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ORIGINAL ARTICLE

Association between the −402GA, −401GT, and −323ins10-bp polymorphisms of factor VII gene and breast cancer

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Abstract
Background and Purpose Recent data have shown that the hemostatic system may play a role in cancer development and progression. To test whether factor VII (FVII) can be a candidate factor for breast cancer, we have evaluated the distribution of FVII gene polymorphisms in breast cancer patients and healthy subjects.
Methods The nested case–control study consisted of 92 women with breast cancer (group 1) and 80 control subjects (in age-matched women) (group 2). Genotyping of the −323ins10-bp, −401GT, and −402GA polymorphisms of the FVII gene was performed by the method of single-strand conformation polymorphism analysis and sequencing.
Results A significant difference was observed in the distribution of the −402GA genotype and allele frequencies in breast cancer and control cases ($p < 0.05$). For other polymorphisms of the FVII gene, the distributions of genotypes and allele frequencies were not significantly different between two groups ($p > 0.05$). There was also a significant difference between the distributions of the haplotypes in breast cancer patients and control subjects ($p < 0.05$).
Conclusion Although the number of cases in this study was small, the preliminary findings revealed a possible contribution of the FVII −402GA polymorphism in the development of breast cancer. However, further case–control studies with larger series are needed to confirm our findings.

Keywords Breast cancer · Risk · Factor VII gene · Polymorphism

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Introduction

It is known that cancer produces a hypercoagulable state, which may lead to thrombosis. In contrast, coagulation and fibrinolysis play an important role in tumor growth, invasion, dissemination, and metastasis [1]. Venous thromboembolism may be the presenting feature of an occult cancer case.

The pathogenesis of activation of the coagulation cascade in cancer is complex and multifactorial [2]. Thrombin can promote angiogenesis and cell proliferation in malignancy. Patients with malignancy including breast cancer have a high frequency of procoagulants and resistance to activated protein C [3, 4]. Persistent activation of the coagulation pathway may play a significant role in the preclinical phase of cancer and is associated with an increased incidence of malignancy, especially of the digestive tract in men [4]. However, whether the polymorphisms of genes encoding hemostatic factors are correlated with tumor development is not well-defined.

Factor VII (FVII) plays an important role in the blood coagulation. FVII binding to tissue factor (TF) starts the extrinsic pathway of the coagulation cascade. The FVII/TF complex initiates fibrin formation which promotes angiogenesis. On the other hand, the FVII/TF is related to cell survival and activated FVII has an anti-apoptotic affect [5]. High plasma levels of FVII can be related to a hypercoagulable state. The common polymorphisms of the

promoter region in the FVII gene; −323ins10-bp, −401GT, and −402GA are associated with FVII blood levels [6–8].

The effect of thrombophilia in the pathogenesis of malignancy is currently under investigation. However, there are no published data related to the effect of FVII gene polymorphisms on the development of breast cancer. We have therefore evaluated the possible association of three common functional polymorphisms in the promoter region of FVII gene; −323ins10-bp, −401GT, and −402GA with breast cancer.

Patients and methods

This nested case–control study consisted of 92 women with breast cancer (group 1). A total of 80 healthy age-matched subjects were included as the control group (group 2). Menopausal status was similar between the groups. None of the women had positive family history for breast cancer or thrombophilia. In addition, the cases had no known risk factors for breast cancer, for example prior hormone-replacement therapy.

All patients and control subjects gave informed consent for their participation in the study. Genomic DNA isolation was performed from peripheral venous blood by standard phenol–chloroform extraction. Genotyping of −323ins10-bp, −401GT, and −402GA polymorphisms of the FVII gene was performed by the method of single-strand conformation polymorphism (SSCP) analysis and sequencing.

Amplification was as follows: initial denaturation for 5 min (min) at 95°C; followed by 34 cycles of 94, 60, and 72°C each for 1 min, and final extension of 10 min at 72°C (Biometra, Germany). The amplified product was 214 bp for SSCP. The SSCP was carried out using the primers: F 5′-GGC CTG GTC TGG AGG CTC TCT TC-3′; R 5′-CGC TGG CAA CAA AAC CGT CCG CTC-3′ [9]. The products were denatured at 99°C for 7 min and then the resulting single-stranded DNA was loaded on to 8% polyacrylamide gel. Electrophoresis was conducted with a sequencing apparatus at 130 V of constant power at 4°C for 10–12 h, depending on the fragment size, and the gel was silver stained.

A 315-bp DNA fragment was amplified for DNA sequencing using the specific primers: F 5′-GTA AGA TGT GGA CCG CTG GA-3′ and R 5′-ACA AAA CCG TCC GCT CTG-3′. Before sequencing the products were purified by using a PCR purification kit (Agencourt, Ampure; Beckman Coulter, Fullerton, CA, USA) and then the DNA sequence analysis was performed using an automatic sequencer (Beckman Coulter CEQ 8000).

All observed genotype and allele frequencies were tested for compliance with Hardy–Weinberg equilibrium. The frequencies of the genotypes and alleles of three polymorphisms of the FVII gene between two groups were compared by use of the Chi-squared test. Haplotype analysis was also carried out and the distribution of the haplotypes between two groups was compared using the Chi-squared test. Statistical significance was defined as $p < 0.05$. Statistical analysis was performed using SPSS software (SPSS, Chicago, IL, USA).

Results

In two groups, the genotype distributions and allele frequencies were in Hardy–Weinberg equilibrium. As shown in Table 1, no differences were observed in the distribution of −401GT and −323ins10-bp genotypes or allele

Table 1 Prevalence of −323ins10-bp, −401 G/T, and −402 G/A polymorphisms and haplotypes of factor VII gene in breast cancer patients (group 1) and control subjects (group 2)

Genotype	Group 1 ($n = 92$)	Group 2 ($n = 80$)	p value
−402GA			
GG	66 (71.7%)	73 (91.3%)	
GA	23 (25%)	7 (8.7%)	0.004
AA	3 (3.3%)	0	
G allele frequency	0.84	0.96	
A allele frequency	0.16	0.04	
−401GT			
GG	56 (60.9%)	41 (51.3%)	
GT	32 (34.8%)	35 (43.7%)	NS
TT	4 (4.3%)	4 (5%)	
G allele frequency	0.78	0.73	
T allele frequency	0.22	0.27	
−323ins10-bp			
w/w	56 (60.9%)	41 (51.3%)	
ins/w	32 (34.8%)	35 (43.7%)	NS
ins/ins	4 (4.3%)	4 (5%)	
w allele frequency	0.78	0.73	
ins allele frequency	0.22	0.27	
Haplotype			
I	36 (39.1%)	34 (42.5)	
II	3 (3.3%)	0	
III	17 (18.5%)	7 (8.8%)	0.017
IV	26 (28.3%)	35 (43.8%)	
V	6 (6.5%)	0	
VI	4 (4.3%)	4 (5%)	

Values are *n* (%)

ins insertion, *w* wild type

Haplotype: I, −402GG/−401GG/−323w/w; II, −402AA/−401GG/−323w/w; III, −402GA/−401GG/−323w/w; IV, −402GG/−401GT/−323ins/w; V, −402GA/−401GT/−323ins/w; VI, −402GG/−401TT/−323ins/ins

frequencies in the breast cancer cases versus control subjects ($p > 0.05$). We found a significant association of −402GA polymorphism with breast cancer risk ($p = 0.004$). The −402 A allele frequency was significantly greater in the breast cancer group than in the control group (0.16 vs. 0.04). Interestingly, two mutations in the promoter region of the FVII gene (−401GT and −323ins10-bp) occurred simultaneously.

We did not detect haplotypes 402AA/−401GG/−323w/w and −402GA/−401GT/−323ins/w among group 2 cases. There was also a significantly difference between the distribution of the six haplotypes groups in breast cancer patients and control subjects ($p < 0.05$).

Discussion

Little knowledge is available in the literature about the association of cancer risk and inherited thrombophilia. Although a range of studies indicates that the hemostatic system plays a key role in the growth, dissemination, and invasion of tumor cells and in tumor-related angiogenesis, its mechanism is still not known in detail [1, 2]. However, the pathogenesis of hemostatic disorders in cancer is mainly on an acquired basis.

Cancer cells may activate the coagulation system directly, thereby generating thrombin, or indirectly, by expression of procoagulants such as TF on cancer cells or by stimulating mononuclear cells to synthesize procoagulants [10]. Thrombin has a significant stimulating effect on angiogenesis. Francis et al. [11] demonstrated that inhibition of TF/FVIIa-dependent FX activation inhibited tumor seeding in athymic mice. Jiang and coworkers [12] first demonstrated that TF-FVII signaling occurred in human breast cancer cells. The inactivated FVII has potential beneficial anti-cancer effects [13]. Moreover, some components of the hemostatic system may modulate the aggressive behavior of malignant tumor [14]. Recently, a study by Tormene et al. [15] has showed that gynecological cancer patients with factor V Leiden (FVL) or prothrombin (PT) G20210A were associated with an advanced stage of the disease or progression of the tumor. In contrast, when we evaluated the effect of the two commonest thrombophilic mutations, FVL and PT G20210A, on breast cancer prognosis it was found that these polymorphisms were not significantly correlated with disease-free survival in breast cancer patients [16].

It is well known that the presence of FVII is responsible for the blood coagulation abnormality in thrombophilia. High plasma levels of FVII can be related to a hypercoagulable state. Three common polymorphisms of the promoter region in the FVII gene—a decanucleotide insertion at position −323 (−323ins10-bp), a G to T substitution at position −401 (−401G/T), and a G to A substitution at position −402 (−402G/A)—have been described and reported to be associated with FVII blood levels [6–8]. The −323ins10-bp polymorphism may affect the transcription rate of the FVII gene. The −323ins10-bp is functionally relevant [6]; the rare insertion allele of 10-bp reduces promoter activity, compared with the common allele, and is related to low plasma levels of FVII. The −401 T allele is also found to be associated with significantly lower plasma levels of FVII than the common −401 G allele, but the rare −402 A allele was associated with significantly higher F VII levels than the common −402 G allele [8].

Different genetic and environmental factors play a role in the development of breast cancer.

Whether the FVII gene polymorphism affects cancer incidence is unknown. To the best of our knowledge, no data regarding the prevalence of FVII gene polymorphisms in breast cancer cases are available in the literature. We, therefore, performed a case–control study to analyze the possible association of the polymorphisms with breast cancer.

In this study, we demonstrated, for the first time, the possible association of FVII −402GA polymorphism with the presence of breast cancer. Two common polymorphisms, −323ins10-bp and −401GT in promoter region of FVII gene were not involved in breast cancer pathogenesis. It can be said that further studies are needed to confirm our findings.

However, we are aware of the limitations of this study. First, we did not determine FVII plasma levels in our cases and control subjects. Another limitation is its small size. Further studies with larger series are necessary to consider the effect of FVII gene polymorphism on breast cancer risk before reaching final conclusion. It is also of great importance to carry out further genetic studies regarding the contribution of other thrombosis-related factors to breast oncogenesis.

Interestingly, the −401GT polymorphism showed complete allelic association with the −323ins10-bp polymorphism. Previously, other authors have also demonstrated the similar finding that the −401GT polymorphism is in strong linkage disequilibrium with the −323ins10-bp polymorphism [7, 8].

Despite the relatively small sample size, several interesting conclusions can be drawn on the basis of our results. The fact that significantly increased incidence of the −402 A allele was observed in breast cancer patients suggests that this allele is probably related to breast cancer. Another interesting observation is that the haplotype II (−402AA/−401GG/−323w/w) and haplotypeV (402GA/−401GT/−323ins/w) were not observed in control subjects.

In conclusion, we have evaluated, for the first time, the possible contribution of the common promoter region polymorphisms of the FVII gene in the development of breast cancer. Our preliminary results indicate that increased prevalence of the −402GA polymorphism may occur directly in malignant tumorogenesis in the human breast. However, further case–control studies with larger series are needed to confirm our findings.

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