RESEARCH ARTICLE

Vascular Endothelial Growth Factor (VEGF) Gene Polymorphisms and Breast Cancer Risk in a Chinese Population

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Abstract

Vascular endothelial growth factor (VEGF) is a potent regulator of angiogenesis and thereby involved in the development and progression of solid tumours. Associations between three VEGF gene polymorphisms (-634 G/C, +936 C/T, and +1612 G/A) and breast cancer risk have been extensively studied, but the currently available results are inconclusive. Our aim was to investigate associations between three VEGF gene polymorphisms and breast cancer risk in Chinese Han patients. We performed a hospital-based case-control study including 680 female incident breast cancer patients and 680 female age-matched healthy control subjects. Polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) analysis was performed to detect the three VEGF gene polymorphisms. We observed that women carriers of +936 TT genotypes [odds ratio (OR) =0.46, 95% confidence interval (CI) = 0.28, 0.76; *P*=0.002] or 936 T-allele (OR=0.81, 95% CI= 0.68, 0.98; *P*=0.03) had a protective effect concerning the disease. Our study suggested that the +1612G/A polymorphism was unlikely to be associated with breast cancer risk. The -634CC genotype was significantly associated with high tumor aggressiveness [large tumor size (OR=2.63, 95% CI=1.15, 6.02; *P*=0.02) and high histologic grade (OR=1.47, 95% CI= 1.06, 2.03; *P*=0.02)]. The genotypes were not related with other tumor characteristics such as regional or distant metastasis, stage at diagnosis, or estrogen or progesterone receptor status. Our study revealed that the VEGF -634 G/C and +936 C/T gene polymorphisms may be associated with breast cancer in Chinese Han patients.

Keywords: VEGF - single nucleotide polymorphism - breast cancer risk - Han Chinese

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Introduction

Breast cancer is the most frequently diagnosed cancer and the leading cause of cancer death among females, accounting for 23% of the total cancer cases and 14% of the cancer deaths (Jemal et al., 2011). Breast cancer is now also the leading cause of cancer death among females in economically developing countries, a shift from the previous decade during which the most common cause of cancer death was cervical cancer (Jemal et al., 2011; Wang et al., 2013). The mechanism of breast carcinogenesis is still not fully understood. Breast cancer may result from multiple environmental, dietary, hereditary, racial and socioeconomic risk factors (Ronco et al., 2012a; Ronco et al., 2012b; Zhang et al., 2012; Zhou et al., 2012a; Zhou et al., 2012b; Shamsi et al., 2013; Sun et al., 2013).

Angiogenesis is an important step in the development of cancer and is necessary for primary tumor growth, invasiveness, and metastasis (Ferrara et al., 2003). The vascular endothelial growth factor (VEGF) is one of the most potent endothelial cell mitogens and plays and important role in angiogenesis (Belinsky et al., 2005; Roy et al., 2006). VEGF is a major mediator of breast cancer angiogenesis. The VEGF gene is located on chromosome 6p21.3 and consists of eight exons that exhibit alternative splicing to form a family of proteins (Vincenti et al., 1996). Several single-nucleotide polymorphisms (SNPs) in the VEGF gene have been shown to affect the expression of the gene.(Watson et al., 2000; Bae et al., 2008) Three potentially functional SNPs (-634 G/C, +936 C/T, and +1612 G/A) in the 3-untranslated region (UTR) of the VEGF gene, were shown to affect VEGF plasma levels. VEGF gene polymorphisms have been reported to be associated with cancers of the prostate (Wang et al., 2012), lung (Lee et al., 2005), colorectum (Dassoulas et al., 2009), gastric and bladder (Garcia-Closas et al., 2007; Zhou et al., 2011).

The associations between three VEGF gene polymorphisms (-634 G/C, +936 C/T, and +1612 G/A) and breast cancer risk have been extensively studied, and the

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currently available results are inconclusive (Krippl et al., 2003; Jin et al., 2005; Lu et al., 2005; Jacobs et al., 2006; Kataoka et al., 2006; Eroglu et al., 2008; Jakubowska et al., 2008; Langsenlehner et al., 2008; Schneider et al., 2008; Wehrschuetz et al., 2009; Gu and Wang, 2011; Jin et al., 2011; Liu et al., 2011; Oliveira et al., 2011; Qiu et al., 2011; Wang et al., 2011; Yang et al., 2011; Rodrigues et al., 2012). The aim of this study was to investigate the association between three VEGF gene polymorphisms and breast cancer risk in Chinese Han patients.

Materials and Methods

Study Subjects

Between June 2010 and May 2012, we performed a hospital-based case-control study including 680 female incident breast cancer patients and 680 female age-matched healthy control subjects from the West China Hospital in Sichuan University (Chengdu, China) were enrolled as the study group. The Chinese Han population was collected from the same geographic region. Written informed consent was obtained from all subjects according to the Declaration of Helsinki, and the study protocol was approved by West China Hospital Research Ethics Board. The clinicopathologic findings of the cancer group were collected. Smoking status was defined as never (smoked less than 100 cigarettes in lifetime) and ever. Education was defined as more than middle school and less than middle school. Menopausal status was defined as pre-menopausal and post-menopausal. Tumor size was defined as in situ, <20, 21-50 and >50. Tumor histologic grade was classified as grade 1, 2, and 3. The tumor stages were usually expressed as a number on a scale of 0 through IV. We also defined the status of estrogen receptors, progesterone receptor, distant metastasis, and regional lymph node metastasis.

Genotyping analysis

The extraction of genomic DNA from the peripheral blood lymphocytes was performed using QIAamp DNA blood mini kit (QIAGEN Inc., Valencia, CA, USA). The VEGF -634 G/C, +936 C/T, and +1612 G/A gene polymorphisms were then determined using a polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) assay. PCR primers were designed based on a Genbank reference sequence, the PCR primers designed for -634 G/C, +936 C/T, and +1612 G/A were 5'-GTA GCA AGA GCT CCA GAG AGA AGT-3' (forward) and 5'-TGG ACG AAA AGT TTC AGT GCG ACG-3' (reverse); 5'-CTC GGT GAT TTA GCA GCA AG-3' (forward) and 5'-CTC GGT GAT TTA GCA GCA AG-3' (reverse); 5'-CAC ATG CTG CAC GCG CAT CTC A-3' (forward) and 5'-ACC CCA GGA AGG GGA GCA GGA-3' (reverse), respectively. The PCR reactions were carried out in a 20-μl reaction volume containing 100 ng genomic DNA, 25 pmol/l each primer, 0.2 mmol/l deoxyuceotide triphosphates, 10 mmol/l Tris–HCl (pH 8.3), 50 mmol/l KCl, 1.5 mmol/l $MgCl_2$, and 1 unit of Taq polymerase (Takara Shuzo Co., Otsu, Shiga, Japan). The PCR cycle conditions consisted of an initial denaturation step at 94°C for 5 min, followed by 35 cycles of 30 s at 94°C, 30 s at 62°C, 30 s at 72°C, and a final elongation at 72°C for 10 min. The PCR products were digested overnight with the appropriate restriction enzymes (New England Biolabs, Beverly, MA, USA), which were BsmFI, NlaIII, and MnlI for the -634 G/C, +936 C/T, and +1612 G/A polymorphisms, respectively. The digested PCR products were resolved on a 3% agarose gel and stained with ethidium bromide for visualization under UV light. For quality control, the genotyping analysis was done blind as regards the subjects. The selected PCR-amplified DNA samples were also examined by DNA sequencing to confirm the genotyping results.

Statistical Analysis

The VEGF allele and genotype frequencies in patients were compared to controls using the x^2 test. Crude odds ratios (OR) and adjusted ORs for sex and age with 95% confidence interval (CI) were as calculated by logistic regression analysis. A *P*-value was considered significant at a level of < 0.05. The Hardy-Weinberg test of genetic equilibrium was applied using the chi-square test to ensure

Table 1. Characteristics of the breast cancer patients and the disease-free individuals

	Cases	Controls	*P*
Number of subjects	680	680	
Age (year)	49.2(8.2)	48.8(7.8)	0.36
Body mass index (kg/m^2)	25.8(5.3)	26.1(5.4)	0.3
Education (more/less than middle school)	613/67	610/70	0.79
Smoking status (Ever/Never)	58/622	52/628	0.55
Family History (Yes/No)	138/542	65/615	<0.001
Menopausal status (Pre-/Post-menopausal)	578/102	582/98	0.76
Tumor size (mm)			
In situ	8		
<20	456		
21-50	199		
>50	17		
Histologic grade			
Grade 1	135		
Grade 2	308		
Grade 3	237		
Stage			
0	9		
I	370		
II	272		
III	18		
IV	11		
Regional lymph node metastasis			
Negative	477		
Positive	203		
Distant metastasis			
Negative	669		
Positive	11		
Estrogen receptors			
Positive	484		
Negative	196		
Progesterone receptor			
Positive	425		
Negative	255		

Continuous variables: mean values ± standard deviation, *P* value from t tests; Categorical variables: numbers and percentages, *P* values from x^2 test

that there was no significant difference between observed and expected genotype frequencies. Data were analyzed using the SPSS statistical package software version 17 (SPSS Inc., Chicago, IL U.S.A.).

Results

Characteristics of participants

Characteristics of the breast cancer patients and the disease-free individuals are shown in Table 1. Cases and controls were similar with respect to the distribution of age (*P*=0.36), body mass index (*P*=0.30), education (*P*=0.79), smoking status (*P*=0.55), and menopausal status (*P*=0.76). Cases were also more likely to have a positive family history of breast cancer than controls, and this difference was highly significant (*P*<0.001).

VEGF -634 G/C gene polymorphisms and breast cancer

Our study suggested that the -634 G/C polymorphism was unlikely to be associated with breast cancer risk (Table 2). The -634CC genotype was significantly associated with high tumor aggressiveness [large tumor size (OR=2.63, 95% CI=1.15, 6.02; *P*=0.02) and high histologic grade (OR=1.47, 95% CI= 1.06, 2.03; *P*=0.02)] (Table 3). The genotypes were not related with other tumor characteristics such as regional or distant metastasis, stage at diagnosis, or estrogen or progesterone receptor status (Table 3).

Table 2. VEGF Gene Polymorphisms among Breast Cancer Patients and Controls

Genotype	Cases n (%)	Controls n (%)	OR (95%CI)	*P*
-634 GG	338(49.7)	341(50.1)	1.00(Reference)	
-634 GC	205(30.1)	204(30.0)	1.01(0.79,1.30)	0.91
-634 CC	137(20.2)	135(19.9)	1.02(0.77,1.36)	0.87
-634 G allele frequency	881(64.8)	886(65.1)	1.00(Reference)	
-634 C allele frequency	479(35.2)	474(34.9)	1.02(0.87,1.19)	0.84
+936 CC	446(65.6)	426(62.6)	1.00(Reference)	
+936 CT	210(30.9)	204(30.0)	0.98(0.78,1.24)	0.89
+936 TT	24(3.5)	50(7.4)	0.46(0.28,0.76)	0.002
+936 C allele frequency	1102(81.0)	1056(77.6)	1.00(Reference)	
+936 T allele frequency	258(19.0)	304(22.4)	0.81(0.68,0.98)	0.03
+1612 GG	478(70.3)	482(70.9)	1.00(Reference)	
+1612 GA	138(20.3)	141(20.7)	0.99(0.76,1.29)	0.92
+1612 AA	64(9.4)	57(8.4)	1.13(0.78,1.65)	0.52
+1612 G allele frequency	1094(80.4)	1105(81.3)	1.00(Reference)	
+1612 A allele frequency	266(19.6)	255(18.7)	1.05(0.87,1.28)	0.59

VEGF +936 C/T gene polymorphisms and breast cancer

We observed that women carriers of +936 TT genotypes (OR=0.46, 95% CI= 0.28, 0.76; *P*=0.002) or 936 T-allele (OR=0.81, 95% CI= 0.68, 0.98; *P*=0.03) had a protective effect concerning this disease (Table 2). When stratified by the tumor size, histologic grade, stage, regional lymph node metastasis, distant metastasis, estrogen receptors, and progesterone receptor of breast cancer, no statistically significant result was observed.

VEGF +1612 G/A gene polymorphisms and breast cancer

Our study suggested that the +1612G/A polymorphism was unlikely to be associated with breast cancer risk (Table 2). When stratified by the tumor size, histologic grade, stage, regional lymph node metastasis, distant metastasis, estrogen receptors, and progesterone receptor of breast cancer, no statistically significant result was observed.

Discussion

The associations between VEGF gene polymorphisms and breast cancer risk have been extensively studied, and the currently available results are inconclusive. Our results were consistent with some previous study. A case-control study of 235 patients and 235 controls

Table 3. Stratification Analysis of VEGF-634 G/C Genotype Frequency in Breast Cancer Patients

Variable	Cases	GG			GC			CC		
		n (%)	OR (95%CI)	*P*	n (%)	OR (95%CI)	*P*	n (%)	OR (95%CI)	*P*
Tumor size (mm)	680	338(49.7)	1(Reference)		205(30.1)	1(Reference)		137(20.2)	1(Reference)	
In situ	8	4(50.0)	1.01(0.30,3.36)	0.99	2(25.0)	0.83(0.18,3.94)	0.81	2(25.0)	1.24(0.26,5.91)	0.79
<20	456	239(52.4)	1.05(0.86,1.29)	0.61	148(32.5)	1.08(0.84,1.37)	0.55	69(15.1)	0.75(0.55,1.03)	0.07
21-50	199	90(45.2)	0.91(0.69,1.21)	0.51	52(26.1)	0.87(0.62,1.22)	0.41	57(28.7)	1.42(1.01,2.01)	0.04
>50	17	5(29.4)	0.59(0.22,1.62)	0.31	3(17.7)	0.58(0.17,2.02)	0.4	9(52.9)	2.63(1.15,6.02)	0.02
Histologic grade	680	338(49.7)	1(Reference)		205(30.1)	1(Reference)		137(20.2)	1(Reference)	
Grade 1	135	68(50.4)	1.01(0.74,1.39)	0.93	45(33.3)	1.11(0.76,1.60)	0.6	22(16.3)	0.81(0.50,1.32)	0.39
Grade 2	308	158(51.3)	1.03(0.82,1.30)	0.79	105(34.1)	1.13(0.86,1.48)	0.37	45(14.6)	0.72(0.50,1.04)	0.08
Grade 3	237	112(47.3)	0.95(0.73,1.23)	0.7	55(23.2)	0.77(0.55,1.07)	0.12	70(29.5)	1.47(1.06,2.03)	0.02
Stage	680	338(49.7)	1(Reference)		205(30.1)	1(Reference)		137(20.2)	1(Reference)	
0	9	5(55.6)	1.12(0.37,3.36)	0.84	2(22.2)	0.74(0.16,3.44)	0.7	2(22.2)	1.10(0.24,5.16)	0.9
I	370	181(48.9)	0.98(0.79,1.23)	0.89	115(31.1)	1.03(0.79,1.34)	0.82	74(20.0)	0.99(0.73,1.35)	0.96
II	272	137(50.4)	1.01(0.79,1.29)	0.91	79(29.0)	0.96(0.72,1.29)	0.81	56(20.6)	1.02(0.73,1.44)	0.9
III	18	9(50.0)	1.01(0.45,2.26)	0.99	6(33.3)	1.11(0.43,2.82)	0.83	3(16.7)	0.83(0.24,2.85)	0.76
IV	11	6(54.5)	1.10(0.40,2.99)	0.86	3(27.3)	0.91(0.25,3.27)	0.88	2(18.2)	0.90(0.20,4.12)	0.89
Regional lymph node metastasis	680	338(49.7)	1(Reference)		205(30.1)	1(Reference)		137(20.2)	1(Reference)	
Negative	477	233(48.8)	0.98(0.80,1.21)	0.87	142(29.8)	0.99(0.77,1.26)	0.92	102(21.4)	1.06(0.80,1.41)	0.68
Positive	203	105(51.7)	1.04(0.79,1.36)	0.77	63(31.0)	1.03(0.74,1.42)	0.86	35(17.3)	0.86(0.57,1.28)	0.45
Distant metastasis	680	338(49.7)	1(Reference)		205(30.1)	1(Reference)		137(20.2)	1(Reference)	
Negative	669	332(49.6)	1.00(0.83,1.20)	0.99	202(30.2)	1.00(0.80,1.25)	0.99	135(20.2)	1.00(0.77,1.30)	0.99
Positive	11	6(54.5)	1.10(0.40,2.99)	0.86	3(27.3)	0.90(0.25,3.27)	0.88	2(18.2)	0.90(0.20,4.12)	0.89
Estrogen receptors	680	338(49.7)	1(Reference)		205(30.1)	1(Reference)		137(20.2)	1(Reference)	
Positive	484	243(50.2)	1.01(0.82,1.24)	0.92	149(30.8)	1.02(0.80,1.30)	0.87	92(19.0)	0.94(0.71,1.26)	0.69
Negative	196	95(48.5)	0.98(0.74,1.29)	0.86	56(28.6)	0.95(0.68,1.33)	0.75	45(22.9)	1.14(0.79,1.65)	0.49
Progesterone receptor	680	338(49.7)	1(Reference)		205(30.1)	1(Reference)		137(20.2)	1(Reference)	
Positive	425	212(49.9)	1.00(0.81,1.24)	0.97	131(30.8)	1.02(0.80,1.31)	0.86	82(19.3)	0.96(0.71,1.29)	0.78
Negative	255	126(49.4)	0.99(0.77,1.28)	0.96	74(29.0)	0.96(0.71,1.30)	0.81	55(21.6)	1.07(0.76,1.51)	0.7

suggested that the VEGF wild 936CC and the variant 634CC genotypes constitute inherited determinants of sporadic breast cancer and sporadic breast cancer aggressiveness in Brazil (Oliveira et al., 2011). The results found that the 936CT+TT genotypes of VEGF reduced BRCA1-associated breast cancer risk in Polish women (Jakubowska et al., 2008). A population-based case-control study conducted in urban Shanghai, China from 1996 to 1998, suggested that the VEGF C936T polymorphism might be a susceptibility factor for breast cancer among Chinese women (Kataoka et al., 2006). A large case-control study found that the VEGF -634CC genotype and the VEGF -2578/-634 CC haplotype were significantly associated with high tumor aggressiveness (large tumor size and high histologic grade) and the VEGF -2578AA genotype and the VEGF -2578/-634 AG haplotype with low histologic grade tumors (Jin et al., 2005). A large case-control study in 500 women with breast cancer and 500 sex- and age-matched healthy control subjects found that carriers of a VEGF 936T-allele are at decreased risk for breast cancer in Austria (Krippl et al., 2003). But some meta-analyses found that the VEGF gene 936 C/T polymorphism may not contribute to breast cancer susceptibility (Gu and Wang, 2011; Qiu et al., 2011; Yang et al., 2011). A meta-analysis involving 16,703 individuals also suggested that the VEGF +936C/T, -1154A/G, -2578C/A, -634G/C and -460T/C may be not associated with risk of breast cancer development based on the currently available studies, especially for Caucasians (Wang et al., 2011).

The mechanism by which the VEGF gene polymorphisms affect breast cancer risk is currently unclear. Several lines of compelling evidence from in vitro and in vivo experiments have shown that VEGF overexpression is associated with tumor growth and metastasis, whereas the inhibition of VEGF signaling results in suppression of both tumor induced angiogenesis and tumor growth (Ferrara, 2002). Some studies have shown that high levels of VEGF expression and increased microvessel density in tumors were associated with advanced stage disease and worse prognosis for various types of tumors (Perrone et al., 2004; Stefanou et al., 2004; Iordache et al., 2010). Several polymorphisms in the VEGF gene have been reported to affect the expression of the gene. The VEGF -634CC have been shown associated with a higher VEGF production. Renner et al. firstly identified VEGF +936 C/T gene polymorphism and found VEGF +936 T allele lead to significant lower plasma VEGF levels in healthy young men (Renner et al., 2000).

Strength of this study was a relatively large sample size. But some shortcomings of our study should be mentioned. Firstly, this is a hospital based case control study, so the selection bias may not be avoidable and the subjects may not be representative of the general population. Secondly, these results should be interpreted with caution because the population only from China, which reduces the possibility of confounding from ethnicity, but it does not permit extrapolation of the results to other ethnic groups. Thirdly, further study is required to delineate the precise mechanisms involved and, potentially, to facilitate the design of effective clinical trials. Finally, the study is based on unadjusted estimates.

In conclusion, our study revealed that the VEGF -634 G/C and +936 C/T gene polymorphisms may be associated with breast cancer in Chinese Han patients. More well designed studies with larger sample size on different ethnicities are needed to further assess the associations.

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