ORIGINAL ARTICLE

Observational Study

Prognostic significance of vascular endothelial growth factor polymorphisms in colorectal cancer patients

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Abstract

AIM
To investigate the associations of the genetic polymor-

phisms of vascular endothelial growth factor A (*VEGF-A*) -1498C>T and -634G>C, with the survival of patients with colorectal cancer (CRC).

METHODS
A prospective cohort consisting of 131 Brazilians patients consecutively operated on with a curative intention as a result of sporadic colorectal carcinoma was studied. DNA was extracted from peripheral blood and its amplification and allelic discrimination for each genetic polymorphism was performed using the technique of polymerase chain reaction (PCR) in real-time. The real-time PCR technique was used to identify the *VEGF-A* -1498C>T (rs833031) and -634G>C (rs2010963) polymorphisms. Genotyping was validated for *VEGF-A* -1498C>T polymorphism in 129 patients and for *VEGF-A* -634G>C polymorphism in 118 patients. The analysis of association between categorical variables was performed using logistic regression, survival by Kaplan-Meier method and multivariate analysis by the Cox regression method.

RESULTS
In the univariate analysis there was a significant association (OR = 0.32; *P* = 0.048) between genotype CC of the *VEGF-A* -1498C>T polymorphism and the presence of CRC liver metastasis. There was no association between *VEGF-A* -1498C>T polymorphism and *VEGF-A* -634G>C polymorphism with further clinical or anatomopathologic variables. The genotype CC of the *VEGF-A* -1498C>T polymorphism was significantly correlated with the 5-year survival (*P* = 0.032), but not significant difference (*P* = 0.27) was obtained with the *VEGF-A* -634G>C polymorphism with the 5-year survival in the univariate analysis. The genotype CT (HR = 2.79) and CC (HR = 4.67) of the polymorphism *VEGF-A* -1498C>T and the genotype CC (HR = 3.76) of the polymorphism *VEGF-A* -634C>G acted as an independent prognostic factor for the risk of death in CRC patients.

CONCLUSION
The CT and CC genotypes of the *VEGF-A* -1498C>T and the CC genotype of the *VEGF-A* -634C>G polymorphisms are prognostic factors of survival in Brazilians patients with sporadic colorectal carcinoma.

Key words: Colorectal cancer; Genetic polymorphisms; Vascular endothelial growth factor-A; Colorectal surgery; Genetic variation

Core tip: Vascular endothelial growth factor A (VEGF-A) affects the tumor biological behavior and phenotype. An applied research with relevant achievement that will possibly be favored by such information is the pharmacogenetics impact of *VEGF-A* polymorphisms. VEGF-A is a significant goal in the anticancer therapy and results about *VEGF-A* polymorphisms may enhance the targeted therapies. This approach will be of great help to the suitability of individual therapies and improve the quality of post operative treatment. Moreover, since polymorphisms often show a discrepancy between ethnic groups, more studies are also warranted to clarify the association between the *VEGF-A* polymorphisms and the CRC in diverse ethnic populations.

INTRODUCTION

Colorectal cancer (CRC) is one of the most common malignancies in the world. Despite advances in diagnostic and treatment modalities, patients still face a poor prognosis, and a more individualized treatment approach appears necessary[1-4].

Unlike genetic mutations, polymorphisms represent variations of the naturally occurring DNA sequence. Polymorphisms are found in at least 1% of the healthy population[5]. The vast majority (90%) of DNA polymorphisms is single nucleotide (SNPs)[6,7] and functionally neutral. However, certain polymorphisms have effects on the regulation of gene expression or on the function of the encoded protein[8] and thereby influence the susceptibility and severity of the disease[9,10]. Thus, the polymorphisms may modify the route of angiogenesis and the susceptibility and severity of malignancies[11].

Angiogenesis is a sequence of processes starting with vessel dilatation and pericyte recruitment in the pre-existing vessels, followed by endothelial cell proliferation, formation of new vessels and recruitment of perivascular cells[9]. In the progress toward malignancy, the normal cells must switch to an angiogenic phenotype to attract the nourishing vasculature that they depend on for their growth[9,11].

Malignant tumors depend on angiogenesis for their growth and metastasis[9,11]. It is generally assumed that microvessel formation around the tumor is stimulated by various angiogenic growth factors secreted by the tumor cells[12]. Among them, the vascular endothelial growth factor (VEGF), one of the most potent endothelial cell mitogens, is considered one of the strongest promoters of angiogenesis in CRC[11,12]. The VEGF is vital for the invasion and metastasis of neoplasms through the formation of new blood vessels from mature endothelial cells[13,14]. Moreover, studies have shown that the genetic polymorphisms can be used to predict the clinical outcomes of gastrointestinal[14,15], breast[16], ovary[17] and pancreatic cancer[18].

The cancer targeted therapy with the use of antiangio-

genic agents is aimed at inhibition of angiogenic function and tumor dissemination since neoangiogenesis stimulates the growth and invasion of adjacent tissues by tumor cells[9,12]. On the other hand, the inhibition of VEGF limits the tumor growth[9,13]. In colorectal carcinoma, VEGF levels are increased and they are related to further spread and poor prognosis[12,14]. The antiangiogenic agent bevacizumab is a humanized monoclonal immunoglobulin G antibody against recombinant VEGF activity and it is used in the treatment of metastatic CRC[13,14].

Clinical studies have shown that an association between the level of *VEGF* expression and increased microvessel density in tumors is correlated with an advanced stage of CRC, and with shorter survival. Therefore, it is important to determine the presence of metastasis, and patients with CRC and *VEGF* over-expression have higher tumor progression and poor prognoses[19-21]. Increased *VEGF* expression in CRC may predict the risk of multiple liver metastasis and play a role in the spread of CRC cells to the lymph nodes[22]. Due to these properties, VEGF has been used as a therapeutic target for the creation of anticancer drugs and is considered a potential prognostic marker of CRC[13,23].

The *VEGF-A* gene is located on chromosome 6p21.3 and consists of 8 exons separated by 7 introns that exhibit alternative splicing to form a family of proteins[24]. This gene is a member of the platelet-derived growth factor/*VEGF* family and encodes a protein that is often found as a disulfide-linked homodimer.

The human *VEGF-A* gene is highly polymorphic, with more than 15 SNPs described[24,25], thus enabling wide variation in its expression between individuals from different ethnic groups, and there are few studies involving Latinos, Hispanics[20], and particularly Brazilians.

VEGF-A is a dimeric glycoprotein and is considered to be the main, dominant inducer of the growth of blood vessels. VEGF-A is essential for adults during organ remodeling and diseases that involve blood vessels in wound healing, tumor angiogenesis, diabetic retinopathy and age-related macular degeneration.

The -634G>C genetic polymorphism in the promoter region and the -1498C>T genetic polymorphism in the 3'-untranslated region were found to be associated with variations in VEGF-A protein synthesis[24,26,27]. Actually, the *VEGF-A* -634G/C polymorphism appears to be associated with a higher *VEGF-A* expression[28,29].

The participation of common genetic polymorphisms of *VEGF-A* in the prognoses of patients with CRC is not yet clearly established[19,20,23,25,29]. Furthermore, studies investigating the association between *VEGF-A* genetic polymorphisms and CRC risk report conflicting results[30] and the specific associations still remain controversial[31].

Since *VEGF-A* is known to be a potent pro-angiogenic factor, we evaluated the potential association of two *VEGF-A* genetic polymorphisms (-634G>C and -1438C>T) with the clinicopathologic variables and its possible implication for prognosis in a population of Brazilian patients operated on CRC.

MATERIALS AND METHODS

Study design and sample
The present study was conducted according to the ethical principles of the Declaration of Helsinki from the World Medical Association and has been approved by the Institutional Research Ethics Committees.

The study was conducted as a prospective cohort study (observational study) of 131 adult patients of both genders with CRC, without a distinction of ethnicity and operated on consecutively with a curative intention in the period from 2008 to 2011.

The patients were 80 (61.1%) males and 51 (38.9%) females, with a mean age of 58.3 ± 12.5 years (20 to 85 years) and median age of 58 years. Regarding the ethnicity, 121 (92.4%) patients were white and 10 (7.6%) afrodescendants.

The following patients were excluded from the study: Those with familial adenomatous polyposis, colorectal neoplasia other than carcinoma, inflammatory bowel disease, other hereditary CRC syndromes, submitted to neoadjuvant treatment or with synchronous/metachronous tumors elsewhere, except for basal cell carcinoma of skin, and those who were impossible to contact, whether the patients or the patient's relatives, to obtain the necessary information.

Collection and processing of biological material
Peripheral blood samples were collected by venipuncture into tubes containing 0.1% EDTA and kept in the refrigerator at 4 ℃ for up to 48 h. If DNA extraction did not take place within this period, the samples were frozen for a maximum of seven days at a temperature of -20 ℃. DNA extraction was performed using the method of salting out and storing the material in a freezer at -80 ℃.

Surgery data
Regarding the anatomical distribution of the tumors, 98 (74.8%) was found in the colon: 41 (31.3 %) in the right colon, 7 (5.3%) in transverse and 50 (38.2%) in the left colon. In the remaining 33 (25.2%) patients, the tumors were located in the rectum. A right colectomy was performed in 42 (32.1%) cases, a left colectomy in 45 (34.3%), rectosigmoidectomy in 41 (31.3%) and an abdominoperineal resection in 3 (2.3%).

Genotyping
The real-time PCR technique was used to identify genetic polymorphisms in *VEGF-A* (rs833061 and rs2010963) genes. DNA was amplified using Taqman assays C_10 (rs833061) and C_10 (rs2010963 (Applied Biosystems, Life Technologies Corporation, Foster City, CA, United States) and VIC/FAM dyes (FAM™/ROX™ and VIC®/ROX™ Dye Normalization Plates, Applied Biosystems, Life Technologies Corporation, Foster City, CA,

United States). The dye was used as ROX™ passive reference. In each PCR reaction, the following were used: 10 μL of TaqMan Universal Master Mix Ⅱ (Applied Biosystems, Life Technologies Corporation, Foster City, CA, United States), 1.0 μL of TaqMan Pre-Designed SNP Genotyping Assays 20 × solution (Applied Biosystems, Life Technologies Corporation, Foster City, CA, United States), 30-50 ng of genomic DNA and a final volume of 20 μL of Nuclease-Free Water (Promega, Madison, WI, United States). The equipment used for amplification and allelic discrimination was the Fast ABI-7500 (Applied Biosystems, Life Technologies Corporation, Foster City, CA, United States). Protocols for genotyping were performed according to the manufacturer involving the cycling amplification for 10 min at 95 ℃ and then 40 cycles for 15 s at 92 ℃ and for 1 min at 60 ℃. At that time, there was the post-run for 1 min allele determination. Negative controls (no template control – NTC) were used, results were analyzed and 10% of all samples were genotyped more than once to ensure there was no contamination. Genotyping was validated for *VEGF-A* -1498C>T polymorphism in 129 patients and for *VEGF-A* -634G>C polymorphism in 118 patients.

Statistical analysis

The estimation of the sample power, calculated as the ability to detect a hazard ratio (HR) ⩾ 1.7 with an alpha value of 0.05 and a statistical power of 80% provided a minimum sample size of 112 individuals. The estimation was performed using the Stata 11.0 (Stata Statistical Software, StataCorp LP, College Station, TX, United States).

The variable survival was well-defined as the time elapsed between the date of the surgery and the event of interest represented by the patient's death by CRC. Operationally, this variable is composed of the duration of the follow-up after the operation that was previously established with a maximum duration of 60 mo or the occurrence of death. The clinical variables analyzed were gender, age and presence of metastasis and/or relapse after the operation.

The analyzed histopathological variables were the anatomical site of the CRC in the large intestine, adjacent invasion, degree of cellular differentiation, venous/lymphatic/perineural invasion, and staging of the neoplasia according to the 2010 TNM staging system[32].

Patients who survived until the end of the study follow-up (60 mo) were considered "censored". In cases in which the event of interest has not occurred after exceeding the maximum observation period of the study (60 mo), loss of observation or occurrence of death were also considered "censored".

The univariate analysis was made by means of a log-rank test. The significant statistical level was considered as 5% (*P* < 0.05).

The Cox regression analysis was used to identify the independent effect of the prognostic factors (independent variables). For the selection of independent variables to be included in the multivariate models of Cox regression,

Table 1 Frequency of vascular endothelial growth factor A -1498C > T and vascular endothelial growth factor A -634G > C genetic polymorphisms in blood samples of patients with resected colorectal carcinoma

Polimorphism	Genotype	*n* (%)
VEGF-A -1498C>T (*n* = 129)	CC	27 (20.9)
	CT	58 (44.9)
	TT	44 (34.1)
VEGF-A -634G>C (*n* = 118)	CC	26 (22.0)
	CG	42 (35.6)
	GG	50 (42.4)

VEGF-A: Vascular endothelial growth factor A.

each individual variable in the Cox model was tested and the following criteria for inclusion in the multivariate model were verified: Variables with a descriptive level of significance lower than 20% (*P* < 0.20) in the univariate model and the genetic variations (polymorphisms) used in this study. For the selection of a final model, the automatic selection backwards in Stata 11.0 (Stata Statistical Software, StataCorp LP, College Station, TX, United States) was used.

RESULTS

The follow-up period of 131 patients ranged from 1.8 to 60 mo, with a mean of 33.8 (± 21.9) mo and a median of 34.0 mo. Liver metastasis was found in 26 (19.1%) patients. The involvement of regional lymph nodes occurred in 63 (48.1%) patients and the invasion of adjacent organs was found in 26 (19.8%) cases. At the end of this follow-up period, 70 (53.4%) patients were alive without the disease, 14 (10.7%) were alive with the disease, 42 (32.1%) died with CRC, 3 (2.3%) had died of causes unrelated to CRC and 2 (1.5%) were lost in the follow-up. Thus, censures occurred in 84 (67.9%) patients with a complete observation after 60 mo. The estimate of overall survival at 5 years was 60.7%, with an average of 45.2% (95%CI: 41.5 to 49.0) and a median of 33.0%.

The results of the genotypes' frequency of the *VEGF-A* -1498C>T and *VEGF-A* -634G>C genetic polymorphisms are shown in Table 1. The stages of the CRC and their respective polymorphisms of *VEGF* -634G>C and the *VEGF* -1498C>T genes are described in Tables 2 and 3.

In the univariate analysis there was a significant association (OR = 0.32; *P* = 0.048) between genotype CC of the *VEGF-A* -1498C>T polymorphism and the presence of CRC liver metastasis. There was no association between *VEGF-A* -1498C>T polymorphism with further clinical or anatomopathologic variables (Table 4). The *VEGF-A* -634G>C polymorphism showed no significant association between clinical or anatomopathologic variables in the univariate analysis.

Following the previously established criteria, the genotype CC of the *VEGF-A* -1498C>T polymorphism was significantly correlated with the 5-year survival (*P*

Table 2 TNM staging of colorectal tumors and their respective polymorphisms of vascular endothelial growth factor -634G > C gene (n = 118)

Stage (n)	C/C n (%)	G/C n (%)	G/G n (%)
I + II (55)	10 (18.2)	22 (40.0)	23 (4I.8)
III + IV (63)	16 (25.4)	20 (31.7)	27 (42.9)
Total	26 (22.0)	42 (35.6%)	50 (42.4)
P	-	0.27	0.53
OR (95%CI)	1	0.56 (0.21-1.53)	0.73 (0.27-1.92)

Cox multiple regression test. OR: Odds ratio.

Table 3 TNM staging of colorectal tumors and their respective polymorphisms of vascular endothelial growth factor -1498C > T gene (n = 129)

Stage (n)	C/C n (%)	G/C n (%)	G/G n (%)
I + II (61)	20 (32.8)	9 (14.8)	32 (52.5)
III + IV (68)	24 (35.3)	18 (26.5)	26 (38.2)
Total	26 (22.0)	42 (35.6)	50 (42.4)
P	-	0.31	0.33
OR (95%CI)	1	1.66 (0.61-4.51)	0.67 (0.30-1.48)

Cox multiple regression test. OR: Odds ratio.

= 0.032) (Table 5), but not significant difference (*P* = 0.27) was obtained in relation to the *VEGF-A* -634G>C polymorphism with the 5-year survival in the univariate analysis.

In the multivariate analysis, the genotypes CT (HR = 2.79; 95%CI: 1.01-7.66) and CC (HR = 4.67; 95%CI: 1.51-14.43) of *VEGF-A* -1498C/T polymorphism and the genotype CC (HR = 3.76; 95%CI: 1.29-10.93) of *VEGF-A* -634C>G polymorphism were associated with a reduced 5-year survival (Table 6; Figures 1 and 2). The *VEGF-A* -1498C/T and the *VEGF-A* -634G>C polymorphisms showed no significant association between clinical or anatomopathologic variables in the multivariate analysis.

DISCUSSION

The selection of studies was carried out by publications on the participation of these SNPs in CRC[25-30]. Due to involvement of angiogenesis in neoplasms, *VEGF-A* may influence the biology and the phenotype of the CRC[33-36]. The results regarding the association of polymorphisms of *VEGF-A* with CRC prognosis are controversial[13,29,36-38].

In the present analysis, we examined whether two common *VEGF-A* -1498C>T and *VEGF-A* -634G>C polymorphisms were related to prognoses and clinico-pathologic features of CRC patients whose tumors had been surgically resected with curative intent.

We used blood samples of patients with colorectal carcinoma because the majority of polymorphism analyses have been carried out on germline DNA extracted from peripheral blood as it is easily obtained

Table 4 Univariate analysis of vascular endothelial growth factor A -1498C > T genetic polymorphism and the presence of liver metastasis in patients with resected colorectal carcinoma (n = 129)

Liver metastasis	Genotype TT n (%)	Genotype CC n (%)	Genotype CT n (%)
Yes (25)	7 (28.0)	10 (40.0)	8 (32.0)
No (104)	37 (35.6)	17 (16.3)	50 (48.1)
P	0.34	0.048	0.765
OR (95%CI)	1	0.32 (0.10-0.98)[1]	1.18 (0.39-3.55)

[1]Significant. OR: Odds ratio.

Table 5 Univariate analysis of vascular endothelial growth factor A -1498C > T genetic polymorphism and 5-year survival in patients with resected colorectal carcinoma (n = 129)

Polymorphism	Genotype	n (%)	5-yr survival (%)	P
VEGF-A -1498C>T (n = 129)	CC	27 (20.9)	46.4	0.032[1]
	CT	58 (44.9)	61.7	
	TT	44 (34.1)	67.1	

[1]Significant Mantel-Cox log-rank test.

Table 6 Multivariate analysis of vascular endothelial growth factor A -1498C > T genetic polymorphism (n = 129) and vascular endothelial growth factor A -634C > G (n = 118) genotypes and 5-year survival in patients with resected colorectal carcinoma

Variables	Category	HR (95%CI)	P
VEGF-A -1498C>T	TT	1 (reference)	-
	CT	2.79 (1.01- 7.66)	0.047[1]
	CC	4.67 (1.51-14.43)	0.007[1]
VEGF-A -634C>G	GG	1 (reference)	-
	CG	1.44 (0.58-3.55)	0.433
	CC	3.76 (1.29-10.93)	0.015[1]

[1]Significant Cox multiple regression test.

and generates large amounts of high quality DNA. Fixation in paraffin-embedded tissue can cause cross-linking and damage of DNA isolated damaging the amplification reaction and primer pattern recognition, besides mutation artifacts, *e.g.*, artificial C-T or G-A transitions[39].

The results of the present study did not support an association of the *VEGF-A* -1498C>T and *VEGF-A* -634G>C polymorphisms with tumor size, histological grading, tumor stage, lymph node metastasis and age at diagnosis in CRC cases. In the same way, Hofmann *et al*[28] and Dassoulas *et al*[36] found no correlation between the *VEGF-A* -634G>C and *VEGF-A* -1498C>T polymorphisms and the tumor characteristics. On the other hand, Chae *et al*[40] showed that the T allele was related to the advanced stage of CRC.

Kim *et al*[29] reported that -634GC and -634CC genotypes were associated with a favorable prognosis compared to -634GG genotype *VEGF-A* polymorphism
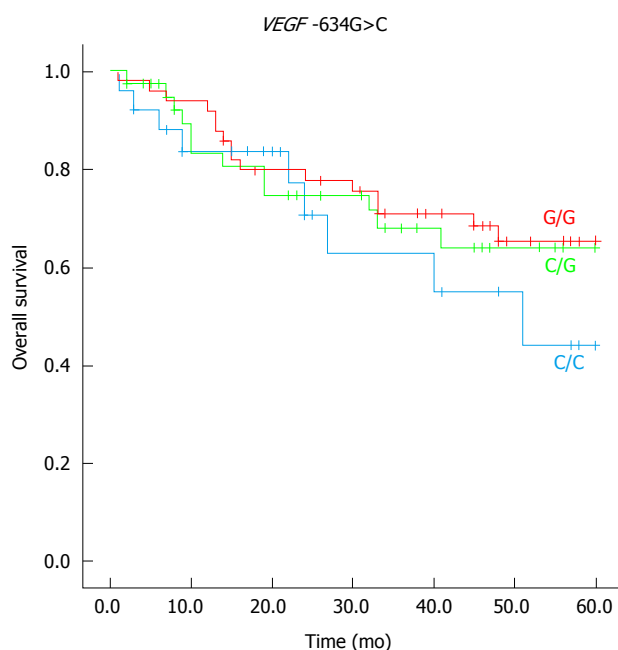
Figure 1 The Kaplan-Meier survival curves as a function of vascular endothelial growth factor A-1498C>T genetic polymorphism genotypes in colorectal cancer patients. VEGF: Vascular endothelial growth factor.
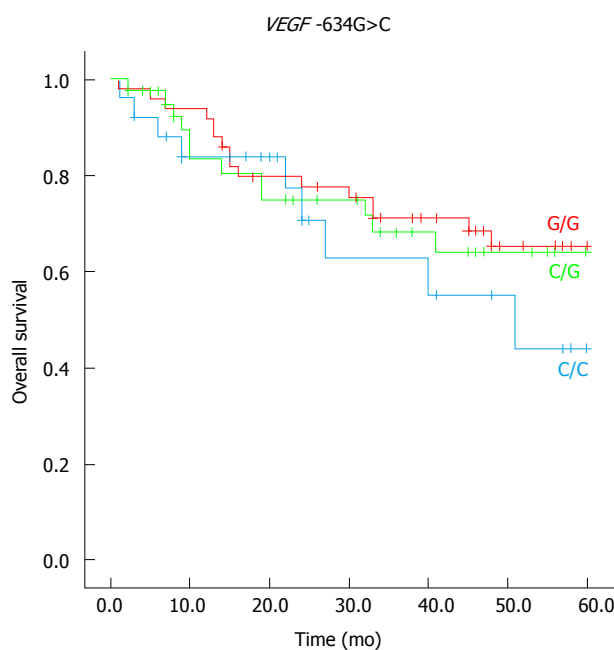

Figure 2 The Kaplan-Meier survival curves as a function of vascular endothelial growth factor A -634G>C genetic polymorphism genotypes in colorectal cancer patients. VEGF: Vascular endothelial growth factor.

in CRC patients. Particularly, these authors reported that -634G>C *VEGF-A* polymorphism is an independent prognostic factor for CRC. Watson *et al*[24] observed that there is a significant correlation between production of VEGF-A protein from peripheral blood mononuclear cells and *VEGF* -634G-A>C polymorphism. These authors also reported the decreased production of VEGF-A protein in patients with homozygotes CC *VEGF-A* gene and increased production of VEGF-A protein in homozygotes GG *VEGF-A* genes. In the present series, the CC genotype of *VEGF-A* -634C>G polymorphism was significantly related to survival in patients with resected colorectal carcinoma. In accordance with Kim *et al*[29], our results pointed out that 634C>C polymorphism was an independent prognostic factor and it was associated with a worse 5-year survival rate compared to the *VEGF-A* GC genotype. Dassoulas *et al*[36] analyzed DNA extracted from paraffin-embedded tissue from 312 Greek patients with CRC in all stages and evaluated the prognostic value of five *VEGF-A* polymorphisms, including the - 634G>C and - 1498C>T polymorphisms. They reported that -634 CC genotype was associated with a poor prognosis in the Greek population, which agree with the results we found with Brazilian patients in the present series. Dassoulas *et al*[36] concluded that, in Greek patients with CRC, *VEGF-A* - 634G>C and - 1498C>T, polymorphisms were independent markers of prognosis. Hansen *et al*[41] demonstrated obvious relationships between genetic variations in the *VEGF-A* gene and response to first-line capecitabine in patients with metastatic colorectal cancer, which translated to a significant difference in progression-free survival.

Chae *et al*[40] analyzed the associations of *VEGF-A* -634G>C polymorphism in patients with CRC. These

authors observed that there was no significant correlation between the genotype GC with TNM stage Ⅲ/Ⅳ, lymph node involvement and distant metastasis in CRC. On the other hand, Jang *et al*[42] genotyped the *VEGF-A* -634G>C polymorphism in 350 CRC cases from the Korean population. The results suggest that this genetic polymorphism variant is not a potential genetic marker for CRC prognosis.

However, Hansen *et al*[41] reported opposite results. The authors studied CRC in Danish patients with stages Ⅱ and Ⅲ and found that -634GC heterozygote genotype exhibited lower free disease and survival rates compared to the corresponding wild-type homozygote genotypes. This result was the opposite of what we found in the present study.

Kjaer-Frifeldt *et al*[43] found in a multivariate analysis that *VEGF-A* -1498C>T and *VEGF-A* -634G>C polymorphisms were independent prognostic factors for the risk of death of patients by CCR, as we observed in our cases.

The difference between the results of these studies is not sufficiently clear. The discrepancy between the studies of *VEGF-A* polymorphism and CRC prognosis can be attributed to the differences in disease status, race and size of the sample studied[29,36]. Another possible explanation for these results is the DNA sequence variations in the *VEGF-A*, variation in the gene *locus*, action by several other genes and environmental characteristics. All these variables may alter VEGF-A production and/or activity, thereby causing inter-individual differences in the lymphangiogenesis and lymphatic tumor spread and, thus, in the development and progression of the tumors[29,36,39]. The differential role that individual polymorphisms of *VEGF-A* may play

in the biological activity of VEGF-A protein secreted by intratumoral variability of *VEGF-A* genetic expression is enhanced by these findings. In a large meta-analysis, involving 27 studies Des Guetz *et al*[19] demonstrated that *VEGF-A* overexpression is significantly correlated with poor overall survival and with an increased risk of relapse in CRC patients.

The differences between the results of published studies can be attributed to the different sources of DNA, the different ethnic backgrounds of the patients studied, the number of patients tested, the different designs of the studies, a lack of prospective randomized trials, laboratory tests, numerous genetic polymorphisms and errors in the interpretation of results[44-46].

In summary, in the univariate analysis we found an association between genotype CC of the *VEGF-A* -1498C>T polymorphism and the occurrence of hepatic metastasis of CRC. In the multivariate analysis, genotypes CT and CC of *VEGF-A* -1498C>T polymorphism and genotype CC of *VEGF-A* -634C>G genetic polymorphism are independent prognostic factors for the risk of death in Brazilian patients with sporadic CRC.

The study of *VEGF-A* polymorphisms can bring new impacts on pharmacogenetics as VEGF-A is an important target in antineoplastic therapy, and the results of the *VEGF-A* polymorphisms may enhance the targeted therapies. This approach will be of great help to physicians in terms of tailoring individual therapies and enhancing the quality of patients' postoperative treatment. Moreover, since genetic polymorphisms often show a discrepancy between ethnic groups, more studies are also warranted to clarify the association between the *VEGF-A* polymorphisms and the CRC in diverse ethnic populations.

In conclusion, our data suggested that the CT and CC genotypes of the *VEGF-A* -1498C>T polymorphisms and the CC genotype of the *VEGF-A* -634C>G polymorphisms are independent prognostic factors for the risk of death in Brazilian patients with sporadic colorectal carcinoma.

COMMENTS

Background

Polymorphisms are naturally occurring DNA sequence variations, which differ from the gene mutations. The functional polymorphisms could contribute to the difference between individuals according to the susceptibility and severity of diseases. Polymorphisms alone or in combination with environmental factors may affect the angiogenic pathway and, thereby, the susceptibility and severity of cancer. The vascular endothelial growth factor (VEGF), one of the most potent endothelial cell mitogens, is considered one of the strongest promoters of angiogenesis in colorectal cancer (CRC). Given these characteristics, VEGF is a potential marker for determining the prognosis of CRC and it has also been used as a therapeutic target for the new molecular anticancer drugs such as bevacizumab. *VEGF-A* is considered to be the main, dominant inducer of the growth of blood vessels. The *VEGF-A* -634G/C polymorphism appears to be associated with a higher *VEGF-A* expression.

Research frontiers

Studies have shown that the genetic polymorphisms can be used to predict the clinical outcomes of gastrointestinal, breast, ovary and pancreatic cancers. The human *VEGF-A* gene is highly polymorphic, with more than 15 single nucleotide polymorphisms (SNPs) described, thus enabling wide variation in its expression between individuals from different ethnic groups, and there are few studies involving Latinos, Hispanics, and particularly Brazilians.

Innovations and breakthroughs

The contribution of common *VEGF-A* genetic polymorphisms to the CRC prognosis remains unclear. Furthermore, studies investigating the association between *VEGF-A* genetic polymorphisms and CRC risk reporting conflicting results and the specific associations still remain controversial. Because VEGF-A is known to be a potent proangiogenic factor, the authors evaluated the potential association of two *VEGF-A* genetic polymorphisms (-634G>C and -1438C>T) with the clinicopathologic variables and its possible implication for prognoses in a population of Brazilian patients who had surgical procedures to remove CRC. The authors found an association between genotype CC of the *VEGF-A* -1498C>T genetic polymorphism and the occurrence of hepatic metastasis of CRC. Moreover, genotypes CT and CC of *VEGF-A* -1498C>T genetic polymorphism and genotype CC of *VEGF-A* -634C>G genetic polymorphism were independent prognostic factors for the risk of death in Brazilian patients with sporadic CRC.

Applications

An important translational research field that will benefit from this knowledge is the pharmacogenetics field, in which researches study the impact of *VEGF-A* polymorphisms. VEGF-A protein is an important target in anticancer therapy and findings about *VEGF-A* polymorphisms may enhance the targeted therapies. This approach will be of great help to physicians in terms of tailoring individual therapies and enhancing the quality of patients' postoperative treatments.

Terminology

Polymorphism is the occurrence of two or more clearly different forms or alternative phenotypes in the population of a species. Genetic polymorphism is the occurrence together in the same population of two or more genetically determined phenotypes in such proportions that the rarest of them cannot be maintained merely by recurrent mutation. Most genetic polymorphisms are functionally neutral, but some have effects on the regulation of the gene expression or on the function of the coded protein. SNPs are a genetic polymorphism between two genomes that is based on substitution, deletion, insertion, or exchange of a single nucleotide. Angiogenesis is a sequence of processes starting with vessel dilatation and pericyte recruitment in the pre-existing vessels, followed by endothelial cell proliferation, formation of new vessels, and recruitment of perivascular cells.

Peer-review

This is an excellent article and the findings will definitely add to the existing knowledge.

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