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ARTICLE

HLA Class II Polymorphism: Protective or Risk Factors to Breast Cancer in Tunisia?

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HLA system plays a key role in the tumor cells' escape from immune surveillance. Herein is the first report on the correlation of the susceptibility to breast cancer with HLA class II markers in Tunisia. Molecular typing of HLA-DRB1 and -DQB1 loci was undertaken for 70 Tunisian female patients. Comparison of allele and haplotype distribution between patients and 70 female control subjects reveals a negative association between HLA-DRB1*07-DQB1*02 and the incidence of breast cancer in the Tunisian population. (Pathology Oncology Research Vol 12, No 2, 79–81)

Key words: HLA, DRB1, DQB1, breast cancer

Introduction

The process of oncogenesis depends on the genetic instability of tumors and interactions with their immunological environment. Considering the importance of immune surveillance during tumorigenesis,[13,17] some individuals who inherit specific alleles or haplotypes of the highly polymorphic HLA genes may be exposed or may resist to specific types of cancers.[2,3,21,23,25] In fact, besides its crucial role in human clinical transplantation, the HLA system plays a key role in the mounting and recruiting of the cytotoxic T lymphocytes against tumor antigens that may contribute to tumor evasion from the destructive immune responses.

The major histocompatibility complex in humans, HLA, is a highly variable genetic system that is constituted by six main polymorphic loci, A, B, C, DRB1, DQB1 and DPB1, located on the short arm of chromosome 6.[9,22] During the last decade, molecular typing techniques have been developed to identify HLA alleles at DNA level with a high-resolution power, and DNA mutations now allow the identification of a high number of alleles according to the loci (121, 69, 429, 751, 219 and 429 alleles described in 2006 for the loci DPB1, DQB1, DRB1, B, C and A, respectively (www.ebi.ac.uk/imgt/hla/)).

Among the different HLA loci, DRB1 and DQB1 have been accurately tested at the molecular level since the 11th international histocompatibility workshop. The DRB1 and DQB1 loci are only separated by 85 kb on the chromosome, and almost no cases of recombination between these two loci have been reported in family studies. Therefore, HLA-DRB1 and DQB1 alleles generally exhibit a highly significant level of linkage disequilibrium.[5]

Until now, few reports have been carried out on the association of HLA antigens and the susceptibility to breast cancer.[1,4,6,15,24] Some of these studies are limited to a small number and lack enough power to reach significance. Three reports have analyzed the polymorphism of HLA-class II genes in breast cancer as a genetic risk factor.[8,10,14] The DRB1*11 allele was defined as protector in Caucasian population, [10] while DRB1*12 was positively associated with breast cancer for Iranian women.[14] Other studies examined single nucleotide polymorphisms (SNPs) in the HLA class III region.[11,19,20]

In order to better understand the role played by the HLA system in the process of oncogenesis, it would be very interesting to search for an association between the HLA polymorphism genes and breast cancer risk in different populations. Such studies will allow us to detect the hid-

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den alleles that can be considered as risk factors in breast cancer development.

Our study is a detailed molecular analysis of HLA DQB1, DRB1 alleles and HLA DRB1-DQB1 haplotypes for Tunisian patients with breast cancer and ethnically matched controls.

Materials and Methods

Population samples

Blood samples were collected from 70 female patients with breast cancer admitted in Salah Azeiz Oncology Institute from April to August, 2001. The age of patients ranged between 27 and 67 years. Seventy healthy female blood donors from the Tunisian Center of Blood Transfusion were considered as a control group. All subjects were unrelated volunteers originated from Tunisia. Five milliliters of venous blood with EDTA, as anticoagulant, were collected from each subject.

DNA typing

Genomic DNA was extracted from peripheral blood according to the method of Maniatis.[18] The second exon of both DRB1 and DQB1 loci and exon 3 of DQB1 were amplified by a polymerase chain reaction (PCR) using specific primers provided by Innolipa kits from Innogenetics (Ref: 80617 and 80337 for DRB1 and DQB1 loci, respectively). The HLA allelic polymorphism of these two loci was then analyzed by RDB hybridization[7,16] using Innolipa kits from Innogenetics (Ref: 80635 and 80336 for DRB1 and DQB1 loci, respectively).

Statistical analysis

HLA-DRB1 and -DQB1 allele and haplotype frequencies were directly estimated by the following equation [f= (n/N)x100] where "n" is the number and "N" is the total number of alleles or haplotypes. Allelic and haplotype frequencies in cancer patients and controls were compared using Pearson's χ^2 test or Fisher's exact test (when the number of subjects in a cell is <5). Odds ratios are given with 95% confidence limits. The EPI INFO 6 package program was used for this statistics analysis.

Results

HLA-DRB1 and -DQB1 typing has been undertaken for 70 patients with breast cancer and 70 healthy matched donors. For the DRB1 loci, the results showed a total of 11 alleles in patients with breast cancer and 12 alleles in the matched controls (*Table 1*). For DQB1 locus only five alleles were found (*Table 2*). No significant correlation with incidence of breast cancer was observed for these two loci.

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

DRB1 alleles	*Patients N=70 (%)*	*Controls N=70 (%)*	*P value*
DRB1*01	7 (5)	8 (5.71)	NS
DRB1*03	29 (20.71)	19 (13.57)	NS
DRB1*04	13 (9.28)	17 (12.14)	NS
DRB1*07	14 (10)	23 (16.42)	NS
DRB1*08	3 (2.14)	1 (0.71)	NS
DRB1*09	1 (0.71)	0	NS
DRB1*10	4 (2.85)	1 (0.71)	NS
DRB1*11	26 (18.57)	29 (20.71)	NS
DRB1*12	4 (2.85)	5 (3.57)	NS
DRB1*13	23 (16.42)	15 (10.71)	NS
DRB1*14	0	1 (0.71)	NS
DRB1*15	16 (11.42)	20 (14.28)	NS
DRB1*16	0	1 (0.71)	NS

Nominal value for comparison, P= 0.05; degree of freedom = 1; NS: not significant

Table 2. **HLA-DQB1* alleles in breast cancer patients and normal controls**

DQB1 alleles	*Patients N= 70 (%)*	*Controls N=70 (%)*	*P value*
DQB1*02	38 (27.14)	40 (28.57)	NS
DQB1*03	46 (31.96)	45 (32.14)	NS
DQB1*04	5 (3.57)	6 (4.28)	NS
DQB1*05	17 (12.14)	18 (12.85)	NS
DQB1*06	34 (24.28)	31 (22.14)	NS

Nominal value for comparison, P= 0.05; degree of freedom = 1; NS: not significant

The results of HLA-DRB1-DQB1 haplotypes are shown in *Table 3*. As indicated, a negative significant association exists between HLA-DRB1*07-DQB1*02 haplotype and the occurrence of the disease [9/140 (6.42%) haplotypes in patients, versus 22/140 (15.71%) in control group] (P= 0.013; OR= 0.37; 95% confidence interval: 0.15-0.88).

Conclusion

Our study is the first report on the correlation of the susceptibility to breast cancer with HLA class II markers in Tunisian patients. The results revealed a negative association between HLA-DRB1*07-DQB1*02 and the occurrence of breast cancer. This association could be linked to the role of HLA class II molecules in the anti-tumoral response, or explained by linkage disequilibrium between these markers and the susceptibility or protective genes.

In fact, recent research has focused on the functional characteristics and the biological significance of MHC class II gene products in relation to processing and presen-

Table 3. **HLA-DRB1*-DQB1* haplotypes in breast cancer patients and normal controls**

DRB1-DQB1 haplotypes	*Patients N=70 (%)*	*Controls N= 70 (%)*	*P value*
DRB1*01 -DQB1*05	6 (4.28)	8 (5.71)	NS
DRB1*01-DRB1*03	1 (0.71)	0	NS
DRB1* 03-DQB1*02	27 (19.28)	18 (12.85)	NS
DRB1*03 -DQB1*04	2 (1.42)	0	NS
DRB1*03-DQB1*06	0	1 (0.71)	NS
DRB1*04 -DQB1*03	11 (7.85)	12 (8.57)	NS
DRB1*04 -DQB1*04	2 (1.42)	5 (3.57)	NS
DRB1*07 -DQB1*02	9 (6.42)	22 (15.71)	P = 0.013
DRB1*07 -DQB1*03	5 (3.57)	1 (0.71)	NS
DRB1*08 -DQB1*03	2 (1.42)	0	NS
DRB1*08 -DQB1*04	1 (0.71)	1 (0.71)	NS
DRB1*09 -DQB1*02	1 (0.71)	0	NS
DRB1*10 -DQB1*05	4 (2.85)	1 (0.71)	NS
DRB1*11 -DQB1*03	22 (15.71)	22 (15.71)	NS
DRB1*11 -DQB1*05	4 (2.85)	7 (5)	NS
DRB1*12 -DQB1*03	2 (1.42)	5 (3.57)	NS
DRB1*12 -DQB1*05	2 (1.42)	0	NS
DRB1*13 -DQB1*02	1 (0.71)	0	NS
DRB1*13 -DQB1*03	3 (2.14)	5 (3.57)	NS
DRB1*13 -DQB1*06	19 (13.57)	10 (7.14)	NS
DRB1*14 -DQB1*05	0	1 (0.71)	NS
DRB1*15 -DQB1*05	1 (0.71)	0	NS
DRB1*15 -DQB1*06	15 (10.71)	20 (14.28)	NS
DRB1*16 -DQB1*05	0	1 (0.71)	NS

Nominal value for comparison, P= 0.05; degree of freedom = 1; NS: not significant

tation of tumor antigens and their role in anticancer strategies against solid tumors. Indeed, Feinmesser and his coworkers have demonstrated that it is likely that the inherited individual specificities affect the quality and quantity of anti-tumor immune response.[12]

Our study reveals a new association between HLA markers and breast cancer, and proves that it would be worthwhile to investigate the association of these markers with breast cancer risk in different world populations.

It is important to note that although we have examined a reasonable number of women with breast cancer, clearly more extensive studies need to be conducted on a large sample of patients and controls, in order to confirm this association and to detect other important alleles or haplotypes that could be considered as risk factors in the occurrence or presentation of breast cancer.

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