GENETIC TESTING AND MOLECULAR BIOMARKERS
Volume 26, Number 5, 2022

Pp. 298–306
DOI: 10.1089/gtmb.2021.0213

Investigation of Vascular Endothelial Growth Factor Polymorphisms on Risk, Metastasis, Laterality, and Prognosis of Colorectal Cancer in Turkish Subjects

Mehtap Cevik,[1] Esat Namal,[2] Nur Dinc-Sener,[2] Ulkuhan Iner-Koksal,[3] Cavlan Ciftci,[4] and Belgin Susleyici[1]

Objectives: Tumor angiogenesis is known to support the spread and invasion of tumor cells, allow distant organ metastasis and to result in poorer prognoses and increased mortality. Since vascular endothelial growth factor-A (VEGF-A) is the major regulator of angiogenesis, in the present study the associations of the *VEGF-A* +405G>C and −460C>T polymorphisms with risk, primary tumor location, prognosis and metastasis of colorectal cancer (CRC) were investigated in Turkish subjects.
Material and Methods: A total of 153 subjects consist of 74 controls and 79 CRC diagnosed patients were included in the study. *VEGF-A* +405G>C and −460C>T polymorphisms were analyzed using the Agena MassARRAY platform.
Results: The *VEGF* +405GC+CC genotypes were found to be significantly associated with left colon cancer (unadjusted OR = 5.208 95% CI: 1.064–25.496, $p = 0.04$). The *VEGF* −460TT and CT+TT genotypes were associated with reduced liver metastasis risk (OR = 0.080 95% CI: 0.009–0.689 $p = 0.02$ and OR = 0.191 95% CI: 0.039–0.925, $p = 0.04$, respectively). Patients with the *VEGF* +405GG genotype showed longer progression-free survival in response to bevacizumab treatment (Log rank = 6.92, $p = 0.03$).
Conclusion: According to our results, the *VEGF* +405G>C and −460C>T polymorphisms were found to be associated with CRC prognosis, sidedness and metastases. Our findings need to be replicated in further studies.

Keywords: VEGF, +405G>C, −460C>T, bevacizumab, colorectal cancer, metastasis

Introduction

Colorectal cancer (CRC) is the third most common cancer and the fourth leading cause of cancer-related death (Torre *et al.*, 2015). By 2030, the global burden of CRC is expected to increase by 60% with 2.2 million new cases and 1.1 million deaths (Ferlay *et al.*, 2013). One of the main causes of the high mortality in CRC is metastasis (Mlecnik *et al.*, 2016). While 90% of CRC patients with early diagnosis can survive >5 years, unfortunately this rate remains around 10% for metastatic patients (Levin *et al.*, 2008). Therefore, metastasis is considered as a keystone in tumor development because it leads tumor cells to spread.

Angiogenesis is a multistep biological process that leads to the formation of new capillary blood vessels from existing vascular systems and is controlled by local or systemic chemical signals (Hanahan and Folkman, 1996; Risau, 1997; Carmeliet, 2000). Angiogenesis, which is necessary for the development and maintenance of homeostasis in a healthy individual, is also an important and fundamental process in tumor development. The formed new vessels as a result of tumor angiogenesis provide oxygen and nutrients to growing tumors, supporting the spread and invasion of the tumor cells to the nearby normal tissue and allow distant organ metastasis (Mousa, 2000; Rajabi and Mousa, 2017; Li *et al.*, 2018).

[1]Department of Molecular Biology, Marmara University Faculty of Science and Letters, Istanbul, Turkey.
[2]Department of Medical Oncology, Demiroglu Bilim University Faculty of Medicine, Istanbul, Turkey.
[3]Istanbul Bagcilar Training and Research Hospital, Istanbul, Turkey.
[4]Department of Cardiology, Demiroglu Bilim University Faculty of Medicine, Istanbul, Turkey.

The vascular endothelial growth factor (VEGF) signaling pathway plays a key role in angiogenesis (Li *et al.*, 2018). VEGF family consists of five secretory proteins (VEGF-A, VEGF-B, VEGF-C, VEGF-D, and PlGF) and three tyrosine kinase receptors (VEGF receptor [VEGFR]-1, VEGFR-2, and VEGFR-3) (Ferrara *et al.*, 2003; Macarulla *et al.*, 2020). VEGF-A ligand is known to be the most important member of the VEGF system. Circulating VEGF-A is secreted by many cells, including malignant cells, and binds to two VEGF receptors (VEGFR-1 and VEGFR-2) that results in promoting the survival, proliferation, migration, and differentiation of endothelial cells (Dvorak, 2002; Hicklin and Ellis, 2005).

VEGF-A levels in tissues have been associated with cancer development, poor prognosis, poor survival, and therapy sensitivity in many *in vitro* and *in vivo* studies (Fu *et al.*, 2014; Bendardaf *et al.*, 2017; Dinami *et al.*, 2020; Lacin and Yalcin, 2020; Van Cutsem *et al.*, 2020; Mashima *et al.*, 2021). Several variations on *VEGF* gene have potential to alter VEGF signaling and may directly affect VEGF levels in tissue and plasma (Koukourakis *et al.*, 2004; Sa-Nguanraksa *et al.*, 2013; O-Charoenrat, 2014; Innocenti *et al.*, 2018).

Since overexpression of VEGF is known to result in tumor growth, metastasis, and poor prognosis (Frezzetti *et al.*, 2017; Li *et al.*, 2017; Cheng *et al.*, 2018; Sopo *et al.*, 2019), it would not be surprising that *VEGF* gene variations may have an influence on development, prognosis, and even treatment of cancer through VEGF expression.

We believe that it is very important to understand genetic mechanisms and the effects of their variations that may be effective in tumor formation, metastasis, drug response, and prognosis for successful CRC treatment. Following the idea, in the present study, we investigated the relationship of *VEGF-A* +405G>C and −460C>T polymorphisms on risk, primary tumor location, prognosis, and metastasis of CRC in Turkish subjects. Additionally, the relationships between the *VEGF* gene variations and prognosis of patients receiving anti-VEGF therapy were also investigated.

Materials and Methods

Subjects

The present investigation was approved by the Marmara University Local Ethics Committee with the protocol number 09.2018.174. Cases were selected randomly from histologically confirmed CRC patients without former inflammatory bowel disease or any of the known hereditary cancer history. Controls were selected randomly from volunteers without CRC and other malignancy or autoimmune disorder history. Both case and control subjects signed a well-written consent complying with the ethical criteria of the Helsinki Declaration. Five milliliters of EDTA blood samples was recruited from each patient at Group Florence Nightingale Sisli Hospital, Medical Oncology Department, and control subjects were recruited in the same hospital at other departments. Following the sampling, the blood samples were cold transferred to the Marmara University Molecular Metabolism Research Laboratory for genomic DNA isolation.

The 5-Fluorouracil or Capecitabine-based therapy regimens were applied in combination with oxaliplatin, irinotecan, cetuximab, or bevacizumab for treatment of CRC patients. Overall and progression-free survival times of patients were defined as the date from the beginning of the treatment until death from any cause, and survivors were censored at the date of last contact.

Genotyping

Genomic DNA was isolated from peripheral blood using the Kurabo, Quick Gene DNA Whole Blood Kit. *VEGF-A* rs2010963 (+405G>C or −634G>C) and rs8333061 (−460C>T or −1498 C>T) polymorphisms were genotyped in the study. Genotyping was performed with Agena MassARRAY platform. The platform combines iPLEX and Mass ARRAY technology (Agena Bioscience, San Diego, CA), which is based on Matrix-assisted laser desorption/ionization-time of flight Mass Spectrometry assay. Assay Design Suite software version 2.0 was utilized to design forward, reverse, and single base extension primers. PCR was conducted with 10 ng/μL DNA samples, deoxyribonucleotide triphosphates, forward and reverse primers, reaction buffers, and DNA polymerase (Agena iPLEX Gold Genotyping Kit).

PCR conditions were performed according to the protocol reported by Gabriel *et al.* (2009). Following the final PCR, for desalting the iPLEX Extension reaction, PCR products were treated with resin and then transferred to a 384-well Spectro-CHIP using Mass ARRAY Nanodispenser. Spectro-CHIPs were transferred to MALDI-TOF mass spectrometry analyzer and the data analyzed by Typer Analyzer v 4.0 software. Assay plate included both positive and negative controls.

Statistical analyses

Statistical analyses were performed using IBM SPSS statistics version 26. Data are expressed as numbers and percentage for discrete variables as mean ± standard error (min–max) for continuous variables. Nonparametric Mann–Whitney *U* test was used to compare continuous variables. χ^2 test was performed to analyze the accordance of genotype distributions with Hardy–Weinberg equilibrium and compared as a function of sex between case and controls. The comparisons of the genotype distributions between dichotomized groups (case/control, liver metastatic/nonmetastatic, left/right colon) were performed with χ^2 tests. Fisher's exact test was considered if the number of the subjects were <5. Binary logistic regression analysis was used to estimate odds ratio (OR) and 95% confidence intervals (CI).

Binary logistic regression analysis was performed as both unadjusted (case/control, right/left colon, liver metastatic/nonmetastatic groups) and adjusted in sex, age, and smoking (case/control group). Log-Rank test was used for comparisons of survival times. Survival times (month) were represented as mean ± standard error estimate (95% CI). *p*-values below 0.05 were considered as statistically significant.

Results

A total of 153 subjects, consisting of 74 controls (31 female, 43 male) and 79 CRC diagnosed patients (33 female, 46 male), were included in the study. Clinicopathological characteristics of the study group are summarized in Table 1.

Comparative genotype frequencies of *VEGF* +405G>C and −460C>T polymorphisms between cases and controls are given in Table 2. While the genotype distributions of the

Table 1. Clinicopathological Characteristics of the Study Group

	Case, n *(%)*	*Control,* n *(%)*	p
Sex	33 (41.8)	31 (41.9)	0.98
Female	46 (58.2)	43 (58.1)	
Male			
Age	59.78 ± 1.30 (28–82)	53.75 ± 1.9 (22–85)	0.05
BMI	26.26 ± 0.5 (18.25–40.57)	27.28 ± 0.5 (19.05–41.62)	0.09
PFS	11.87 ± 8.5 (3–44)		
OS	22.63 ± 17.48 (2–91)		
Primary tumor location			
Right	18 (22.8)		
Left	61 (77.2)		
Metastasis			
No	15 (19.0)		
Liver	61 (80.3)		
Peritoneal	3 (4.7)		
KRAS mutant			
Yes	25 (31.6)		
No	54 (68.4)		
Dead	31 (42.5)		
Irınotecan	23 (31.9)		
Bevacizumab	23 (31.9)		
Oxaliplatin	52 (72.2)		
Cetuximab	16 (22.2)		
Capecitabine	22 (27.8)		
5-FU	57 (72.2)		

Categorical variables were expressed as *n* (%). Age, weight, BMI, PFS, OS values are expressed as mean ± standard error (Min–Max).
BMI, body mass index; 5-FU, 5-Fluorouracil; OS, overall survival; PFS, progression-free survival.

–460C>T polymorphism were in accordance with the Hardy–Weinberg equilibrium in cases and controls, +405G>C polymorphism deviated in controls.

The frequencies of *VEGF* +405G>C and –460C>T genotypes in cases and controls were not statistically significant ($p = 0.136$ and $p = 0.219$, respectively). The relationships between *VEGF* +405G>C and –460C>T polymorphisms and CRC risk are given in Supplementary Table S1. *VEGF* +405G>C and –460C>T polymorphisms were not found to be associated with CRC risk.

The genotype frequencies of *VEGF* +405G>C and –460C>T polymorphisms based on primary tumor localization are given in Table 3. In basic comparisons between right and left colon cancer patients, *VEGF* +405G>C genotype distributions were not found to be statistically significant. However, since GC+CC genotype frequencies were higher in the left colon than right colon ($p = 0.08$), association of GC+CC genotypes and left colon cancer was additionally examined with binary logistic regression analysis (Table 4). *VEGF* +405GC+CC

Table 2. Distribution of Genotype and Allele Frequencies of *VEGF* +405G>C and –460C>T Polymorphisms in Cases and Controls

	Case					*Control*					
	Allele frequency					*Allele frequency*					p[a]
+405G>C	*n*: 40 (%)	Wt	Mut	χ^2	p^b	*n*: 53 (%)	Wt	Mut	χ^2	p^b	
GG	11 (27.5)	0.538	0.463	0.125	0.72	22 (41.5)	0.575	0.425	6.255	0.01	0.136
GC	21 (52.5)					17 (32.1)					
CC	8 (20.0)					14 (26.4)					
GG+GC/CC	32 (80.0)					39 (73.6)					0.623
GC+CC/GG	29 (72.5)					31 (58.5)					0.193
–460C>T	*n*: 74 (%)	Wt	Mut	χ^2	p^b	*n*: 62 (%)	Wt	Mut	χ^2	p^b	
CC	27 (36.5)	0.635	0.365	2.045	0.15	15 (24.2)	0.540	0.460	2.513	0.11	0.219
CT	40 (54.1)					37 (59.7)					
TT	7 (9.5)					10 (16.1)					
CC+CT/TT	67 (90.5)					52 (83.9)					0.301
CT+TT/CC	47 (63.5)					47 (75.8)					0.139

Categorical variables were expressed as *n* (%).
p^a shows chi-square analysis results for comparisons of genotype distributions between case and control groups.
p^b and χ^2 show accordance with Hardy–Weinberg equilibrium.
$p < 0.05$ was considered statistically significant.
Mut, Mutant allele; VEGF, vascular endothelial growth factor; Wt, Wild-type allele.

Table 3. Distribution of *VEGF* +405G>C and −460C>T Genotype Frequencies Based on Primary Tumor Localization and Presence of Liver Metastasis

	Primary tumor location			*Liver metastasis*		
	Left colon (%)	*Right colon (%)*	p	*No (%)*	*Yes (%)*	p
VEGF +405G>C						
GG	6 (19.4)	5 (55.6)	0.10	0 (0.0)	10 (31.3)	0.20
GC	18 (58.1)	3 (33.3)		5 (83.3)	15 (46.9)	
CC	7 (22.6)	1 (11.1)		1 (16.7)	7 (21.9)	
GG+GC/CC	24 (77.4)	8 (88.9)	0.66	5 (83.3)	27 (78.1)	0.77
GC+CC/GG	25 (80.6)	4 (44.4)	0.08	6 (100.0)	22 (68.8)	0.17
VEGF −460C>T						
CC	17 (30.4)	10 (55.6)	0.15	2 (13.3)	25 (44.6)	**0.04**
CT	33 (58.9)	7 (38.9)		10 (66.7)	28 (50.0)	
TT	6 (10.7)	1 (5.6)		3 (20.0)	3 (5.4)	
CC+CT/TT	50 (89.3)	17 (94.4)	0.49	12 (80.0)	53(94.6)	0.10
CT+TT/CC	39 (69.6)	8 (44.4)	0.09	13 (86.7)	31 (55.4)	**0.04**

Categorical variables were expressed as *n* (%). $p < 0.05$ was considered statistically significant.
Statistically significant values were stressed as bold.

genotypes were found to be significantly associated with left colon cancer (unadjusted OR = 5.208 95% CI: 1.064–25.496, $p = 0.04$).

Similarly, *VEGF* −460C>T genotype distributions were not significantly different between right and left colon patients. However, in binary logistic regression analysis 460CT+TT genotypes were partially associated with left colon cancer, although not as much as +405G>C polymorphism (unadjusted OR = 2.868, 95% CI: 0.964–8.532, $p = 0.06$) (Table 4).

The distributions of *VEGF* +405G>C and −460C>T genotype frequencies according to the presence of liver metastasis are given in Table 3. Patients with a variant allele carrying *VEGF* −460CT+TT genotypes was found to be higher in nonmetastatic compared with liver metastatic patients ($p = 0.04$). Compared with the CC genotype, TT and CT+TT genotypes were associated with reduced liver metastasis risk (Table 5) (OR = 0.080 95% CI: 0.009–0.689 $p = 0.02$ and OR = 0.191 95% CI: 0.039–0.925, $p = 0.04$, respectively).

VEGF +405G>C genotype distributions were not significantly different between liver and nonmetastatic patients ($p = 0.20$).

The associations between *VEGF* +405G>C and −460C>T polymorphisms and progression-free and overall survival of CRC patients are summarized in Supplementary Table S2.

Table 4. The Associations of *VEGF* +405GC+CC and −460CT+TT Genotypes and Left Colon Cancer Risk

	OR (95% CI)	p
VEGF +405G>C		
GG	Reference	
GC+CC	5.208 (1.064–25.496)	**0.04**
VEGF −460C>T		
CC	Reference	
CT+TT	2.868 (0.964–8.532)	0.06

Statistically significant values were stressed as bold.
CI, confidence interval; OR, odds ratio.

No statistically significant relationship was found between *VEGF* +405G>C and −460C>T polymorphisms and survival of CRC patients. However, although small sample size, we subcategorized our study group based on anti-VEGF agent bevacizumab use as BEV^+ (bevacizumab prescribed) and BEV^- (as no bevacizumab prescription) and further investigated associations of patients' survival and *VEGF* +405G>C and −460C>T polymorphisms between these groups.

In general, survival times did not differ significantly between BEV^+ and BEV^- groups (Log rank = 0.221 $p = 0.638$ for progression-free survival; Log rank = 1.288 $p = 0.256$ for overall survival, data not given in the table).

The associations of *VEGF* +405G>C genotypes and progression-free survival of CRC patients in BEV^+ and BEV^- groups are summarized in Table 6. *VEGF* +405 GG genotype resulted with longer progression-free survival in BEV^+ patients compared with other genotypes (Log rank = 6.92, $p = 0.03$). Similarly, patients with GC+CC genotypes had shorter progression survival times compared with patients with GG genotype (Log rank = 4.61, $p = 0.03$). No statistically significant relationship was found between +405G>C polymorphism and progression-free survival in BEV^- group.

VEGF −460C>T polymorphism was not found to be associated with progression-free survival in both BEV^+ and BEV^- groups (shown in Supplementary Table S3). No association was detected in both *VEGF* +405G>C and −460C>T polymorphisms and overall survival times of the patients between BEV^+ and BEV^- groups (shown in Supplementary Table S4).

Table 5. The Association of *VEGF* −460C>T Polymorphism and Liver Metastasis

VEGF *−460C>T*	*OR (95% CI)*	p
CC	Reference	
CT	0.224 (0.045–1.122)	0.07
TT	0.080 (0.009–0.689)	**0.02**
CT+TT	0.191 (0.039–0.925)	**0.04**

Statistically significant values were stressed as bold.

Table 6. Effects of *VEGF* +405G>C and −460C>T Polymorphisms on Progression-Free Survival of Bevacizumab-Prescribed and Unprescribed Colorectal Cancer Patients

	BEV^+	*Log rank*	p	BEV^-	*Log rank*	p
VEGF +405G>C						
GG	43.50 ± 0.35 (42.81–44.19)	6.92	**0.03**	11.86 ± 1.31 (9.30–14.42)	0.34	0.84
GC	10.69 ± 1.90 (6.96–14.42)			18.00 ± 3.31(11.51–24.49)		
CC	6.50 ± 1.50 (3.56–9.44)			18.00 ± 0.00 (18.00–18.00)		
GG+GC/CC	22.36 ± 6.27 (10.07–34.6)	3.64	0.06	18.19 ± 2.53 (13.23–23.16)	0.33	0.57
GC+CC/GG	9.74 ± 1.59 (6.63–12.85)	4.61	**0.03**	18.85 ± 2.40 (14.15–23.55)	0.11	0.74

Values were represented as mean ± standard error estimate (95% CI).
Statistically significant values were stressed as bold.
BEV^+, bevacizumab-prescribed CRC patients. BEV^-, CRC patients with no bevacizumab prescription; CRC, colorectal cancer.

Discussion

The importance of tumor angiogenesis in growth and survival of solid tumors is well known (Hasina and Lingen, 2001; Gupta and Qin, 2003). In addition, angiogenesis contributes to the development of cancer by facilitating the metastatic spread of tumor cells (Kumar *et al.*, 2004). Therefore, angiogenesis is a keystone in a successful metastasis. For this reason, we investigated the effects of *VEGF* polymorphisms in CRC metastasis in the present study. *VEGF* −460TT and CT+TT genotypes were associated with reduced liver metastasis risk (OR = 0.080 95% CI: 0.009–0.689 $p = 0.02$ and OR = 0.191 95% CI: 0.039–0.925, $p = 0.04$, respectively). *VEGF* +405G>C polymorphism was not found to be associated with metastasis in our study group.

Similar to our results, do Espírito Santo *et al.* (2017) evaluated the association between *VEGF* +405G>C and −460C>T polymorphisms and liver metastasis and reported −460C>T polymorphism was associated to be reduced liver metastasis (OR = 0.32; $p = 0.048$). The researchers did not report the same association for +405G>C polymorphism (do Espírito Santo *et al.*, 2017). Chae *et al.* (2008) on the other hand, subcategorized their study groups based on the presence of distant organ metastasis, and they detected +405GC genotype to be nearly associated with reduced metastasis risk ($p = 0.05$), but not record the same association with CC genotype. Koutras *et al.* (2012) reported that *VEGF* +405G>C and −460C>T polymorphisms were not associated with liver metastasis.

Although the presence of conflicting studies, results from our study and do Espírito Santo *et al.* may indicate that *VEGF* −460C>T variation displays metastasis-preventing attitude in CRC. Since it is known that *VEGF* −460TT genotype results with decreased VEGF mRNA expressions compared with TC and CC genotypes in CRC (Yamamori *et al.*, 2004), the *VEGF* −460C>T single nucleotide polymorphism might be protective against liver metastasis in CRC. However, further *in vivo* and *in vitro* studies are needed to understand by which mechanisms *VEGF* −460C>T polymorphism protects against metastasis.

CRCs may have different histological, pathological, molecular, or genetic features depending on the location of primary tumor (Benedix *et al.*, 2010; Lee *et al.*, 2015; Plastiras *et al.*, 2019). Although tumors in the right colon are rare compared with left, right colon cancers have worse prognosis and higher mortality (Benedix *et al.*, 2010; Stintzing *et al.*, 2017). Therefore, we believe primary tumor location in CRC has the potential to be one of the evaluation criteria of cancer development and progression. It is known that variations on gene regions that encode some members of VEGF signaling pathway, including VEGF-A, affect CRC depending on primary tumor location (Riera *et al.*, 2018; Grassadonia *et al.*, 2019). Since VEGF expression levels are known to vary depending on the location in colon (Bendardaf *et al.*, 2008; Szajewski *et al.*, 2014), we thought that genetic variations of *VEGF* might be associated with occurrence of tumors in a specific location.

Only a limited number of studies have focused on the relationship of angiogenesis-related *VEGF* polymorphisms and location of primary tumor. It is difficult to get clear results due to different classification criteria of study groups in these limited studies. Slattery *et al.* (2014) subclassified their study group based on primary tumor location as rectum and colon, and reported *VEGF* +405GC+CC genotypes to be associated with rectal cancer ($p = 0.007$). Similarly, Jang *et al.* considered the primary tumor locations as colon and rectum, but unlike Slattery *et al.* (2014) and they did not detect a significant relationship between *VEGF* +405G>C polymorphism and primary tumor localization (Jang *et al.*, 2013b). In another study, study group was subclassified as colon, rectum and rectosigmoid, and no correlation was found between both +405G>C and −460C>T polymorphisms and the primary tumor location (Koutras *et al.*, 2012).

In our study, we subcategorized our study group as right (cecum, ascending colon, hepatic flexure, and transverse colon) or left (splenic flexure, descending colon, sigmoid colon, and rectum) colon patients. *VEGF* +405GC+CC genotypes were found to be associated with left colon cancer (OR = 5.208 95% CI: 1.064–25.496, $p = 0.04$). Since rectum is involved in the left colon, our results partially support the study conducted by Slattery *et al.* (2014). But still, further studies with larger sample size are needed to validate our results.

In the present study, we also investigated the association of *VEGF* +405G>C and −460C>T polymorphisms with CRC risk in Turkish subjects. Although *VEGF* +405GC genotype was found to be nearly associated with increased CRC risk in both unadjusted (OR = 2.471 95% CI: 0.941–6.490, $p = 0.066$) and the adjusted models (OR = 2.643 95% CI: 0.916–7.624, $p = 0.072$), results were not statistically significant. Similar (Dassoulas *et al.*, 2009; Antonacopoulou *et al.*, 2012; Jang *et al.*, 2013a) and opposite results (Chae *et al.*, 2008; Zhao *et al.*, 2012; Guo *et al.*, 2014) were reported by researchers in different populations. No significant

relationship was found between *VEGF* −460C>T polymorphism and CRC risk in Turkish subjects. In a different study conducted with CRC patients and healthy controls in the Turkish population, *VEGF* −460C>T genotype distributions also were not found significantly different in cases and controls (Jannuzzi *et al.*, 2015). Dassoulas *et al.* (2009) reported no association between *VEGF* −460C>T polymorphism and CRC risk; whereas other studies reported the opposite (Maltese *et al.*, 2009; Zhao *et al.*, 2012).

Bevacizumab, a recombinant humanized IgG1 monoclonal antibody, is the first antiangiogenic agent approved for metastatic CRC treatment (Hurwitz *et al.*, 2004). Favorable outcomes in progression-free survival, overall survival, and response rate have been achieved in metastatic CRC patients treated with chemotherapy+bevacizumab compared with chemotherapy alone (Botrel *et al.*, 2016; Ruan *et al.*, 2018).

Although clinical use is increasing, the efficacy or drug resistance of bevacizumab varies widely among patients. The different bevacizumab responses mainly occur due to genetic variations, which effects function of the proteins that involve directly or indirectly in angiogenesis (Novillo *et al.*, 2020). These variations are also associated with the etiology and clinical outcomes of metastatic CRC (Loupakis *et al.*, 2013; Ulivi *et al.*, 2015).

The efficiency of bevacizumab treatment at the population level is limited due to multiple resistance mechanisms. Since the molecular genetic mechanisms underlying the different bevacizumab responses have not yet been clarified enough, attempts to individualize treatment for bevacizumab remain insufficient. Thus, we finally evaluated the effects of *VEGF* +405G>C and −460C>T polymorphisms on progression-free and overall survival times of patients regarding bevacizumab use.

According to our results, progression-free survival times of CRC patients with *VEGF* +405GG genotype was found to be longer only in the BEV^+ group compared with the GC+CC genotypes (Log rank = 4.61, $p = 0.03$, respectively). Our results may indicate that bevacizumab treatment results in longer progression-free survival in CRC patients with *VEGF* +405GG genotype. Studies focused on relationship between *VEGF* +405G>C polymorphism and CRC survival show heterogeneity in terms of both content and results. Dassoulas *et al.* (2009) reported that a 6-year survival of CRC patients with +405CC genotype was lower compared with other genotypes. Hansen *et al.* (2011) found the +405GC genotype to be associated in borderline with progression-free survival in mCRC patients treated with first-line capecitabine and oxaliplatin. Do Espírito Santo *et al.* (2017) found +405CC genotype to be associated with 5-year survival of CRC patients in multivariate analysis, but the researchers did not detect any significant relationship in univariate analysis.

In many studies conducted with CRC patients, where BEV was prescribed in combination with fluorouracil, irinotecan, or oxaliplatin-based chemotherapies, no significant relationship was found between *VEGF* +405G>C polymorphism and progression-free and/or overall survival (Loupakis *et al.*, 2011a, 2011b; Pander *et al.*, 2011; Koutras *et al.*, 2012; Papachristos *et al.*, 2019). Many studies indicate that *VEGF* +405GG genotype causes decreased VEGF protein levels and angiogenicity in tumor cells (Awata *et al.*, 2002; Koukourakis *et al.*, 2004). It is also known that those patients with low or no VEGF expression show better survival compared with patients with high VEGF expression (Lee *et al.*, 2000; Ferroni *et al.*, 2005; Kuramochi *et al.*, 2006; Altomare *et al.*, 2007). Therefore, it may be predicted that *VEGF* +405GG genotype affects CRC prognosis through VEGF expression levels. However, since *VEGF* +405G>C polymorphism was not found to be related with VEGF protein levels in CRC patients under FOLFOXIRI+BVZ treatment (Loupakis *et al.*, 2011a), further studies in larger study groups are needed to fully elucidate the role of *VEGF* +405GG polymorphism in CRC prognosis.

The heterogeneous results may occur due to differences in size, content, analysis methods, and observation times of the studies, or due to differences in ethnicity, genetic backgrounds, drug regimens, or disease characteristics of the patients in study groups. For this reason, it is difficult to define the association of *VEGF* +405G>C and −460C>T polymorphisms with survival clearly. However, if each study group is defined in detail and the factors that have potential effects on survival are standardized, perhaps a common denominator might be found and the effects of these polymorphisms on survival processes might be clarified.

Conclusion

In the present study, we investigated the associations of *VEGF* +405G>C and −460C>T polymorphisms with CRC risk, primary tumor location, metastasis, and prognosis as a response to bevacizumab therapy. While *VEGF* +405G>C polymorphism was associated with left colon cancer and progression-free survival as a response to bevacizumab treatment, *VEGF* −460C>T polymorphism was found to be related to liver metastasis in CRC patients. It is obvious that our study has limitations such as small sample size, heterogeneous treatment regimens, and these limitations may be limiting our results. However, we suggest that our findings would contribute to the understanding of the genetic background of CRC and treatment process by providing data on personalized therapy regimens. Thus, our conclusions should be verified in further studies with larger populations.

Authors' Contributions

M.C. contributed to the experimental molecular genetic analysis, study design, and literature. E.N., U.I.K., N.D.S., and C.C. as the clinicians selected and provided the patient to participate in this study, and blood samples and clinical data of the patients. B.S. contributed to the whole study management, development of the final protocol of the experiments, interpretation of the results, and organization of the article. All authors were involved in preparing the article.

Ethics Approval and Consent to Participate

This study was approved by the Marmara University Local Ethics Committee with the protocol number 09.2018.174. Informed consent was obtained from all individual participants included in the study.

Availability of Data and Materials

The datasets used and/or analyzed during this study are available from the corresponding author on reasonable request.

Author Disclosure Statement

No competing financial interests exist.

Funding Information

This study was supported by the Marmara University Scientific Research Projects Coordination Unit. Grant No: FEN-C-DRP-110718-0407.

Supplementary Material

Supplementary Table S1
Supplementary Table S2
Supplementary Table S3
Supplementary Table S4

References

Altomare DF, Rotelli MT, Pentimone A, *et al.* (2007) Tissue factor and vascular endothelial growth factor expression in colorectal cancer: relation with cancer recurrence. Colorectal Dis 9:133–138.

Antonacopoulou AG, Kottorou AE, Dimitrakopoulos FID, *et al.* (2012) VEGF polymorphisms may be associated with susceptibility to colorectal cancer: a case-control study. Cancer Biomark 10:213–217.

Awata T, Inoue K, Kurihara S, *et al.* (2002) A common polymorphism in the 5′-untranslated region of the VEGF gene is associated with diabetic retinopathy in type 2 diabetes. Diabetes 51:1635–1639.

Bendardaf R, Buhmeida A, Hilska M, *et al.* (2008) VEGF-1 expression in colorectal cancer is associated with disease localization, stage, and long-term disease-specific survival. Anticancer Res 28:3865–3870.

Bendardaf R, El-Serafi A, Syrjänend K, *et al.* (2017) The effect of vascular endothelial growth factor-1 expression on survival of advanced colorectal cancer patients. Libyan J Med 12:1290741.

Benedix F, Kube R, Meyer F, *et al.* (2010) Comparison of 17,641 patients with right-and left-sided colon cancer: differences in epidemiology, perioperative course, histology, and survival. Dis Colon Rectum 53:57–64.

Botrel TEA, de Oliveira Clark LG, Paladini L, Clark OAC (2016) Efficacy and safety of bevacizumab plus chemotherapy compared to chemotherapy alone in previously untreated advanced or metastatic colorectal cancer: a systematic review and meta-analysis. BMC Cancer 16:1–19.

Carmeliet P (2000) Mechanisms of angiogenesis and arteriogenesis. Nat Med 6:389–395.

Chae YS, Kim JG, Sohn SK, *et al.* (2008) Association of vascular endothelial growth factor gene polymorphisms with susceptibility and clinicopathologic characteristics of colorectal cancer. J Korean Med Sci 23:421–427.

Cheng JZ, Chen JJ, Xue K, *et al.* (2018) Clinicopathologic and prognostic significance of VEGF, JAK2 and STAT3 in patients with nasopharyngeal carcinoma. Cancer Cell Int 18: 1–9.

Dassoulas K, Gazouli M, Rizos S, *et al.* (2009) Common polymorphisms in the vascular endothelial growth factor gene and colorectal cancer development, prognosis, and survival. Mol Carcinog 48:563–569.

Dinami R, Porru M, Amoreo CA, *et al.* (2020) TRF2 and VEGF-A: an unknown relationship with prognostic impact on survival of colorectal cancer patients. J Exp Clin Cancer Res 39:1–13.

do Espírito Santo GF, Galera BB, Duarte EC, *et al.* (2017) Prognostic significance of vascular endothelial growth factor polymorphisms in colorectal cancer patients. World J Gastrointest Oncol 9:78.

Dvorak HF (2002) Vascular permeability factor/vascular endothelial growth factor: a critical cytokine in tumor angiogenesis and a potential target for diagnosis and therapy. J Clin Oncol 20:4368–4380.

Ferlay J, Soerjomataram I, Ervik M, *et al.* (2013) GLOBOCAN 2012 v1. 0 (2013) Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11 (Internet). International Agency for Research on Cancer, Lyon, France.

Ferrara N, Gerber HP, LeCouter J. (2003) The Biology of VEGF and its Receptors. Nat Med 9:669–676.

Ferroni P, Spila A, Martini F, *et al.* (2005) Prognostic value of vascular endothelial growth factor tumor tissue content of colorectal cancer. Oncology 69:145–153.

Frezzetti D, Gallo M, Maiello MR, *et al.* (2017) VEGF as a potential target in lung cancer. Expert Opin Ther Targets 21: 959–966.

Fu ZZ, Sun XD, Li P, *et al.* (2014) Relationship between serum VEGF level and radiosensitivity of patients with nonsmall cell lung cancer among asians: a meta-analysis. DNA Cell Biol 33:426–437.

Gabriel S, Ziaugra L, Tabbaa D (2009) SNP genotyping using the Sequenom MassARRAY iPLEX platform. Curr Protoc Hum Genet 60:2–12.

Grassadonia A, Di Marino P, Ficorella C, *et al.* (2019) Impact of primary tumor location in patients with RAS wild-type metastatic colon cancer treated with first-line chemotherapy plus anti-EGFR or anti-VEGF monoclonal antibodies: a retrospective multicenter study. J Cancer 10:5926.

Guo Q, Dai SB, Shen F, *et al.* (2014) VEGF +405G/C (rs2010963) polymorphisms and digestive system cancer risk: a meta-analysis. Tumor Biol 35:4977–4982.

Gupta MK, Qin RY (2003) Mechanism and its regulation of tumouqr induced angiogenesis. World J Gastroenterol 9: 1144–1155.

Hanahan D, Folkman J (1996) Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. Cell 86: 353–364.

Hansen TF, Spindler KG, Andersen RF, *et al.* (2011) The predictive value of genetic variations in the vascular endothelial growth factor A gene in metastatic colorectal cancer. Pharmacogenomics J 11:53–60.

Hasina R, Lingen MW (2001) Angiogenesis and oral cancer. J Dent Educ 65:1282–1290.

Hicklin DJ, Ellis LM (2005) Role of the vascular endothelial growth factor pathway in tumor growth and angiogenesis. J Clin Oncol 23:1011–1027.

Hurwitz H, Fehrenbacher L, Novotny W, *et al.* (2004) Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. New Eng J Med 350:2335–2342.

Innocenti F, Jiang C, Sibley AB, *et al.* (2018) Genetic variation determines VEGF-A plasma levels in cancer patients. Sci Rep 8:1–9.

Jang MJ, Jeon YJ, Kim JW, *et al.* (2013a) Association of VEGF and KDR single nucleotide polymorphisms with colorectal cancer susceptibility in Koreans. Mol Carcinog 52: 60–69.

Jang MJ, Kim JW, Jeon YJ, *et al*. (2013b) Prognostic significance of vascular endothelial growth factor gene polymorphisms in patients with colorectal cancer. Int J Clin Oncol 18: 1032–1041.

Jannuzzi AT, Özhan G, Yanar HT, Alpertunga B (2015) VEGF gene polymorphisms and susceptibility to colorectal cancer. Genet Test Mol Biomark 19:133–137.

Koukourakis MI, Papazoglou D, Giatromanolaki A, *et al*. (2004) VEGF gene sequence variation defines VEGF gene expression status and angiogenic activity in non-small cell lung cancer. Lung cancer 46:293–298.

Koutras AK, Antonacopoulou AG, Eleftheraki AG, *et al.* (2012) Vascular endothelial growth factor polymorphisms and clinical outcome in colorectal cancer patients treated with irinotecan-based chemotherapy and bevacizumab. Pharmacogenomics J 12:468–475.

Kumar V, Abbas AK, Fausto N (2004) Robbins and Cotran Pathologic Basis of Disease. 7th ed. Saunders; Elsevier Inc., Philadelphia.

Kuramochi H, Hayashi K, Uchida K, *et al.* (2006) Vascular endothelial growth factor messenger RNA expression level is preserved in liver metastases compared with corresponding primary colorectal cancer. Clin Cancer Res 12: 29–33.

Lacin S, Yalcin S (2020) The prognostic value of circulating VEGF-A level in patients with hepatocellular cancer. Technol Cancer Res Treat 19:1533033820971677.

Lee GH, Malietzis G, Askari A, *et al*. (2015) Is right-sided colon cancer different to left-sided colorectal cancer?–a systematic review. Eur J Surg Oncol 41:300–308.

Lee JC, Chow NH, Wang ST, Huang SM (2000) Prognostic value of vascular endothelial growth factor expression in colorectal cancer patients. Eur J Cancer 36:748–753.

Levin B, Lieberman DA, McFarland B, *et al.* (2008) Screening and surveillance for the early detection of colorectal cancer and adenomatous polyps, 2008: a joint guideline from the American Cancer Society, the US Multi-Society Task Force on Colorectal Cancer, and the American College of Radiology. CA Cancer J Clin 58:130–160.

Li S, Wang L, Meng Y, *et al*. (2017) Increased levels of LAPTM4B, VEGF and survivin are correlated with tumor progression and poor prognosis in breast cancer patients. Oncotarget 8:41282.

Li T, Kang G, Wang T, Huang HE (2018) Tumor angiogenesis and anti-angiogenic gene therapy for cancer. Oncol Lett 16: 687–702.

Loupakis F, Cremolini C, Fioravanti A, *et al.* (2011a) Pharmacodynamic and pharmacogenetic angiogenesis-related markers of first-line FOLFOXIRI plus bevacizumab schedule in metastatic colorectal cancer. Br J Cancer 104:1262–1269.

Loupakis F, Cremolini C, Yang D, *et al.* (2013) Prospective validation of candidate SNPs of VEGF/VEGFR pathway in metastatic colorectal cancer patients treated with first-line FOLFIRI plus bevacizumab. PLoS One 8:e66774.

Loupakis F, Ruzzo A, Salvatore L, *et al.* (2011b) Retrospective exploratory analysis of VEGF polymorphisms in the prediction of benefit from first-line FOLFIRI plus bevacizumab in metastatic colorectal cancer. BMC Cancer 11:1–9.

Macarulla T, Montagut C, Sánchez-Martin FJ, *et al.* (2020) The role of PIGF blockade in the treatment of colorectal cancer: overcoming the pitfalls. Expert Opin Biol Ther 20: 15–22.

Maltese P, Canestrari E, Ruzzo A, *et al.* (2009) VEGF gene polymorphisms and susceptibility to colorectal cancer disease in Italian population. Int J Colorectal Dis 24: 165–170.

Mashima T, Wakatsuki T, Kawata NJ, *et al.* (2021) Neutralization of the induced VEGF-A potentiates the therapeutic effect of an anti-VEGFR2 antibody on gastric cancer in vivo. Sci Rep 11:1–12.

Mlecnik B, Bindea G, Kirilovsky A, *et al.* (2016) The tumor microenvironment and Immunoscore are critical determinants of dissemination to distant metastasis. Sci Transl Med 8:327ra26.

Mousa SA (2000) Mechanisms of Angiogenesis: Potential Therapeutic Targets. Eurekah.com/Landes Bioscience, Georgetown, WA, USA.

Novillo A, Gaibar M, Romero-Lorca A, *et al.* (2020) Efficacy of bevacizumab-containing chemotherapy in metastatic colorectal cancer and CXCL5 expression: six case reports. World J Gastroenterol 26:1979.

O-Charoenrat P (2014) Vascular endothelial growth factor polymorphisms affect gene expression and tumor aggressiveness in patients with breast cancer. Mol Med Rep 9:1044–1048.

Pander J, Wessels JA, Gelderblom H, *et al*. (2011) Pharmacogenetic interaction analysis for the efficacy of systemic treatment in metastatic colorectal cancer. Ann Oncol 22: 1147–1153.

Papachristos A, Kemos P, Katsila T, *et al.* (2019) VEGF-A and ICAM-1 gene polymorphisms as predictors of clinical outcome to first-line bevacizumab-based treatment in metastatic colorectal cancer. Int J Mol Sci 20:5791.

Plastiras A, Iosif E, Georgiou G, *et al.* (2019) Right versus left Colon cancer: is there a difference in outcomes?. World J Colorectal Surg 8:14.

Rajabi M, Mousa SA (2017) The role of angiogenesis in cancer treatment. Biomedicines 5:34.

Riera P, Virgili AC, Salazar J, *et al.* (2018) Genetic variants in the VEGF pathway as prognostic factors in stages II and III colon cancer. Pharmacogenomics J 18:556–564.

Risau W (1997) Mechanisms of angiogenesis. Nature 386:671–674.

Ruan WC, Che YP, Ding L, Li HF (2018) Efficacy and toxicity of addition of bevacizumab to chemotherapy in patients with metastatic colorectal cancer. Comb Chem High Throughput Screen 21:718–724.

Sa-Nguanraksa D, Chuangsuwanich T, Pongpruttipan T, *et al.* (2013) Vascular endothelial growth factor 634G/C polymorphism is associated with increased breast cancer risk and aggressiveness. Mol Med Rep 8:1242–1250

Slattery ML, Lundgreen A, Wolff RK (2014) VEGFA, FLT1, KDR and colorectal cancer: assessment of disease risk, tumor molecular phenotype, and survival. Mol Carcinog 53:E140–E150.

Sopo M, Anttila M, Hämäläinen K, *et al.* (2019) Expression profiles of VEGF-A, VEGF-D and VEGFR1 are higher in distant metastases than in matched primary high grade epithelial ovarian cancer. BMC Cancer 19:1–12.

Stintzing S, Tejpar S, Gibbs P, *et al*. (2017) Understanding the role of primary tumour localisation in colorectal cancer treatment and outcomes. Eur J Cancer 84:69–80.

Szajewski M, Kruszewski WJ, Lakomy J, *et al.* (2014) VEGF-C and VEGF-D overexpression is more common in left-

sided and well-differentiated colon adenocarcinoma. Oncol Rep 31:125–130.

Torre LA, Bray F, Siegel RL, *et al.* (2015) Global cancer statistics, 2012. CA Cancer J Clin 65:87–108.

Ulivi P, Scarpi E, Passardi A, *et al.* (2015) eNOS polymorphisms as predictors of efficacy of bevacizumab-based chemotherapy in metastatic colorectal cancer: data from a randomized clinical trial. J Transl Med 13:1–10.

Van Cutsem E, Paccard C, Chiron M, Tabernero J (2020) Impact of prior bevacizumab treatment on VEGF-A and PlGF levels and outcome following second-line aflibercept treatment: biomarker post hoc analysis of the VELOUR trial. Clin Cancer Res 26:717–725.

Yamamori M, Sakaeda T, Nakamura T, *et al.* (2004) Association of VEGF genotype with mRNA level in colorectal adenocarcinomas. Biochem Biophys Res Commun 325:144–150.

Zhao Z, Ba C, Wang W, *et al*. (2012) Vascular endothelial growth factor (VEGF) gene polymorphisms and colorectal cancer: a meta-analysis of epidemiologic studies. Genet Test Mol Biomark 16:1390–1394.

Address correspondence to:
Belgin Susleyici, PhD
Department of Molecular Biology
Marmara University Faculty of Science and Letters
Goztepe Campus
Kadikoy
Istanbul 34722
Turkey

E-mail: belginsusleyici@gmail.com