Cytokine Gene Polymorphisms and Breast Cancer Susceptibility

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ABSTRACT: Human breast cancer (BC) is characterized by a considerable clinical heterogeneity. Steroid hormone receptor expression and growth factor receptor expression have been considered suitable diagnostic and prognostic markers, whereas mutations of oncosuppressor and gatekeeper genes have been found associated with an increased risk for this malignancy. To evaluate the role that polymorphisms of genes involved in the regulation of inflammatory response might play in BC susceptibility, we investigated associations between cytokine functionally relevant polymorphisms in 84 BC patients compared to 110 age- and sex-matched controls. TNF-α (-308G/A), TGF-β1 (+869C/T), IL-10 (−1117G/A; −854C/T; −627C/A), and IFN-γ (874T/A) single nucleotide polymorphisms (SNPs) were identified by sequence-specific primers (SSP)-PCR or restriction fragment length polymorphism (RFLP)-PCR. Genotype or haplotype distributions for each polymorphisms were consistent with the HWE in these populations. We were unable to demonstrate differences in genotype or allele frequencies between patient and control groups. Data obtained in this study indicate that none of the cytokine SNPs studied is likely to have

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predisposing or protective effects on BC susceptibility. On the other hand, both positive and negative association with BC have been reported for some of the studied genotypes by different research groups. In conclusion, further studies involving larger numbers of subjects are required.

KEYWORDS: **breast cancer; cytokine polymorphisms; susceptibility**

INTRODUCTION

The clinical features of human breast cancer (BC) are characterized by a considerable heterogeneity. Biological markers as steroid hormone receptor expression, angiogenesis, cell proliferation, and cytokine production regulation have been found associated with differences in development and clinical course of this malignancy.[1] Some single nucleotide polymorphisms (SNPs) in nontranscribing region of cytokine genes, as –308G/A of TNF-α, or the IL-10 promoter region haplotypes composed by −1117G/A, −819C/T, and −627C/A SNPs, or the intronic +874T/A SNP of IFN-γ gene, may influence a differential production of the respective cytokine, modifying affinity of regulatory elements to transcription factors.[2] Other SNPs as the +869 C/T (Leu10Pro) of the TGF-β1 gene modify the sequence of critical elements in protein sequence, as the so-called signal peptide of the TGF-β1,[3] influencing cytokine production. These polymorphisms have been investigated by different research groups considering that a genetic regulation of cytokine production might play a role in cancer susceptibility.[3–5] Here are reported the genotype frequencies of some cytokine functional polymorphisms evaluated in a group of patients affected by BC compared to sex- and age-matched control.

MATERIALS AND METHODS

Eighty-four women with BC were recruited from the Department of Oncologic and Surgical Disciplines at the University of Palermo. The control group consisted of 226 unrelated sex- and age-matched healthy subjects, recruited in the same geographic area. The DNA was extracted with the salting out technique. Cytokine polymorphisms were identified by sequence-specific primers or RFLP-PCR. The three Caucasian haplotypes composed by −854C/T, −627C/A, and −1117G/A IL-10 SNP[6] were identified using the haplotype-specific typing method described by Koss *et al.*[7] The Leu10Pro polymorphism was analyzed by SSP-PCR methodology employing two primers sense-specific for +869T and +869C, a primer antisense common to two alleles according to Gewaltig *et al.*[8] +874T/A IFN-γ alleles were identified using the amplification refractory mutation system methodology described by Pravica *et al.*[9] In the TNF-α promoter region, -308G/A SNP

TABLE 1. Genotype frequencies (percentage) of haplotypes obtained by combination of polymorphisms −627C/A, −854C/T, and −1117G/A of IL-10 gene and +869C/T, TGF-β1, +874T/A IFN-γ, −308G/A, and TNF-α SNPs in a group of patients with BC and in a group of control women

		Patients $N = 84$	Controls $N = 106$
	GCC/GCC	16 (19.05)	21 (19.81)
	GCC/ACC	24 (28.57)	24 (22.64)
IL-10	GCC/ATA	16 (19.05)	21 (19.81)
−1117G/A	ACC/ACC	9 (10.71)	14 (13.21)
−854C/T haplotypes	ACC/ATA	14 (16.67)	14 (13.21)
−627C/A	ATA/ATA	5 (5.95)	12 (11.32)
	+869CC	41 (48.81)	35 (33.02)
TGF-β1	+869CT	27 (32.14)	52 (49.06)
+869C/T	+869TT	16 (19.05)	19 (17.92)
	+874TT	30 (35.71)	39 (36.79)
IFN-γ	+874TA	29 (34.52)	38 (35.84)
+874T/A	+874AA	25 (29.76)	29 (27.36)
	−308GG	71 (84.52)	79 (74.53)
TNF-α	−308GA	12 (14.29)	26 (24.53)
−308G/A	−308AA	1 (1.19)	1 (0.94)

was performed as described by Lio *et al*.[10] Comparisons were made among individual genotype frequencies and genotype distributions in the patient group and in the control population, using 2 × 2 and 2 × 3 contingency tables and χ^2 analysis.

RESULTS

Clinical and biological characteristics of the patient were analyzed, evaluating estrogen and progesterone receptor status, lymph node involvement, and tumor size. No associations were found among cytokine polymorphisms and clinical status or grading (data not shown). The genotype distributions for each polymorphism were consistent with the HWE in both the populations. TABLE 1 shows the genotype and haplotype frequencies of cytokine regulatory SNPs in BC patients and healthy controls.

As reported by Eskdale *et al*.,[6] −854C/T and −627C/A IL-10 SNPs are in tight linkage, the linkage of allele −854C with allele −627C and of allele −854T with allele −627A, and the presence of only three different allele combinations of −1117G/A, −854C/T, and −627C/A allows identification of the GCC, ACC, and ATA haplotypes in Caucasians. No significant differences were observed among haplotype combination frequencies in patient versus control groups. Similar results were obtained evaluating +874T/A SNP at the

first intron of IFN-γ genes, +869C/T (Leu10Pro) TGF-β1, and −308G/A SNP at TNFA gene regulatory region.

DISCUSSION

Both tumor and free stromal cells are able to produce cytokines that seem to affect the complex phenomena occurring at the tumor–host interface, thus leading to tumor invasion.[11] Serum levels of many cytokines have been measured in patients with tumors. In particular, abnormal circulating levels or mRNA of IL-10 were observed in ovarian cancer, prostate carcinoma, and BC patients.[12–15] The concentration of circulating IL-10 correlated with the severity of BC as reported by Merendino *et al*.[15] IL-10 has been demonstrated to affect macrophage functions in different ways, weakening and turning off tumor-associated inflammation. The studies on genetic regulation of IL-10 production in patients affected by BC show disagreement between different distributions of IL-10 −1117G/A SNPs in both control and patient populations. We investigated the above-cited polymorphism and IL-10, −854C/T, −627C/A SNPs, in linkage with −1117G/A SNP.[6]

We observed a nonsignificant association between haplotype combination of IL-10 SNPs, as identified by Eskdale *et al*.,[6] and the risk of BC. Thus, our data are at variance with those described by Giordani *et al*.[16] on a population of BC patients of Southern Italy, and so are not clear on the role of IL-10 SNPs studied in susceptibility to BC. We also investigated the polymorphism Leu10Pro of TGF-β1 signaling peptide, extensively described in genetic BC studies. Two large contrasting findings associate the Pro/Pro phenotype with a 64% decreased[17] and 21% increased[18] BC risk. Our data appear to conflict with both large studies, showing nonassociation with TGF-β1 Leu10Pro SNP. On the other hand, our data are confirmed by a large study in Germany.[19]

IFN-γ is a member of a family of cytokines with immunomodulatory and antiproliferative activity. This activity is observed in murine model and *in vitro* on breast tumor–derived cell lines.[20,21] Nevertheless, nonassociation was demonstrated when we investigated the possible correlation of IFN-γ +874T/A SNP with susceptibility to BC.

As well known, TNF-α causes cytolysis and cytostasis of BC cell lines and hemorrhagic necrosis of transplanted tumors.[22] It also mediates IL-1-induced upregulation of HLA class II and is involved in apoptotic pathways. Tumors infiltrating lymphocytes and cells of tumor stroma are able to produce TNF-α.[23] On the other hand, both positive and negative association with BC have been reported for −308G/A genotypes by different research groups.[22,24] The limited sample size characterizing our and other studies could be an important factor explaining the variability among different results obtained for cytokine polymorphisms in the evaluation of susceptibility factors for BC.

In conclusion, further studies are required above all with larger cohorts of patients, before definitive conclusions can be drawn.

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