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ARTICLE

HLA-DRB 1 Alleles and the Susceptibility of Iranian Patients with Breast Cancer

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Breast cancer is considered a major malignancy among women worldwide. The contribution of genetic elements to the onset of familial breast cancer has already been established. The current study investigate the alfele frequency of HLA-DRB 1 in 36 primary operable female breast cancer patients from southern Iran by polymerase chain reaction using sequence specific primers (PCR-SSP). Results were compared with those of 36 female control subjects. Statistical analysis was performed and P values were determined for each character. Our results indicated that the frequency of HLA-DRB 1*12 allele is significantly higher in the patient group ($p<0.03$) compared to the control group. In addition, HLA-DRB1*11 appeared to be as the most frequent allele in the control group (29.2%) and had approximately the same distribution among the patient group (22.5%). (Pathology Oncology Research Vol 7, No 1, 39–41, 2001)

Keywords: breast cancer, HLA-DRB1, PCR-SSP

Introduction

Breast cancer is one of the most common fatal and malignant diseases of women with an increasing incidence in recent years. It is second to lung cancer as a major cause of cancer death in women and is also a leading cause of cancer death at ages 15-54.[1] Although the major etiology of the disease has not yet been known, several factors such as genetic, hormonal, environmental, occupational, and even infectious agents could contribute to the etiology of this disease. In recent years, a large number of data have been published on the role of genetic factors which may contribute to the process of malignant transformation of breast carcinoma (for review see 2). In inherited and familial breast cancer, mutations, deletions and other genetic alterations in the BRCA1 and BRCA2 genes have been considered to be among important genetic risk factors in the etiology of breast cancer in different races and ethnic groups worldwide.[3]

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Considering the fact that the majority of breast carcinoma cases occur sporadically with no familial history of breast or ovarian tumor, search for other genetic risk factors particularly in the sporadic cases is still continuing. The products of the major histocompatibility complex (MHC) genes play a key role in the mounting and recruiting of the cytotoxic T lymphocytes against tumor antigens. These lymphocytes are MHC restricted class I molecules which recognize processed tumor antigenic peptides only in the context of products of MHC class I genes. The loss and the aberration of the expression of HLA class I in many solid tumors have been extensively studied.[4,5] These abnormalities in the mechanisms of expression have always been considered as one of the pathways by which tumor evades from the destructive immune responses. For such reasons, many investigators focus their attention on the role and the significance of HLA-class I in solid tumors, both in understanding the alterations in expression and in allele or locus association with the disease.[6,7,8]

Reports on the association of HLA-class II in breast carcinoma, particularly as a genetic risk factor is limited. In this study, we report the significance of an HLA-DRB 1 allele as an important risk factor for Iranian patients with breast cancer.

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Materials and Methods

Patients and subjects

From 1996 to 1998, thirty-six blood samples were collected from female patients with breast cancer admitted in Nemazi and Faghihi Hospitals (Shiraz University Hospitals, Shiraz-Iran). Thirty-six healthy female blood donors, referred to the Shiraz Blood Transfusion Center (Shiraz-Iran) were considered as control group. Ten milliliters of venous blood with EDTA as anticoagulant, were collected from each subject.

Genomic DNA extraction

Extraction of DNA was done according to a modified Graham and Miller method.[9,10] Briefly, red cells were lysed using RBC lysis buffer-I containing 0.144 M NH_4Cl & 1 mM $NaHCO_3$ and RBC lysis buffer-II containing 0.3 M sucrose, 10 mM Tris-HCl (pH 7.5), 5 mM $MgC1_2$ and 1% Triton-X-100. For destruction of WBCs a lysis buffer containing 0.075 mM NaCI & 0.024 mM Na-EDTA was used. Subsequently, 125 µl of 10% SDS and 1 ml of 5 M $NaC1O_4$ were added. For salting out of proteins, 6 M NaCl was used. DNA was precipitated with isopropanol and washed twice with 70% ethanol.

HLA typing

HLA-DRB1 typing was performed by PCR-SSP according to the Olerup and Zetterquist method.[11] DNA was amplified using 18 PCR reactions for each individual. Each reaction was performed in a total volume of 20 µl containing 17 µl PCR mixture (50 mM KCl, 10 mM $MgC1_2$,10 mM Tris-HCI; pH 8.3, 0.001 (w/v) gelatin, 200 mM of each dNTPs, 1 mM of specific primers and 0.2 mM of the internal control primers), 1 µl template DNA and 2 µl of Taq DNA polymerase (0.5 U/µ1). DNA samples were amplified for 30 cycles. Each cycle consisted of denaturation at 94 °C for 30 seconds, annealing at 55 °C for one minute and extension at 72 °C for one minute. The extension was continued for a further 10 minute at 72 °C. PCR products were electrophoresed on 1.5% agarose gel and the presence of specific DNA bands were analyzed under UV light.

Results

The results of HLA-DRB 1 typing are shown in *Table 1*. As indicated a strong and significant association exists between HLA-DRB1*12 allele and the occurrence of the disease (8/36 in patients verses 1/36 in the control group) ($P < 0.03$). HLA DRB1*11 was found with a higher frequency among control group, but as shown, the frequency of this allele was also found to be high among the patients. HLA-DRB1*11, DRB1*7 and DRB1*04 had higher frequencies in the control group compared to the patient groups. However, these increased frequencies were not statistically significant.

Discussion

In 1995 Casoli and his co-workers, in a short communication, reported a correlation between a decrease in the frequency of HLA-B7 and HLA-DR4 and susceptibility to breast cancer.[12] There is no other report so far available about the association of the frequency of any particular HLA-class II allele and the susceptibility or resistance to the development of breast cancer. Our serological assessment of HLA-class I, indicated no association between any particular HLA-class I allele and breast cancer (data not shown). However, PCR-SSP analysis of HLA-DRBl alleles indicated a significant association between HLA-DRB1*12 allele and susceptibility to breast cancer ($P < 0.027$; $RR = 2.00$). We have previously shown that this allele has a very low frequency in normal Iranian male and female population (manuscript submitted). Although, our control group in the current study consisted only of female subjects, we have observed an identical frequency of this allele as the one we noticed for a large group of normal male and female subjects. This study is the first report on the relationship between the frequency of HLA-DRB1*12 allele and the susceptibility to breast cancer. Most investigators in the field of immunology of solid tumors have focused their attention on the functional characteristics and the biological significance of MHC class I gene products in relation to processing and presentation of tumor antigens to CD8+ T cells. This area of research has provided valuable information on the role of MHC and CD8+ T lymphocytes in immune defense mechanisms against tumor antigens.

Table 1. **HLA-DRB1* alleles in breast cancer patients and normal controls**

DRB1 alleles	*Controls N = 36 (%)*	*Patients N = 36 (%)*	*P value*
01	2 (2.8)	6 (8.3)	NS
03	5 (6.9)	8 (11.1)	NS
04	7 (9.7)	5 (9.6)	NS
07	8 (11.1)	5 (6.9)	NS
08	3 (4.2)	0	NS
09	3 (4.2)	3 (4.2)	NS
10	2 (2.8)	2 (2.8)	NS
11	21 (29.2)	16 (22.2)	NS
12	1 (1.4)	8 (11.1)	<0.03
13	4 (5.6)	2 (2.8)	NS
14	6 (8.3)	8 (11.1)	NS
15	5 (6.9)	5 (6.9)	NS
16	5 (6.9)	4 (5.6)	NS

As the experimental data concerning the capabilities of the MHC class II molecules in processing of endogenous peptides to anti-tumor CD4+ T cells is emerging,[13,14,15] the role and the properties of these molecules in anticancer strategies against solid tumors must be seriously reexamined. In a recent study Feinmesser et al[16] reported a significant correlation between tumor type und the expression of HLA-DR antigens in breast carcinoma. They found a higher expression of HLA-DR and β2 macroglobulin in medullary carcinoma of breast with a relatively favorable prognosis. It is noteworthy that the potential effector activities of CD8+ T cells und the prolongation of antitumor memory is dependent on the state of activation und proliferation of CD4+ T cells which are primed by the tumor encoding antigens in the context of MHC class II gene products. It has been shown that the CD4+ T lymphocytes infiltrating human breast cancer can recognize autologous tumor antigens in a MHC-class-II restricted fashion und secrete cytokines in response to these antigens.[17] In this case, it is likely that the inherited individual specificities affect the quality und quantity of the anti-tumor immune response. A so called „allele effect" may have an influence on CD4+ T cell cytotoxicity against tumor peptides, on the phenotype (secreted cytokine profiles) and on the function of the CD4+ helper T cells. If so, it would be worthwhile to investigate the association of HLA-DR allele frequencies with breast cancer risk in different populations to assess the importunt alleles that could be considered as risk factors in breast cancer development.

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