEGF* gene in men in the SCC development.

No such relationship was found in women with NSCLC. Squamous cell carcinoma is predominantly found in men and is hardly diagnosed in women, as known [22]. Therefore, in our study the associations found for SCC correspond to the data characterizing a group of male patients.

Polymorphic variants of the *KDR* gene do not separately influence the NSCLC development and its most common histological subtypes, but their combinations +936CC *VEGF*/1719TT *KDR* and +936CT *VEGF*/1719TT *KDR* significantly contribute to the risk of the ADC development. It is known that 1719A > T polymorphism of the *KDR* gene leads to the nonsynonymous substitution of amino acids, which affects the binding efficiency of *VEGF* to its VEGFR2 receptor [23].

In addition to the angiogenesis induction in tumors, VEGF promotes the spread of the tumor process. In particular, it increases vascular permeability in tumors, induces serine proteases, inhibits apoptosis of epithelial cells, which

further promotes tumor spreading and metastazing [11] [12] [24]. Among the clinical and morphological parameters characterizing these processes, the present study takes into account a degree of primary tumor (T) spread, the presence of metastases in regional lymph nodes (N) and distant organs (M).

It is known that −634C allele of the *VEGF* gene is associated with a higher serum concentration of VEGF [14]. Among oncological patients with the GG genotype, the decreased expression of the *VEGF* gene is observed, and consequently, the primary lesion prevalence is lower than that in the GC and CC genotype carriers [15] [16], which confirms our results obtained with regard to the association of the heterozygous −634GC genotype of the *VEGF* gene with a higher degree of the primary lesion prevalence in women.

The relationship between −2578CC genotype of the *VEGF* gene with a higher degree of primary tumor spread in the general group of patients with NSCLC and the presence of regional metastases in women is consistent with the data of many studies showing that −2578C allele of the *VEGF* gene correlates with a higher level of *VEGF* expression than the A allele in cases of breast, large intestine, stomach and also lung cancer [16] [17] [25]. The increased *VEGF* expression causes the increased vascular permeability and this leads to the increased interstitial and intratumoral pressure, promoting the penetration of tumor cells into the vascular bed and the metastases' formation [24]. This can explain that in our study regional metastases are less common occurring in the homozygous carriers (−2578AA): a lower *VEGF* expression level in the presence of −2578A allele appears to protect −2578AA genotype carriers from the regional metastases' formation.

Other polymorphic variants of *VEGF* and *KDR* genes were not associated with a degree of the primary lesion spread and the presence of metastases in NSCLC cases.

VEGF triggers neoplastic angiogenesis resulting in the increased microcirculatory tumor vessels. Newly formed vessels start the supply of the tumor tissue with oxygen and nutrients, the tumor grows and continues to produce a large amount of VEGF. Polymorphism −2578C > A of the *VEGF* gene provides for the enhanced expression of a growth factor; +936C > T polymorphism contributes to the increased *VEGF* concentration in the blood plasma and −634C > G polymorphism is associated with the increased *VEGF* expression in patients with cancer diseases [11] [19] [21]. Our study showed that the C allele of −2578C > A polymorphism influences largely the development of regional metastases in women with NSCLC. Analysis of haplotypes showed that this effect is enhanced when combined with the C allele of polymorphism +936C > T, which increases the VEGF concentration in the blood plasma. This in turn contributes to the enhanced *VEGFR2* receptor expression through endothelial cells of tumor vessels, which stimulates cell growth and endothelial cell proliferation [23]. This leads to the increased intratumoral pressure, the penetration of tumor cells into the vascular bed and the emergence of metastases.

5. Conclusion

Thus, the study showed that the development of squamous cell carcinoma is associated with −634CC genotype of the *VEGF* gene and the genotypes containing −2578A allele of the *VEGF* gene reduce the likelihood of this cancer type development. Regardless of patients' gender, the development of adenocarcinoma is associated with +936CC *VEGF*/1719TT *KDR* and +936CT *VEGF*/1719TT *KDR* combinations. In women with non-small cell lung cancer, regardless of the histological type of tumor, −634GC genotype of the *VEGF* gene is associated with a greater degree of the primary lesion spread. Genotype −2578CC of the *VEGF* gene is associated with a higher degree of the primary tumor spread in the general group of patients and with regional metastases in women. Haplotypes −634G/−2578C/+936C are risky for the occurrence of metastases in regional lymph nodes in women.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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