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Clinical profile and role of VEGF-c polymorphism in prognosis and management of breast cancer

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ABSTRACT

Purpose: Angiogenesis is a necessary step in tumor growth and metastasis. Vascular endothelial growth factor (VEGF-c) is a major mediator of breast cancer angiogenesis. Therefore, we investigated the association of polymorphism in the VEGF-c gene with breast cancer risk and prognostic characteristics of the tumors in a case-control study. **Experimental Design:** We examined one polymorphism in the VEGF-c gene (+936C/T) in 75 breast cancer cases and 75 control from Kanpur, Uttar Pradesh, India and adjacent areas together with geographically selected controls. **Results:** None of the polymorphism or any haplotype was significantly associated with either breast cancers. Our study suggests that the +936C/T polymorphism is unlikely to be associated with breast cancer. We also analyzed the cases for genotypes or haplotypes that associated with tumor characteristics. The genotypes and haplotypes were not related with other tumor characteristics such as regional or distant metastasis, stage at diagnosis, or dietary history. **Conclusions:** Although none of the polymorphisms studied in the VEGF-c gene was found to influence susceptibility to breast cancer significantly, some of the VEGF-c genotypes and haplotypes may influence tumor growth through an altered expression of VEGF-c and tumor angiogenesis.

Key words: Polymorphism, VEGF-c, Breast cancer, Indian population

Introduction

Angiogenesis is an important step in the development of cancer and is necessary for primary tumor growth, invasiveness, and metastasis.[1] Vascular endothelial growth factor (VEGF-c) is believed to be important for the process of initiation of angiogenesis and is a major mediator of breast cancer angiogenesis.[2] Overexpression of VEGF-c has been shown in various cancers.[3] Several polymorphisms in the VEGF-c gene have been reported to affect the expression of the gene. The 2578CC, 2549 del/del, 1154GG, and 634CC have been shown associated with a higher VEGF-c production,[4-7] whereas the +936 T allele has been shown to correlate with lower VEGF plasma levels.[8,9] In addition, the 634G/C polymorphism is located within a potential binding site of the MZF1 transcription factor[10] and the +936C/T polymorphism leads to a loss of a potential AP-4 binding site.[8,9] Recent studies have shown that some VEGF-c polymorphisms are associated with the development of cancer. The 1154AA genotype, for example, is associated with a decreased prostate cancer risk and less advanced melanomas[11,12] and the +936 C/T polymorphism with a decreased breast cancer risk.[9] In breast cancer, VEGF-c has been shown to be of prognostic importance.[13] These data suggest that the polymorphisms involved in the angiogenic pathway may affect the progression or aggressiveness of the tumor, including breast tumors. In the present study, we investigated the relationship between genetic polymorphism in the VEGF-c gene and the development of breast cancer in patients from Kanpur and adjacent areas. Polymorphisms were located in the 3'untranslated region at position +936, according to the numbering used by Renner *et al*. [Figure 1].[8] This polymorphism was selected for further analysis to evaluate the possible influence on the risk to breast cancer and the prognostic characteristics of the tumors.

The translation starting site is marked by the ATG codon. The polymorphisms within the box are in the complete linkage. The +936 C/T polymorphism was studied in the Kanpur, India breast cancer cases and controls.

Methods

Totally, 75 breast cancer cases together with ethnically and geographically selected controls were used in the study. The study populations consisted of North Indian were incident cases collected during the years 2013 to 2015 according to the criteria described earlier[14] through the outpatient and indoor patient surgery department clinics (LLR Hospital, Kanpur, India) and the Cancer clinics (J.K. Cancer Institute Kanpur, India). About 90% of patients approved participation in the study. The controls were recruited to earlier studies with comparable participation rate. These cases together with controls were analyzed at Department of Biochemistry, GSVM Medical College Kanpur, India. Samples (n = 75) were collected in a hospital-based manner from untreated patients referred to the Department of Surgery for newly diagnosed breast cancer; the controls were selected from the hospital staff nurse and volunteers. Characteristics of the subjects at diagnosis

are shown in Table 2. Altogether, only 2 of 75 breast cancer cases had distant metastasis indicating that even the prevalent cases were recruited relatively shortly after diagnosis, excluding biases due to preferential survival. After DNA digestion, the coded samples were divided on the plates by randomly mixing cases and controls.

Polymerase chain reaction (PCR) amplification

The primer sequence for the polymorphism of VEGF-c gene (+936 C/T) was retrieved from the Ensemble database. Primers were designed for the regions around the polymorphic sites/or for the entire coding regions with the help of the primer blast tool of the NCBI or Gene tool software. Primers were custom synthesized by Micelles Life Sciences, Lucknow, India.

Restriction fragment length polymorphism (RFLP) analysis

The +936C/T polymorphism was analyzed in the sample sets using RFLP analysis. The assays were set up after PCR as described.[16] PCR products were digested with restriction endonucleases, using the buffers and temperatures recommended by the manufacturers. The digested PCR products were resolved on a 10% polyacrylamide gel and stained with ethidium bromide for visualization under ultraviolet light.

Statistical analysis

Genotype data of control samples for polymorphism were analyzed for fitness in the Hardy-Weinberg equilibrium using the online calculator.[23] Chi-square test was used to compare genotype data between cases and controls using Vassar stats online calculator,[24,25] adopting dominant, recessive, codominant, and additive models. $P < 0.05$ was considered statistically significant.

The differences in the genotype and haplotype frequencies of the studied polymorphism in the breast cancer cases and controls were compared for statistical significance by the Yates corrected Chi-square test. Odds ratios and 95% confidence intervals were calculated for associations of genotypic polymorphism between breast cancer cases and healthy controls.

Results

We examined the effect of polymorphism in the VEGF-c gene on breast cancer development [Figure 1]. The polymorphism +936C/T were examined in breast cancer samples and controls using the RFLP assay. The genotype distribution of studied polymorphism followed the Hardy-Weinberg equilibrium in every sample set. The genotype and allele distributions among the breast cancer cases and control subjects are shown in Table 1. The number of samples analyzed for polymorphism was not exactly equal because of unsuccessful amplification of a few samples. No differences in the allele or genotype frequencies between the breast cancer cases and controls were detected in the population. The lack of association remained when the data were stratified by age (data not shown). Haplotypes were created using genotyping data of the polymorphism +936C/T. No significant differences in haplotype frequencies between the breast cancer cases and controls were detected. There were no indications that the polymorphism would have any effects on the other tumor characteristics. The addition of the information of the +936C/T polymorphism data did not change the results (data not shown).

Discussion

Functional polymorphisms, which have an effect on the regulation of gene expression, can contribute to the differences between individuals in susceptibility to and severity of a disease. The effect may be seen by a polymorphism alone, or in combination with other polymorphisms. Several studies have shown that polymorphisms in the promoter as well as in the 5' and 3' untranslated regions of the VEGF-c gene are associated with the production of the VEGF-c protein.[4-10] We analyzed the +936(C/T) polymorphism using RFLP assay. The allele and genotype distributions of the polymorphism were in close agreement with those previously published for healthy Caucasian individuals.[8,11,12,15-22] In a previous study among 500 Caucasian breast cancer cases and 500 controls, Krippl *et al*. have shown a decreased risk of breast cancer in individuals who were +936 T allele carriers.[9] However, the genotypes in patients did not follow the Hardy-Weinberg equilibrium. In another study, no association between the +936(C/T) polymorphism and risk to breast cancer among 862 cases and 713 controls could be observed.[21] Here, we did a case-control study of 75 women with breast cancer. We observed no differences in the allele or genotype frequencies between breast cancer case and control groups, (odds ratio, 1.26; 95% confidence interval, 0.50–1.00; $P = 0.715$). To the best of our knowledge, no other studies on the effect of the other polymorphisms on the risk of breast cancer have been published in Indian population. In this study, no significant

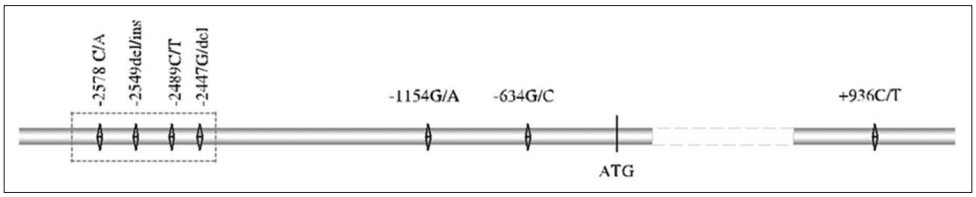

Figure 1: Structure of the vascular endothelial growth factor gene indicating the polymorphism included in the present study

Table 1: Allelic and genotype distribution (+936 C/T) of VEGF-c gene

Groups (%)	CC	CT/TT	*P*-value	OR (95% CI)
Controls (*n*=75)	50 (66.7)	25 (33.3)	Ref.	
Ca breast (*n*=75)	53 (70.7)	22 (29.3)	0.511	1.26 (0.628–2.533)
Groups (%)	**CC**	**CT**	**TT**	***P*-value**
Controls (*n*=75)	50 (66.7)	23 (30.7)	2 (2.6)	Ref.
Ca breast (*n*=75)	53 (70.7)	19 (25.3)	3 (4.0)	0.715

Ref: It is treated as reference to compare between cases and control.
VEGF-c: Vascular endothelial growth factor. OR: Odd ration, CI: Confidence interval

Table 2: Characteristics of the subjects at diagnosis

Characteristics	Breast cancer patients (cases)	Controls
Mean age at diagnosis (years)	46.13	42.11
Distant metastasis		
Negative	73	N/A
Positive	2	N/A
Regional lymph node metastasis		
Negative	65	74
Positive	10	1 (Reactive)
Stage at diagnosis		
I	7	N/A
II	59	N/A
III	7	N/A
IV	2	N/A
Histology		
DCIS	38	N/A
IDC	37	N/A
Tumor size		
T1	7	N/A
T2	34	N/A
T3	32	N/A
T4	2	N/A
Dietary history		
Vegetarian	54	52
Non-vegetarian	21	23

DCIS: Ductal carcinoma *in situ*, IDC: Invasive ductal carcinoma

differences in the allele, genotype, or haplotype distribution of the polymorphism in the VEGF-c gene between breast cancer cases and respective controls were detected. Being the only Indian study thus far, our study provided strong evidence that the +936 C/T allele polymorphism do not modify the risk of breast cancer. This result is not surprising, because VEGF-c, as a key mediator of angiogenesis, is more likely to alter the aggressiveness of the tumor than susceptibility to cancer. In summary, the present study investigated polymorphism in the VEGF-c gene in a case-control study. Our study provided evidence that the +936C/T polymorphism is not associated with breast cancer risk. However, some genotypes and haplotypes in the VEGF-c gene may have an effect on breast tumor growth. Functional studies of the haplotypes and an independent study are needed to confirm our results. The polymorphisms should also be studied in relation to metastasis and survival and whether they influence therapeutic effects.

References

1. Ferrara N, Gerber HP, LeCouter J. The biology of VEGF and its receptors. Nat Med 2003;9:669-76.
2. Morabito A, Sarmiento R, Bonginelli P, Gasparini G. Antiangiogenic strategies, compounds, and early clinical results in breast cancer. Crit Rev Oncol Hematol 2004;49:91-107.
3. Nakamura M, Abe Y, Tokunaga T. Pathological significance of vascular endothelial growth factor A isoform expression in human cancer. Pathol Int 2002;52:331-9.
4. Yang B, Cross DF, Ollerenshaw M, Millward BA, Demaine AG. Polymorphisms of the vascular endothelial growth factor and susceptibility to diabetic microvascular complications in patients with Type 1 diabetes mellitus. J Diabetes Complications 2003;17:1-6.
5. Mohammadi M, Ollier WE, Hutchinson IV. A functional association study of VEGF gene promoter polymorphisms with VEGF expression by stimulated cells. Hum Immunol 2003;64:S125.
6. Shahbazi M, Fryer AA, Pravica V, Brogan IJ, Ramsay HM, Hutchinson IV, *et al.* Vascular endothelial growth factor gene polymorphisms are associated with acute renal allograft rejection. J Am Soc Nephrol 2002;13:260-4.
7. Awata T, Inoue K, Kurihara S, Ohkubo T, Watanabe M, Inukai K, *et al.* A common polymorphism in the 5'-untranslated region of the VEGF gene is associated with diabetic retinopathy in Type 2 diabetes. Diabetes 2002;51:1635-9.
8. Renner W, Kotschan S, Hoffmann C, Obermayer-Pietsch B, Pilger E. A common 936 C/T mutation in the gene for vascular endothelial growth factor is associated with vascular endothelial growth factor plasma levels. J Vasc Res 2000;37:443-8.
9. Krippl P, Langsenlehner U, Renner W, Yazdani-Biuki B, Wolf G, Wascher TC, *et al.* A common 936 C/T gene polymorphism of vascular endothelial growth factor is associated with decreased breast cancer risk. Int J Cancer 2003;106:468-71.
10. Watson CJ, Webb NJ, Bottomley MJ, Brenchley PE. Identification of polymorphisms within the vascular endothelial growth factor (VEGF) gene: Correlation with variation in VEGF protein production. Cytokine 2000;12:1232-5.
11. Howell WM, Bateman AC, Turner SJ, Collins A, Theaker JM. Influence of vascular endothelial growth factor single nucleotide polymorphisms on tumour development in cutaneous malignant melanoma. Genes Immun 2002;3:229-32.
12. McCarron SL, Edwards S, Evans PR, Gibbs R, Dearnaley DP, Dowe A, *et al.* Influence of cytokine gene polymorphisms on the development of prostate cancer. Cancer Res 2002;62:3369-72.
13. Linderholm B, Grankvist K, Wilking N, Johansson M, Tavelin B, Henriksson R. Correlation of vascular endothelial growth factor content with recurrences, survival, and first relapse site in primary node-positive breast carcinoma after adjuvant treatment. J Clin Oncol 2000;18:1423-31.
14. Jin Q, Hemminki K, Grzybowska E, Klaes R, Söderberg M, Zientek H, *et al.* Polymorphisms and haplotype structures in genes for transforming growth factor beta1 and its receptors in familial and unselected breast cancers. Int J Cancer 2004;112:94-9.
15. Kaaks R, Lundin E, Rinaldi S, Manjer J, Biessy C, Söderberg S, *et al.* Prospective study of IGF-I, IGF-binding proteins, and breast cancer risk, in northern and southern Sweden. Cancer Causes Control 2002;13:307-16.
16. Försti A, Jin Q, Grzybowska E, Söderberg M, Zientek H, Sieminska M, *et al.* Sex hormone-binding globulin polymorphisms in familial and sporadic breast cancer. Carcinogenesis 2002;23:1315-20.
17. Försti A, Jin Q, Sundqvist L, Söderberg M, Hemminki K. Use of monozygotic twins in search for breast cancer susceptibility loci. Twin Res 2001;4:251-9.
18. Brogan IJ, Khan N, Isaac K, Hutchinson JA, Pravica V, Hutchinson IV. Novel polymorphisms in the promoter and 5' UTR regions of the human

vascular endothelial growth factor gene. Hum Immunol 1999;60:1245-9.
19. Lambrechts D, Storkebaum E, Morimoto M, Del-Favero J, Desmet F, Marklund SL, *et al.* VEGF is a modifier of amyotrophic lateral sclerosis in mice and humans and protects motoneurons against ischemic death. Nat Genet 2003;34:383-94.
20. Stevens A, Soden J, Brenchley PE, Ralph S, Ray DW. Haplotype analysis of the polymorphic human vascular endothelial growth factor gene promoter. Cancer Res 2003;63:812-6.
21. Balasubramanian SP, Brown NJ, Reed MW. Role of genetic polymorphisms in tumour angiogenesis. Br J Cancer 2002;87:1057-65.
22. Houlston RS, Peto J. The future of association studies of common cancers. Hum Genet 2003;112:434-5.
23. Antoniou AC, Easton DF. Polygenic inheritance of breast cancer: Implications for design of association studies. Genet Epidemiol 2003;25:190-202.
24. Hardy-Weinberg equilibrium in genetic association studies: An empirical evaluation of reporting, deviations, and power. Available from: http://www.ihg.gsf.de/cgi-bin/hw/hwa1.pl.
25. Online Calculator. Available from: http://www.vassarstats.net/tab2x2.html.

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