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ORIGINAL RESEARCH

Association between VEGF –460T/C gene polymorphism and clinical outcomes of nasopharyngeal carcinoma treated with intensity-modulated radiation therapy

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Abstract: Vascular endothelial growth factor (VEGF) is a potent angiogenic factor that plays a critical role in the development, metastasis, and recurrence of tumors. This study aims to determine the correlation of single-nucleotide polymorphisms in the *VEGF* gene with the prognosis of nasopharyngeal carcinoma (NPC). The VEGF –460T/C gene polymorphisms in the genomic DNA of the blood samples of 338 patients with NPC were investigated through polymerase chain reaction and direct DNA sequencing. Results showed a significant association between the –460C-allele carriers and the aggressive forms of NPC as defined by stages N2–3 (odds ratio =1.820, 95% confidence interval [CI]: 1.118–2.962, P=0.015). Furthermore, the VEGF –460T/C polymorphism was significantly associated with 3-year overall survival (OS), distant metastasis-free survival (DMFS), and progression-free survival (PFS) (T/C + C/C vs T/T: 3-year OS 78.8% vs 95.1%, P=0.003; 3-year DMFS 80.2% vs 90.6%, P=0.036; 3-year PFS 73.9% vs 86.7%, P=0.042) but was not associated with the local recurrence-free survival (LRFS) of the patients. The multivariate analysis indicated that the VEGF –460C-allele carrier was an independent significant prognostic factor for OS (hazard ratio [HR] 4.096, 95% CI: 1.333–12.591, P=0.014). N classification was an independent significant prognostic factor for DMFS in patients with locoregionally advanced NPC (HR 3.674, 95% CI: 1.144–11.792, P=0.029). However, neoadjuvant chemotherapy (NACT) followed by concurrent chemoradiotherapy (CCRT) was not superior to CCRT alone in terms of the 3-year OS, LRFS, DMFS, and PFS of patients with VEGF –460T/C polymorphism. In conclusion, the VEGF –460T/C gene polymorphism may negatively affect the clinical outcomes of patients with NPC and may be considered a potential prognostic factor for this disease.

Keywords: vascular endothelial growth factor, gene polymorphism, nasopharyngeal carcinoma, clinical outcomes

Introduction

Nasopharyngeal carcinoma (NPC) is the most common malignancy in the epithelial lining of the nasopharynx. NPC is unique in the aspects of epidemiology because of its prominent geographic distribution. The highest incidence rates of NPC are observed in the southern part of China, especially in Guangdong and Guangxi.[1,2] Radiotherapy is used as a standard treatment for NPC because of the unique anatomical position and moderate radiosensitivity of the tumor. Despite the combined applications of magnetic resonance imaging (MRI), intensity-modulated radiation therapy (IMRT), chemotherapy, and targeted therapy, treatment for NPC still fails, especially when the

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tumor is in the advanced stage.[3] Hence, prognostic predictors, such as gene single-nucleotide polymorphisms (SNPs), must be developed.

The *VEGF* gene is located in chromosome 6p12 and is composed of a 14 kb coding region with eight exons and seven introns. This gene plays a key role in the formation of new blood vessels.[4] In this regard, the *VEGF* gene, as a major angiogenic factor, is thought to be associated with tumor development and metastasis; inhibition of vascular endothelial growth factor (VEGF) signaling can suppress tumor growth and angiogenesis by modulating the blood flow and oxygenation of the tumors.[5] *VEGF* gene polymorphisms affect the aggressiveness and progression of NPC.[6,7] However, the correlation of VEGF –460T/C gene polymorphism with the clinical outcomes in NPC has been rarely investigated. Our previous studies showed that VEGF –460T/C gene polymorphism is associated with the risk of NPC in the Chinese population.[8,9] Thus, we carried out a retrospective study to assess the role of VEGF –460T/C gene polymorphism in the prognostic relevance by correlating it with the survival of NPC patients.

Materials and methods
Patients, treatment, and follow-up
This study included 338 patients diagnosed with NPC at the First Affiliated Hospital of Guangxi Medical University (Nanning, Guangxi Province, People's Republic of China) between December 2012 and December 2013. The inclusion criteria were as follows: 1) the initial diagnosis of NPC was determined by pathologists according to World Health Organization (WHO) classification; 2) Karnofsky performance score (KPS) ≥90; 3) patients who underwent IMRT with platinum-based chemotherapy, along with regular follow-ups at our hospital; and 4) availability of peripheral blood samples. The exclusion criteria were as follows: 1) diagnosis with distant metastasis before treatment; 2) history of any other malignant disease; 3) any prior treatment for NPC; and 4) contraindications of radiotherapy. All the TNM classification was restaged according to the seventh edition of the International Union against Cancer/American Joint Committee on Cancer (UICC/AJCC) classification system. Written informed consents were obtained from all of the patients, and the research protocol was approved by the Ethical Review Committee of the First Affiliated Hospital of Guangxi Medical University. Information that can be used to identify individual participants during or after the data collection was available and can be accessed.

Each subject underwent the following pretreatment evaluations: patient history, physical examinations, hematological and biochemical profiling, chest radiography, abdominal sonography, MRI of the head and neck, and whole-body bone scan. The radiotherapy course was generally uniform. All the patients underwent definitive IMRT according to established methods.[10] The patients also underwent two-to-three cycles of concurrent chemoradiotherapy (CCRT) and were administered cisplatin every 3 weeks. Neoadjuvant chemotherapy (NACT) was adopted in conjunction with CCRT in some patients. The NACT regimen comprised the administration of cisplatin with docetaxel every 3 weeks for two cycles. The chemotherapy was discontinued when the patient showed leukocyte counts lower than 3,000/mm^3, or platelet count lower than 75,000/mm^3. The chemotherapy was continued when the leukocyte and platelet counts reverted to the normal values.

The participants were followed up every 3 months during the first 2 years and then every 6 months thereafter, until the final follow-up or death of the participant. The follow-ups were conducted until February 2016. The median follow-up period was 31 months (range: 9–38 months). The overall survival (OS), local recurrence-free survival (LRFS), distant metastasis-free survival (DMFS), and progression-free survival (PFS) were selected as end points. OS was calculated from the date of enrollment to the date of the confirmed death (from any cause) or the last follow-up. LRFS was calculated from the date of enrollment to the date of the local recurrence or the last follow-up. DMFS was calculated from the date of enrollment to the date of the distant metastasis or the last follow-up. PFS was calculated from the date of enrollment to the date of any form of tumor progression or the last follow-up.

DNA extraction and genotyping
Genomic DNA was extracted from peripheral blood (5 mL) at the time of enrollment for genotyping by using a commercially available kit according to the manufacturer's instructions (Tiangen Biotech, Beijing, Co, Ltd). The selected VEGF SNP (–460T/C) was genotyped through polymerase chain reaction (PCR) and direct DNA sequencing. The PCR primers used for the VEGF –460T/C were 5′-TGTGCAGACGGCAGTCACTA-3′ (upstream primer) and 5′-CCCGCTACCAGCCGACTTT-3′ (downstream primer). The PCR amplifications were performed in a 20 µL reaction volume containing 2 µL of genomic DNA, 0.6 µL of each primer, 10 µL of TaqMan Universal PCR Master Mix (Applied Biosystems), and 6.8 µL of DNA-free water.

PCR was performed under the following conditions: initial denaturation at 94°C for 4 min, followed by 35 cycles of 30 s at 94°C, annealing at 62°C for 30 s, polymerization at 72°C for 45 s, and final holding at 72°C for 2 min. The PCR product was verified and genotyped through DNA sequencing. All the blood samples were genotyped successfully.

Statistical analysis

Chi-square test was performed to determine the association between the SNP and the clinicopathological features of the patients. The odds ratios (ORs) and their corresponding 95% confidence intervals (CIs) were computed. Kaplan–Meier method was used to calculate the survival curves. The effect of the SNP on the clinical outcomes was assessed using the log-rank test. Multivariate analyses were then performed using a Cox proportional hazards model to calculate the hazard ratios (HRs) and the corresponding 95% CIs. All the statistical analyses were performed using the Statistical Product and Service Solutions software (SPSS; version 21.0). Two-sided P-values <0.05 were considered statistically significant.

Results
Patient characteristics

Table 1 shows the clinical characteristics of the 338 patients with NPC. The median age was 45 years (range: 13–76 years).

Table 1 Patient characteristics and clinical features

Patient characteristics	Number (%)
Gender	
Male	232 (68.6)
Female	106 (31.4)
Age	
≤45 years	174 (51.5)
>45 years	164 (48.5)
UICC/AJCC clinical classification	
II	40 (11.8)
III	99 (29.3)
IV	199 (58.9)
T classification	
T2	60 (17.8)
T3	83 (24.5)
T4	195 (57.7)
N classification	
N0	40 (11.8)
N1	141 (41.7)
N2	131 (38.8)
N3	26 (7.7)
Chemotherapy regimens	
NACT + CCRT	139 (41.1)
CCRT alone	199 (58.9)

Abbreviations: AJCC, American Joint Committee on Cancer; CCRT, concurrent chemoradiotherapy; NACT, neoadjuvant chemotherapy; UICC, International Union against Cancer.

IMRT combined with cisplatin-based chemotherapy was administered to all patients. A total of 139/338 (41.1%) patients received NACT. During the follow-up period, 25 patients were lost to follow-up. Twenty patients developed locoregional relapse, and 41 patients presented with distant metastasis. The 20 patients with locoregional relapse comprised 10 patients with nasal or nasopharyngeal relapse and 10 patients with relapse in the base of the skull. Forty-one patients with distant metastasis included 14 patients with pulmonary metastasis, 14 patients with hepatic metastasis, 9 patients with bone metastasis, and 4 patients with metastasis to multiple organs. At the end of the follow-up, 43 patients died from different causes. The following results were obtained: 3-year OS of 87.1%, 3-year LRFS of 93.7%, 3-year DMFS of 85.5%, and 3-year PFS of 80.5%.

Correlation of VEGF –460T/C polymorphism with the clinical features of the patients

The genotyping results showed that the distribution of the VEGF –460C-allele genotypes among the 338 enrolled patients included 9.2% (31/338) CC, 37.9% (128/338) TC, and 52.9% (179/338) TT (homozygous wild allele). Table 2 summarizes the correlation of the VEGF –460T/C polymorphisms with the clinical features of the patients. The –460C-allele was significantly associated with high lymphatic metastasis, including N2–3 stage (OR =1.820, 95% CI: 1.118–2.962, P=0.015). However, this allele was not significantly associated with gender, age, clinical classifications, T classifications, distant metastasis, local recurrence, and disease progression (P>0.05).

Associations of VEGF –460T/C gene polymorphisms with OS, LRFS, DMFS, and PFS of patients with NPC

Patients with VEGF –460T/T, VEGF –460T/C, and VEGF –460C/C genotypes showed 3-year OS of 95.1%, 76.7%, and 86.7%, respectively (P=0.008); and 3-year PFS of 86.7%, 70.8%, and 86.7%, respectively (P=0.047). Furthermore, the same patients had DMFS of 90.6%, 79.9%, and 81.7%, respectively (P=0.081); and LRFS of 92.8%, 95.1%, and 93.3%, respectively (P=0.862). The 3-year OS and PFS in patients with VEGF –460T/C and VEGF –460C/C genotypes were significantly lower than those of the patients with the VEGF –460T/T genotype. The outcomes are shown in Figures 1–4.

The combined effects of the genotypes on the survival of patients with NPC were assessed. The (T/C + C/C) genotype

Table 2 Relationship of VEGF –460T/C genotype and allele with the clinical characteristics of the patients

| Characteristics	Genotype			T-allele	C-allele	OR	95% CI	P-value
	T/T	T/C	C/C					
Gender								0.835
Male	122	87	23	331	133			
Female	56	41	9	153	59	0.946	0.561–1.595	
Age, years								0.259
≤45	83	75	16	241	107			
>45	95	54	15	244	84	0.756	0.465–1.229	
Clinical classification								0.916
II	21	15	4	57	23			
III–IV	158	114	26	430	166	0.960	0.455–2.207	
T classification								0.446
T1–2	35	21	4	91	29			
T3–4	143	108	27	394	162	1.290	0.669–2.488	
N classification								0.015*
N0–1	112	64	5	288	74			
N2–3	66	64	27	196	118	1.820	1.118–2.962	
M classification								0.077
M0	166	104	27	436	158			
M1	12	25	4	49	33	1.842	0.929–3.654	
Local recurrence								0.741
Yes	12	6	2	30	10			
No	166	122	30	454	182	1.192	0.420–3.379	
Disease progression								0.158
Yes	23	37	4	83	45			
No	156	91	27	403	145	0.656	0.365–1.181	

Note: *P<0.05 was considered statistically significant.
Abbreviations: CI, confidence interval; OR, odds ratio; VEGF, vascular endothelial growth factor.

carriers had significantly lower 3-year OS, DMFS, and PFS compared with the wild-type T/T genotype carriers (95.1% vs 78.8%, P=0.003, Figure 5; 90.6% vs 80.2%, P=0.036, Figure 6; 86.7% vs 73.9%, P=0.042, Figure 7, respectively). In addition, LRFS was not significantly different between (T/C + C/C) and T/T genotype carriers (Figure 8).

Univariate and multivariate analyses for determining the prognostic factors of patients with NPC

The univariate analysis indicated that the N classification was associated with OS, PFS, and DMFS (P=0.023, P=0.008, and P=0.014, respectively). Moreover, the VEGF –460
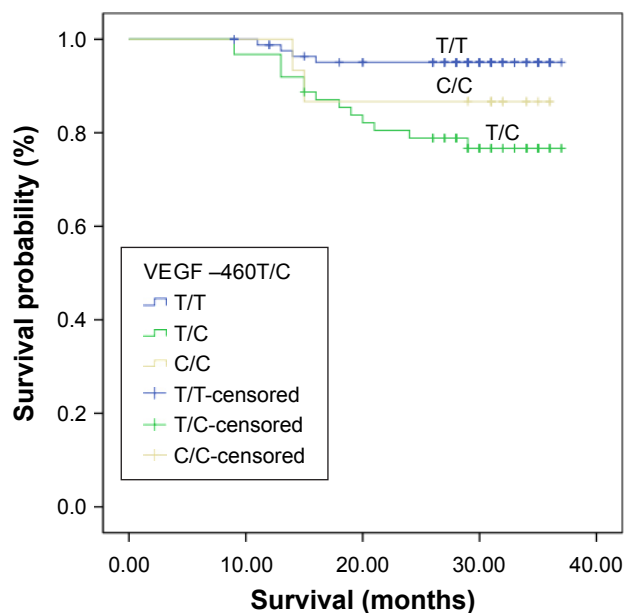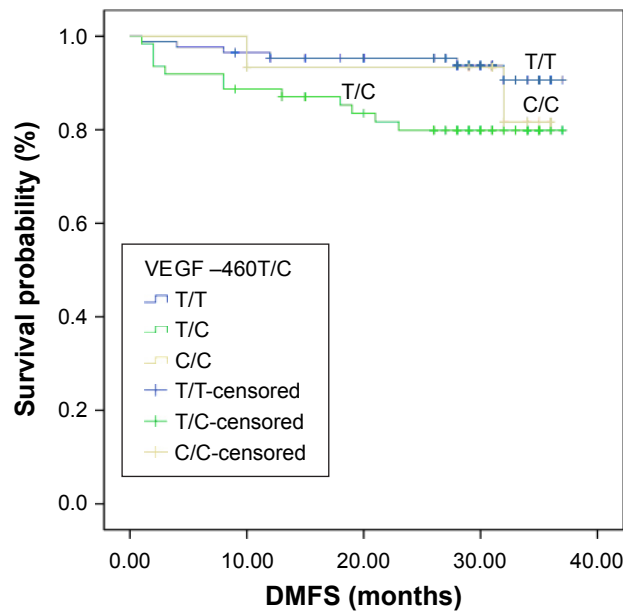

Figure 1 Influence of VEGF –460T/C genotypes on OS.
Abbreviations: OS, overall survival; VEGF, vascular endothelial growth factor.
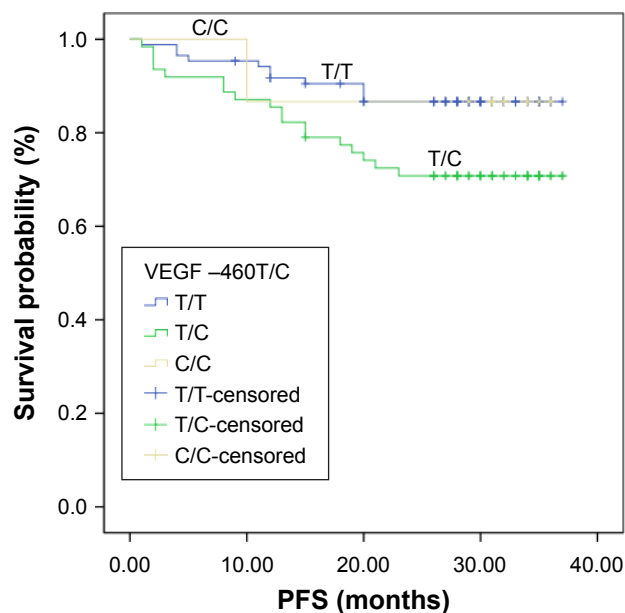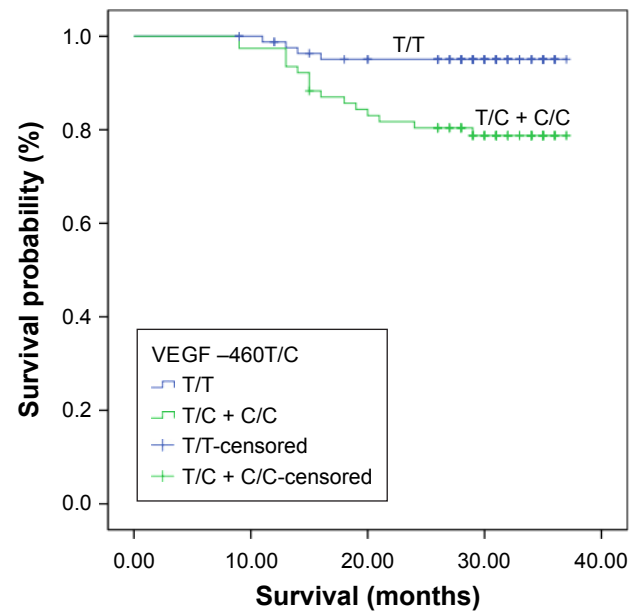

Figure 2 Influence of VEGF –460T/C genotypes on PFS.
Abbreviations: PFS, progression-free survival; VEGF, vascular endothelial growth factor.
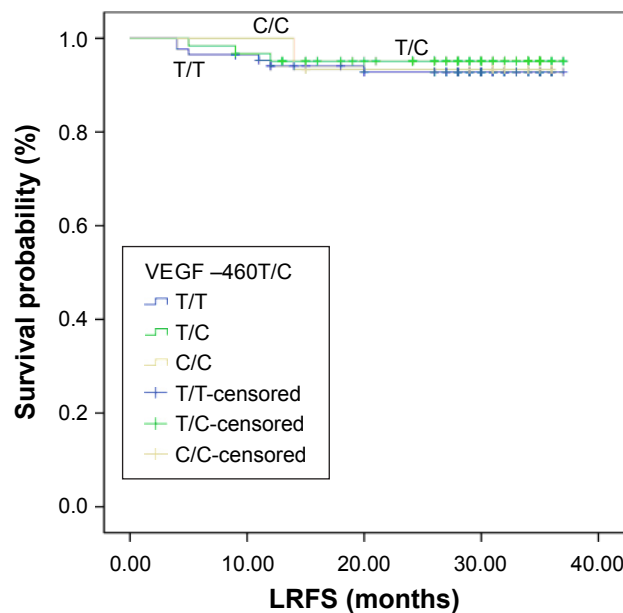
Figure 3 Influence of VEGF –460T/C genotypes on DMFS.
Abbreviations: DMFS, distant metastasis-free survival; VEGF, vascular endothelial growth factor.
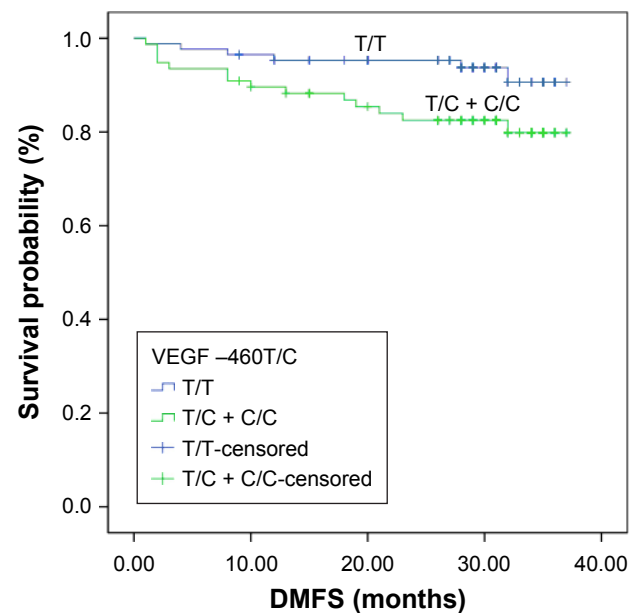

Figure 5 OS for (T/C + C/C) genotype carriers and T/T genotype carriers.
Abbreviations: OS, overall survival; VEGF, vascular endothelial growth factor.

SNPs were significantly associated with OS (*P*=0.008) and PFS (*P*=0.047). Compared with the T/T genotype, the (T/C + C/C) genotype was associated with PFS (*P*=0.042), DMFS (*P*=0.036), and OS (*P*=0.003). The results are shown in Tables 3–6. Compared with patients receiving CCRT, the patients receiving NACT followed by CCRT had no significant improvement in their prognosis in terms of 3-year OS, LRFS, DMPS, and PFS in relation to the VEGF –460T/C polymorphism (*P*>0.05; Table 7).

The multivariate analysis results showed that the VEGF –460T/C polymorphism was an independent significant prognostic factor for OS (HR 4.096, 95% CI: 1.333–12.591, *P*=0.014). The N classification (N2–3 vs N0–1) was an independent significant prognostic factor for DMFS in patients with locoregionally advanced NPC (HR 3.674, 95% CI: 1.144–11.792, *P*=0.029). No significant association was observed between the predictors (gender, age, clinical classifications, T classifications, N classifications,

Figure 4 Influence of VEGF –460T/C genotypes on LRFS.
Abbreviations: LRFS, local recurrence-free survival; VEGF, vascular endothelial growth factor.

Figure 6 DMFS for (T/C + C/C) genotype carriers and T/T genotype carriers.
Abbreviations: DMFS, distant metastasis-free survival; VEGF, vascular endothelial growth factor.
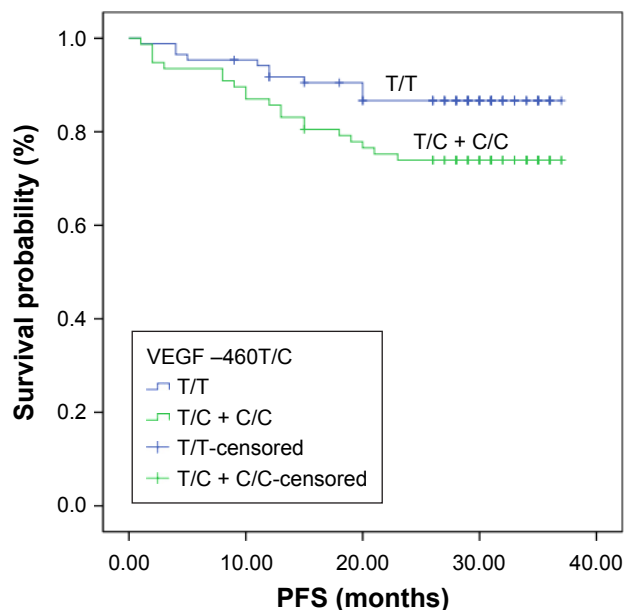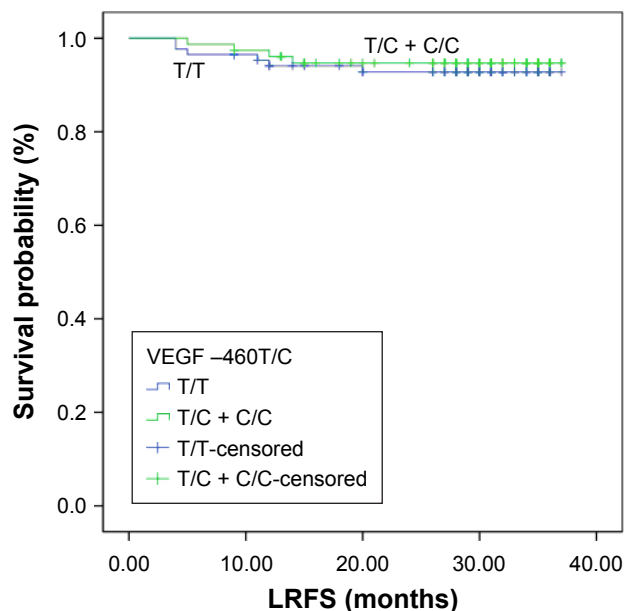
Figure 7 PFS for (T/C + C/C) genotype carriers and T/T genotype carriers.
Abbreviations: PFS, progression-free survival; VEGF, vascular endothelial growth factor.

chemotherapy regimens, and VEGF –460T/C SNP) and LRFS or PFS (Table 8).

Discussion

VEGF plays a pivotal role in prompting tumor angiogenesis, metastasis, and survival through a variety of mechanisms, such as the effects on endothelial cell proliferation, survival, and migration.[11] Several VEGF SNPs were reported

Figure 8 LRFS for (T/C + C/C) genotype carriers and T/T genotype carriers.
Abbreviations: LRFS, local recurrence-free survival; VEGF, vascular endothelial growth factor.

Table 3 Univariate analysis of OS

Variables	Cumulative survival			P-value
	1-year OS	2-year OS	3-year OS	
Gender				
Male	97.3%	87.9%	86.7%	0.872
Female	100.0%	87.9%	87.9%	
Age, years				
≤45	98.8%	86.7%	85.1%	0.515
>45	97.5%	89.3%	89.3%	
Clinical stage				
II	94.7%	94.7%	94.7%	0.311
III–IV	97.9%	88.5%	86.0%	
T classification				
T2	100.0%	93.1%	93.1%	0.314
T3–4	97.7%	86.7%	85.7%	
N classification				
N0–1	97.7%	93.0%	93.0%	0.023*
N2–3	98.6%	81.8%	79.7%	
Chemotherapy regimens				
NACT + CCRT	100.0%	87.6%	87.6%	0.883
CCRT	96.9%	88.2%	86.6%	
–460T/C				
T/T	98.8%	95.1%	95.1%	0.008*
T/C	96.8%	78.9%	76.7%	
C/C	100.0%	86.7%	86.7%	
T/T vs	98.8% vs	95.1% vs	95.1% vs	0.003
T/C + C/C	97.4%	80.4%	78.8%	

Note: *P<0.05 was considered statistically significant.
Abbreviations: CCRT, concurrent chemoradiotherapy; OS, overall survival; NACT, neoadjuvant chemotherapy.

Table 4 Univariate analysis of LRFS

Variables	Cumulative survival			P-value
	1-year LRFS	2-year LRFS	3-year LRFS	
Gender				
Male	95.5%	93.5%	93.5%	0.931
Female	94.1%	94.1%	94.1%	
Age, years				
≤45	96.4%	93.9%	93.9%	0.891
>45	93.5%	93.5%	93.5%	
Clinical stage				
II	100.0%	100.0%	100.0%	0.238
III–IV	94.4%	92.8%	92.8%	
T classification				
T2	96.6%	96.6%	96.6%	0.497
T3–4	94.7%	93.0%	93.0%	
N classification				
N0–1	96.5%	96.5%	96.5%	0.105
N2–3	93.2%	90.2%	90.2%	
Chemotherapy regimens				
NACT + CCRT	97.0%	90.6%	90.6%	0.212
CCRT	95.8%	95.8%	95.8%	
–460T/C				
T/T	94.1%	92.8%	92.8%	0.862
T/C	95.1%	95.1%	95.1%	
C/C	100.0%	93.3%	93.3%	
T/T vs	94.1% vs	92.8% vs	92.8% vs	0.619
T/C + C/C	96.1%	94.7%	94.7%	

Note: P<0.05 was considered statistically significant.
Abbreviations: CCRT, concurrent chemoradiotherapy; LRFS, local recurrence-free survival; NACT, neoadjuvant chemotherapy.

Table 5 Univariate analysis of DMFS

Variables	Cumulative survival			P-value
	1-year DMFS	2-year DMFS	3-year DMFS	
Gender				
Male	92.8%	88.8%	84.5%	0.869
Female	92.2%	90.0%	87.4%	
Age, years				
≤45	91.7%	87.6%	80.2%	0.223
>45	93.7%	90.9%	90.9%	
Clinical stage				
II	97.4%	97.4%	85.3%	0.709
III–IV	92.3%	88.4%	85.8%	
T classification				
T2	93.1%	93.1%	85.9%	0.677
T3–4	92.5%	88.3%	85.5%	
N classification				
N0–1	94.4%	94.4%	92.0%	0.014*
N2–3	90.4%	82.6%	77.0%	
Chemotherapy regimens				
NACT + CCRT	92.4%	89.0%	84.3%	0.814
CCRT	92.8%	89.4%	86.5%	
–460T/C				
T/T	95.3%	95.3%	90.6%	0.081
T/C	88.7%	79.9%	79.9%	
C/C	93.3%	93.3%	81.7%	
T/T vs	95.3% vs	95.3% vs	90.6% vs	0.036*
T/C + C/C	89.6%	82.5%	80.2%	

Note: *P<0.05 was considered statistically significant.
Abbreviations: CCRT, concurrent chemoradiotherapy; DMFS, distant metastasis-free survival; NACT, neoadjuvant chemotherapy.

Table 6 Univariate analysis of PFS

Variables	Cumulative survival			P-value
	1-year PFS	2-year PFS	3-year PFS	
Gender				
Male	91.0%	83.6%	79.8%	0.736
Female	90.2%	82.0%	82.0%	
Age, years				
≤45	89.8%	81.7%	81.7%	0.816
>45	90.5%	79.4%	79.4%	
Clinical stage				
II	94.7%	94.7%	94.7%	0.114
III–IV	90.2%	78.5%	78.5%	
T classification				
T2	89.7%	89.7%	89.7%	0.196
T3–4	90.2%	78.4%	78.4%	
N classification				
N0–1	92.2%	88.7%	88.7%	0.008*
N2–3	87.7%	70.7%	70.7%	
Chemotherapy regimens				
NACT + CCRT	89.3%	76.8%	76.8%	0.433
CCRT	88.6%	83.1%	83.1%	
–460T/C				
T/T	93.7%	86.7%	86.7%	0.047*
T/C	85.5%	70.8%	70.8%	
C/C	86.7%	86.7%	86.7%	
T/T vs	93.7% vs	86.7% vs	86.7% vs	0.042*
T/C + C/C	85.7%	73.9%	73.9%	

Note: *P<0.05 was considered statistically significant.
Abbreviations: CCRT, concurrent chemoradiotherapy; NACT, neoadjuvant chemotherapy; PFS, progression-free survival.

to be associated with variations in VEGF expression in vitro. Among these SNPs, the –460T/C polymorphism is related to high VEGF expression levels.[12–14] Our previous studies also revealed that the VEGF –460T/C gene polymorphism is associated with the risk of NPC and lymphatic metastasis in the Chinese population.[8,9] Currently, the correlation between VEGF SNPs and NPC has been verified in a few studies. Wang et al[6] demonstrated that patients with NPC harboring the –2578CC genotype exhibited increased aggressiveness, large size, poor differentiation, and advanced stage of tumors compared with patients harboring the –2578A-allele. Nasr et al[7] found a significant association between the –2578C-allele carriers and aggressive forms of NPC, which were characterized by large tumors and advanced tumor stages. Furthermore, the VEGF SNPs influenced the prognosis and treatment toxicity in patients with different cancer types treated with CRT.[15–18] However, the association between VEGF –460T/C gene polymorphism and the clinical outcomes in NPC treated with IMRT has been rarely investigated.

In this study, a significant association was found between the –460C-allele carriers and aggressive forms of NPC, which were defined by the N2–3 stage (OR =1.820, 95% CI: 1.118–2.962, P=0.015). The VEGF –460T/C polymorphism

was significantly associated with 3-year OS, 3-year DMFS, and 3-year PFS (T/C + C/C vs T/T: 3-year OS 78.8% vs 95.1%, P=0.003; 3-year DMFS 80.2% vs 90.6%, P=0.036; 3-year PFS 73.9% vs 86.7%, P=0.042) but was not associated with LRFS. The VEGF –460C-allele carrier (T/C + C/C) was an independent significant prognostic factor for the 3-year OS according to the multivariate analysis results (HR 4.096, 95% CI: 1.333–12.591, P=0.014). Our results might support the correlation between the polymorphisms in the VEGF –460T/C gene and poor clinical outcomes of NPC. Our results on the VEGF –460T/C SNP are consistent with the findings of previous studies on different cancer types. An in vitro study demonstrated that the VEGF –460T/C SNP is located in the promoter region and may play a role in the promoter activity. Thus, the VEGF –460C-allele may increase VEGF expression and thus may promote abnormal tumor angiogenesis and growth.[13] High VEGF levels in the tumor microenvironment may cause rapid cancer progression and increased resistance of the tumors to radiotherapy and chemotherapy. Chen et al[12] reported that patients with colorectal cancer harboring the VEGF –460T/C and VEGF –460C/C genotypes had significantly higher circulating VEGF levels, more aggressive tumor behavior, and lower chemotherapy sensitivity and prognosis than those with the wild-type T/T genotype; furthermore, the VEGF –460T/C gene polymorphisms were considered

Table 7 The 3-year OS, LRFS, DMFS, and PFS in the VEGF −460T/C genotype subgroups after chemotherapy regimens

−460T/C genotype	3-year OS		P-value	3-year LRFS		P-value	3-year DMFS		P-value	3-year PFS		P-value
	NACT + CCRT	CCRT		NACT + CCRT	CCRT		NACT + CCRT	CCRT		NACT + CCRT	CCRT	
T/T	93.9%	95.9%	0.707	88.0%	96.1%	0.173	84.7%	96.2%	0.238	84.9%	87.9%	0.754
T/C	79.5%	74.6%	0.700	96.0%	94.7%	0.7815	81.8%	78.1%	0.587	63.3%	75.7%	0.420
C/C	85.7%	87.5%	0.881	85.7%	100.0%	0.285	85.7%	75.0%	0.962	85.7%	87.5%	0.922
T/C + C/C	80.9%	80.0%	0.720	93.6%	95.6%	0.760	83.1%	76.8%	0.597	68.4%	77.8%	0.469

Note: *P*<0.05 was considered statistically significant.
Abbreviations: CCRT, concurrent chemoradiotherapy; DMFS, distant metastasis-free survival; LRFS, local recurrence-free survival; NACT, neoadjuvant chemotherapy; OS, overall survival; PFS, progression-free survival; VEGF, vascular endothelial growth factor.

independent predictors of recurrence and prognosis in colorectal cancer. Masago et al[19] also reported that the VEGF −460CC genotype had a negative prognostic effect on the survival of patients with advanced-stage non-small-cell lung cancer. Further, the survival rates of patients with NPC and overexpressed VEGF in tumor tissues were significantly lower than those of the patients with low VEGF expression.[20] By contrast, Lv et al[21] reported that elevated serum VEGF expression in patients with NPC was closely associated with DMFS and OS but

was not significantly associated with LRFS. The correlation between VEGF and local recurrence of NPC after radiotherapy remains unclear. Radiotherapy could promote VEGF expression and enhance tumor angiogenesis, which may contribute to the radioresistance of NPC in mouse xenograft models. Therefore, radiotherapy combined with anti-VEGF therapy may effectively decrease radiation resistance.[22] Our study also showed no significant association between LRFS and VEGF −460T/C polymorphism. This finding mainly stems from the

Table 8 Multivariate analysis of OS, DMFS, LRFS, and PFS

Variables	HR	95% CI	P-value
Results of the multivariate analysis of OS			
Gender (female vs male)	1.149	0.412–3.205	0.790
Age (>45 vs ≤45 years)	0.967	0.379–2.466	0.944
T classification (T3–4 vs T2)	1.671	0.203–13.786	0.633
N classification (N2–3 vs N0–1)	2.490	0.840–7.377	0.100
Chemotherapy (CCRT vs NACT + CCRT)	1.169	0.450–3.036	0.749
VEGF −460T/C SNP (T/C + C/C vs T/T)	4.096	1.333–12.591	0.014*
Results of the multivariate analysis of DMFS			
Gender (female vs male)	1.169	0.422–3.241	0.764
Age (>45 vs ≤45 years)	0.667	0.254–1.747	0.409
T classification (T3–4 vs T2)	1.906	0.232–15.688	0.549
N classification (N2–3 vs N0–1)	3.674	1.144–11.792	0.029*
Chemotherapy (CCRT vs NACT + CCRT)	1.004	0.390–2.585	0.994
VEGF −460T/C SNP (T/C + C/C vs T/T)	2.442	0.913–6.531	0.075
Results of the multivariate analysis of LRFS			
Gender (female vs male)	0.848	0.203–3.543	0.821
Age (>45 vs ≤45 years)	1.338	0.367–4.872	0.659
T classification (T3–4 vs T2)	0.892	0.096–8.257	0.920
N classification (N2–3 vs N0–1)	2.449	0.600–10.002	0.212
Chemotherapy (CCRT vs NACT + CCRT)	0.434	0.114–1.654	0.222
VEGF −460T/C SNP (T/C + C/C vs T/T)	0.708	0.185–2.713	0.614
Results of the multivariate analysis of PFS			
Gender (female vs male)	0.922	0.404–2.101	0.846
Age (>45 vs ≤45 years)	1.210	0.581–2.518	0.610
T classification (T3–4 vs T2)	1.152	0.253–5.238	0.855
N classification (N2–3 vs N0–1)	2.189	0.967–4.956	0.060
Chemotherapy (CCRT vs NACT + CCRT)	0.763	0.360–1.616	0.480
VEGF −460T/C SNP (T/C + C/C vs T/T)	2.023	0.937–4.368	0.073

Note: *P*<0.05 was considered statistically significant.
Abbreviations: CCRT, concurrent chemoradiotherapy; CI, confidence interval; DMFS, distant metastasis-free survival; HR, hazard ratio; LRFS, local recurrence-free survival; NACT, neoadjuvant chemotherapy; OS, overall survival; PFS, progression-free survival; SNP, single-nucleotide polymorphism; VEGF, vascular endothelial growth factor.

following factors: first, despite more than 80% of the patients being at the T3–4 stage, the application of IMRT and combined CRT greatly improved the local control of the patients, resulting in 3-year LRFS of more than 90%. This effect might be a major reason that the VEGF SNP cannot attain significant effectiveness on LRFS. Second, we only focused on a single functional promoter VEGF SNP, and the results are not comprehensive. The result might have been influenced by the interference caused by the genetic linkages with other functional SNPs. Finally, we assumed that the VEGF –460T/C possessed some additional unknown biological functions. In summary, the VEGF –460T/C polymorphism may play a critical role in lymph node involvement, distant metastasis, and poor prognosis by promoting angiogenesis in NPC. The VEGF –460T/C polymorphism is thus a valuable prognostic marker for patients with NPC. However, the generalizability of our study is limited because we did not directly detect VEGF expression in tumor cells or evaluate the serum VEGF levels in the patients.

IMRT, which is widely used for patients with NPC, delivers a high radiation dose to tumors while maintaining a safe dose to normal tissues surrounding the tumor. This technique also exhibits excellent tumor coverage. Despite these advantages and the improved locoregional control with IMRT, patients are still at high risk of systemic failure and radioresistance.[10] Thus, a combined modality therapy is necessary. In our study, the VEGF –460C-allele carrier (T/C + CC) was regarded as an inferior prognostic factor of survival according to IMRT. As such, we evaluated the influence of combining NACT with CCRT. Our results showed that the combination could not prolong 3-year OS, LRFS, DMFS, and PFS of the patients relative to those treated with CCRT alone. NACT did not improve the survival of the VEGF –460C-allele carriers in NPC, and the role of NACT remains unclear. Song et al[23] also reported that the application of NACT followed by CCRT did not show superior effectiveness, compared with CCRT alone, in patients with NPC because NACT can increase the risk of locoregional recurrences. Qiu et al[24] found that survival rates of patients treated with combined NACT and IMRT were not significantly different from those treated with CCRT plus adjuvant chemotherapy (AC). Meta-analysis results confirmed that integrating NACT to CCRT significantly improved PFS and OS, in contrast to CCRT with or without AC in locoregionally advanced NPC; however, an increased chance of developing acute toxicity, such as grade 3–4 anemia, thrombocytopenia, leukopenia, and fatigue, was observed.[25] Recently, a combined radiotherapy and antiangiogenic therapy has been proposed and considered as a promising method for treatment

of NPC. On the one hand, the proliferation and metastasis of tumor cells rely on angiogenesis induced by VEGF. On the other hand, rapidly growing tumors cause hypoxia, which upregulates VEGF, thereby promoting tumor proliferation, angiogenesis, and increased radioresistance. Thus, treatment that targets tumor angiogenesis can modulate the tumor microenvironment and thus can improve tumor blood flow and oxygenation, leading to enhanced radiosensitivity.[5] Bevacizumab, a recombinant humanized monoclonal antibody against VEGF, has progressed into clinical trials for different tumor types, and has a promising future.[26–28] Some studies also demonstrated that antiangiogenic therapy can normalize tumor vasculature and enhance radiation responses in xenografted human NPC models.[29,30] The therapies that combine antiangiogenic therapy and radiotherapy might be promising strategies to inhibit angiogenesis and prevent adverse NPC outcomes. The result of our study must be viewed cautiously because of some limitations. Particularly, the number of participants was not sufficient, and the details of the underlying mechanisms were not investigated. Thus, our results must be validated and analyzed using larger sample sizes.

Conclusion

This study demonstrated that the VEGF –460T/C polymorphism was associated with poor clinical outcomes in patients with NPC. The VEGF –460T/C polymorphism may be a potential prognostic indicator for patients with NPC and a promising target for treatment. Further studies must determine the effect of VEGF gene polymorphisms associated with combined modality therapy on NPC.

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Author contributions

Yong Zhang, Li Jiang, and Junyin Tan conceived and designed the study. Xiaowei Cheng, Chunlin Wang, Jingshan Chen, and Xiaoqing Huang performed the experiments. Dongmei Xia, Peng Xie, and Rensheng Wang analyzed the data. Junyin Tan, Li Jiang, and Yong Zhang wrote the paper. All authors contributed toward data analysis, drafting and critically revising the paper, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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