GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

3'-UTR polymorphism (rs10434) in the *VEGF* gene is associated with B-CLL in a Chinese population

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Genet. Mol. Res. 14 (2): 4085-4089 (2015)
Received July 29, 2014
Accepted November 24, 2014
Published April 27, 2015
DOI http://dx.doi.org/10.4238/2015.April.27.23

ABSTRACT. We investigated the relationship between a *VEGF* genetic polymorphism and B cell chronic lymphocytic leukemia (B-CLL). A total of 102 patients with B-CLL and 124 healthy subjects were included in this study. All individuals were typed for the rs10434 in the vascular endothelial growth factor (VEGF) gene using the TaqMan technique. We found that the A allele and the AA genotype of rs10434 were more frequent in B-CLL patients than in control subjects (0.54 *vs* 0.34; 27 *vs* 13%; respectively). VEGF alleles and genotypes segregated similarly in patients at different disease stages according to Rai classification. These results suggest a possible association between the VEGF polymorphism and high-risk B-CLL.

Key words: B cell chronic lymphocytic leukemia; VEGF polymorphism; Disease progression

INTRODUCTION

There is strong evidence that altered immunological function is associated with an increased risk of lymphoma. The primary mechanism of an anti-tumor response depends on T-cell activation. Additionally, dysregulation of angiogenesis occurs in various pathologies and is one of the hallmarks of cancer. The importance of this biological process in normal hematopoietic cell development and the pathophysiology of several malignancies have been reported for B cell chronic lymphocytic leukemia (B-CLL) (Aguayo et al., 2000; Shanafelt and Kay, 2006; Ghosh et al., 2010). Patients with CLL have been demonstrated to have detectable levels of plasma and cellular pro- and anti-angiogenic cytokines, as well as abnormal neovascularization in the marrow and lymph nodes (Chen et al., 2000; Smolej et al., 2005; Frater et al., 2008).

Angiogenesis has been established as an important factor in human carcinogenesis, influencing tumor growth and invasion (Folkman, 1995). Vascular endothelial growth factor (VEGF), an important pro-angiogenic molecule, has been shown to parallel selective steps of tumor growth and the development of metastases (Carmeliet, 2005; Kaplan et al., 2005) through a direct autocrine effect on tumor cells (Lichtenberger et al., 2010). Breast and gynecologic cancer are among the best-known malignancies involving lymphangiogenesis, which is the recruitment of blood and lymphatic vessels, to a growing tumor (Schoppmann et al., 2002). This has been confirmed by previous studies demonstrating that VEGF plays an important role in the development of breast (Berezov et al., 2009), reproductive organ (Mazurek et al., 2004; Dobrzycka et al., 2011; Piastowska-Ciesielska et al., 2012), and ovarian cancer (Hefler et al., 2006; Sadłecki et al., 2011), as well as lung (Carrillo-de Santa Pau et al., 2010), colon (Bunger, 2011), and gastric (Hălmaciu et al., 2012) cancer.

In the present study, we investigated the relationship between a genetic polymorphism in VEGF and B-CLL.

MATERIAL AND METHODS

Patients and controls

A total of 102 patients aged 31-81 years (median 64 years) with B-CLL were included in the study. B-CLL was diagnosed according to defined clinical, morphological, and immunological criteria. All patients gave informed consent prior to participating in the study. The study was approved by the appropriate Ethics Committee. According to the modified Rai classification (Hallek et al., 2008), there were 27, 38, and 17 patients in stages 0, I, and II of the disease, respectively. The other 20 patients presented more advanced disease: 11 and 9 patients were in stages III and IV, respectively. In addition, 124 healthy individuals of both genders served as a control group.

VEGF genotyping

As shown in Figure 1, there are 452 single nucleotide polymorphisms (SNPs) for the human *VEGF* gene listed in the National Center for Biotechnology Information SNP database (http://www.ncbi.nlm.nih.gov/SNP). We selected the tagging SNPs based on previous studies (Xiang et al., 2009). Briefly, using the Haploview 4.2 software and the HapMap phrase II database, we identified 1 tagging SNP (rs10434) for Chinese Han using minor allele frequency

≥0.10 and linkage disequilibrium patterns with $r^2 \geq 0.6$ as a cutoff. DNA was isolated from whole blood using a Qiagen DNA Isolation Kit (Qiagen GmbH, Hilden, Germany). *VEGF* alleles were detected using the TaqMan assay as described previously (Tung et al., 2014).

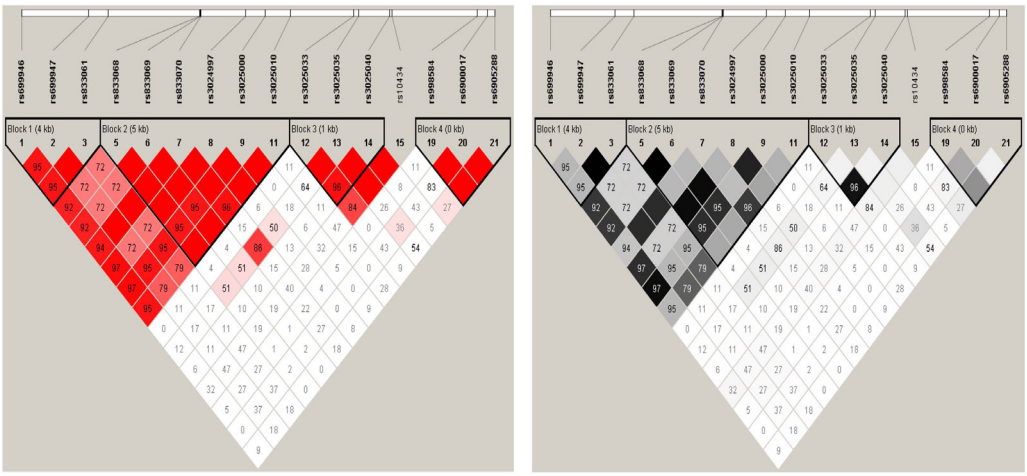

Figure 1. Genetic variation at the human *VEGF* gene. Using the Haploview 4.2 software and the HapMap phrase II database, we scanned 21 genotyped single nucleotide polymorphisms in Chinese Han. Linkage disequilibrium blocks across the locus in Chinese Han.

Statistical analysis

Statistical evaluation was performed using the SPSS 17.0 for Windows software (SPSS, Inc., Chicago, IL, USA). Genotype and allele frequencies were compared between groups using the Fisher exact test or the chi-square test. The odds ratio was calculated using Haldane's modification of Woolf's method, and the significance of its deviation from unity was estimated by the Fisher exact test. Survival analyses were performed using Kaplan-Maier analysis and the log rank test. $P < 0.05$ was considered to be statistically significant, and values from 0.05-0.1 indicated a trend.

RESULTS

The distributions of the alleles and genotypes for all of the studied polymorphisms in B-CLL patients and healthy controls are shown in Table 1. Deviation from Hardy-Weinberg equilibrium was not observed in either group (Table 1).

Table 1. Distribution of genotypes and alleles.

SNP	Genotype/Allele	B-CLL patients (N = 102)	Control subjects (N = 124)	P
rs10434	AA	28 (27%)	16 (13%)	0.007
	AG	44 (43%)	53 (43%)	
	GG	30 (29%)	55 (44%)	
	A	0.54	0.34	0.003
	G	0.46	0.66	

The distributions of alleles and genotypes for all of the studied polymorphisms were similar in patients and controls (Table 1). The presence of the rs10434 A allele and AA genotype was significantly high in B-CLL patients compared to controls (0.54 *vs* 0.34; 27 *vs* 13%; respectively).

The features of the *VEGF* gene polymorphism were subjected to analysis for correlation with clinical data, i.e., gender, age at diagnosis, peripheral lymphocyte doubling time, and time to Rai stage progression. No associations were observed between the *VEGF* gene polymorphism and gender, age at diagnosis, survival, or peripheral lymphocyte doubling time (data not shown).

DISCUSSION

In the present study, we found that the rs10434 polymorphism in the 3'-UTR of the *VEGF* gene is associated with B-CLL risk in a Chinese population. This is the first study to analyze the relationship between the rs10434 polymorphism and risk of B-CLL in a Chinese population.

Recent reports have suggested that VEGF-based autocrine pathway promotes the survival of CLL B cells in part by upregulating anti-apoptotic proteins (Farahani et al., 2005). Moreover, interactions between CLL B cells and their microenvironment alter the secretion of angiogenic factors that result in enhanced leukemic B cell resistance to apoptotic cell death (Gehrke et al., 2011). Among the variety of angiogenic factors involved in the CLL, VEGF was identified (König et al., 1997). VEGF levels are regarded as a prognostic marker of disease progression (Menzel et al., 1996) in patients with B-CLL.

In the present study, we found that the rs10434 AA genotype and the A allele were significantly frequently observed in B-CLL patients compared to control subjects. However, no associations were observed between the *VEGF* gene polymorphism and gender, age at diagnosis, survival, or peripheral lymphocyte doubling time.

There were several limitations to the study. First, the relatively small sample size may have led to low power to detect significant efects. Second, we only detected one polymorphism in the *VEGF* gene. Finally, we have not followed up the patients' outcomes between different genotypes.

In conclusion, the present study indicated that a polymorphism in the *VEGF* gene may be a genetic marker of B-CLL in a Chinese population.

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