ORIGINAL ARTICLE

HLA A genotyping in Tunisian Women with Breast Cancer: Correlations with Genetic Susceptibility and Histoprognostical Parameters of Breast Tumours

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Key words: HLA A alleles, Breast cancer, EE grading, Ganglionic invasion.

Submitted: 06 November 2008; Accepted: 16 December 2008

ISSN: 2070-254X

Abstract

Innumerable reports aimed at discovering the role of HLA in the control of responsiveness and disease susceptibility. Many of these studies allowed defining several HLA associations to cancers in different human populations.
In the present study, HLA A molecular typing using luminex technology was undertaken in 64 Tunisian women with breast cancer and 74 unrelated ethnically matched controls healthy females in order to define alleles of protection or susceptibility to breast tumors in Tunisia.
Our data revealed A*30 as risk factor for breast tumors prevalence. Statistical analysis comparing A*30 frequencies between EE III grade patients and healthy controls showed the positive association between this allele and the worst prognosis of the disease (EE III).
However, our results define HLA A*01 as conferring protection against the most serious presentation of breast tumours in Tunisia.

Introduction

Innumerable reports aimed at discovering the role of HLA in the control of responsiveness and disease susceptibility. Many of these studies allowed defining several HLA associations to cancers in different human populations 1-15 especially breast cancer 3;7;11;12;14;15. As described in literature, some alleles of the studied locus were considered as negatively3-11 or positively 12 associated to the occurrence of breast cancer. Further more, a previous study 14 dealing with the correlations between HLA DRB1, DQB1 and breast cancer susceptibility in Tunisian patients showed that DRB1*07-DQB1*02 haplotype is negatively associated to breast cancer occurrence.
HLA class I molecules play a major role in the anti tumor surveillance by presenting peptides of tumor antigens to CD8+ cytotoxic cells16. Decreased expression of these antigens in tumor cells may contribute to an evasion of immune system and consequently to enhanced tumor growth 17-19.
However, not all tumors expressing low levels of HLA antigens show increased malignancy, probably as a result of the differential activity of the oncogenes involved in malignant transformation or the HLA polymorphism. In fact, HLA system is represented by highly polymorphic genes especially class I.
According to the last updated nomenclature in 2007, 649 alleles were defined for HLA A (http://www.ebi.ac.uk/imgt/hla/stats.html).
This high polymorphism leads to the recognition of a larger panel of antigens. But, the specificity will not be the same according to the expressed allelic version. As a consequence of this variable genetic system, subjects will not have the same immunocompetence against tumor.
In the present study, HLA A molecular typing using luminex technology20. was undertaken in 64 Tunisian women with sporadic breast cancer and 74 unrelated ethnically matched controls healthy females in order to define alleles of protection or susceptibility to breast tumors in Tunisia.

Materials and methods

Studied populations

The present study includes 64 Tunisian women between the age of 27 to 67 years and who are undergoing treatment in the Salah Azaiz oncology institute from April to August 2001. An informed consent, using a standardized written form was obtained from each patient. All has histological verification of sporadic breast cancer. The EE (Elston and Ellis) grading21 was given for only 55 subjects: 6 EE I, 19 EE II, 30 EE III. The axilary lymph nodes were positive for 31 patients, and negative for 16 patients, for the remaining cases, no information was available.
74 Healthy and unrelated women served as controls. All subjects were unrelated volunteers and are originated from Tunisia. Absence of breast cancer familiar tumor attack was verified.
Five milliliters of venous blood with EDTA, as anticoagulant, were collected from each subject.

HLA A typing

Genomic DNA was extracted at phenol chloroform method. The most polymorphic exons of HLA A locus were amplified by PCR SSO using biotinilyed primers given by Labtype amplifying kits (Labtype amplification kit: LABType® SSO, RSSO-1 A, One Lambda).
The obtained amplicons are hybridized to probes immobilised on fluorescent beads

(Labtype hybridization kit: LABType® SSO, RSSO-1 A, One Lambda).
The beads are then red by the apparatus LABScan™ 100 and genotypes deduced by « LABType® SSO Software for Windows® (LTYPPGR) ».

Statistical analysis

HLA-A alleles frequencies were directly estimated by the following equation [f= (nlN)x100] were "n" is the number and "N" is the total number of alleles. Allelic frequencies in cancer patients and controls were compared using Pearson's $\chi 2$ test or Fisher's exact test when appropriate. Odds ratios are given with 95% confidence limits. The EPI INFO 6 package program was used for this statistics analysis (http://www.ensp.fr/services/logiciels/epiinfo_604d_fr.htm).

Results

HLA A and breast cancer occurrence in Tunisia (Table 1)

In order to define possible associations between HLA A alleles and the occurrence of breast cancer, we compared HLA polymorphism between patients and healthy controls.
Among the nineteen HLA A alleles given by molecular typing, HLA A*02 was the most frequent allele both in patients and controls groups (22,66% versus 26,35%).
Whereas HLA A*30 which is highly (10,94%) represented within patients is considered rare in the healthy group (4,05%). After applying statistical analysis, A*30 seems to be positively correlated to the occurrence of breast cancer in Tunisia with p= 0,027; OR=2,91 , CI from 1 to 8,79.

Association of HLA A polymorphism to the EE grading (Table 2)

HLA A alleles frequencies are compared between patients with different EE grading on the one hand and with healthy controls, on the other hand.
HLA A*01 is significantly less frequent among EE III subjects (N=30) than EEI/II (N=25) ones (8,33% versus 24%) with a protection (OR=0,29, CI from 0,08 to 0,98) against the most serious presentation of the disease (EE III grade). This allele shows a significant difference between EE III grade group and healthy women (24% versus 12,16%; p=0,043) with no significant association according to OR confidence interval (OR= 2,28, CI]0,93-5,53[).
Where as HLA A*30 which is higher represented within EEIII women than controls (13,33 versus 4,05%) shows a positive association to the higher grading (EE III) of breast tumour (OR=3,64, CI]1,08- 12,53[).

Is HLA A polymorphism correlated with the ganlionic invasion in breast cancer? (Table 3)

In the aim of focusing for a possible associations of HLA A alleles to the ganglionic envadment in Tunisian women with breast tumours, we compared HLA A alleles frequencies between 31 N+ patients (with ganglionic envadment) and 16 N- patients (without ganglionic envadment) or healthy subjects.
Only HLA A*30 is defined as a risk factor (p=0,01; OR=4,38; CI]1,06- 17,84[) for the occurrence of the breast cancer with no ganglionic invasion.

Discussion

It was largely reported that HLA class I expression may affect considerably tumor progression. In fact, it is well established that a reduced HLA class I antigen expression confers a metastatic advantage19. To the expression level impact, we can associate HLA polymorphism effect since the allelic version may modulate the efficiency of antigen presentation and consequently the immune response, especially anti-tumor surveillance.
In the aim of defining risk or protector factors, many studies have been undertaken in several ethnic groups and prove negative or positive correlations between HLA polymorphism and cancers 1-15.
In the present work we focused on the relationship between HLA class I alleles and breast cancer in the Tunisian population. This is the first report that deals with defining correlations between HLA A and the occurrence of mammary tumors in Tunisian women. Previous studies were interested in HLA DRB1 and DQB114.
Our data revealed A*30 as risk factor for breast tumors prevalence. This correlation is confirmed by the statistical analysis comparing A*30 frequencies between EE III grade patients and healthy controls. In fact, this allele is significantly associated to the worst prognosis of the disease (EE III). However, A*30 is positively associated to the absence of lymph node attack. This is probably due to the easier diagnosis of the worst presented tumours (EEIII) just before that ganglion invasion occurs. In fact, 42,85% of EEIII and A*30 positive subjects are N- (with no lymph node envadment). So, A*30 seems not to contribute genetically in ganglion invasion but only in the susceptibility to the highest histological EE grade (EEIII) of breast cancer in Tunisia.
Furthermore, our results define HLA A*01 as conferring protection against the most serious presentation of breast cancer in Tunisia. This finding is in contrast with a previous serological study defining HLA A1 as protector factor1.Other serological studies have reported positive associations for A24, A28 antigens to mammary tumours in different populations 2;5.
The positive or negative correlations between HLA A alleles and disease are not related to gene polymorphism only, but also to the disequilibrium linkage between this locus and HLA B, Cw or class II loci. So, typing women for the susceptibility alleles in one gene will not be sufficient for disease prognosis. But, it will be more interesting to investigate HLA typing in a larger sample considering class I and II markers in order to search for haplotypes conferring a protection or enhancing breast cancer risk in the studied population. The genetic correlations may be more significant if taking into account the histoloprognostical parameters of the disease.
The definition of protective or risk haplotypes may allow deducing the efficiency of the immune system in tumour evolution because of the key role of HLA molecules in antigen presentation.
Our study revealed new associations between HLA markers and breast cancer and proves that it is worthwhile to investigate the association of these markers with breast cancer risk in different world populations because of the richness of genetic background.

References

1.Feingold N, Degos L, Feingold J: HLA in populations: an approach forgenetical susceptibility to cancer. J Immunogenet 1 : 29-35, 1979
2.Yokoe T, Ishida T, Ogawa T, et al: Relationship of breast cancer and HLA in Japanese females. Gan No Rinsho 36: 29-33, 1990
3.Casoli C, Zanelli P, Adorni A, et al: Serological and molecular study on the HLA phenotype of female breast cancer patients. Eur J cancer 30: 1207-1208, 1994
4.Howell WM, Leung ST, Jones DB, et al: HLA-DRB, -DQA, and -DQB polymorphism in celiac disease and enteropathy-associated T-cell lymphoma. Common features and additional risk factors for malignancy. Hum Immunol 43: 29-37,1995

5.Bouillenne C, Deneufbourg JM. Positive correlation between breast cancer incidence and HLA antigens. Oncology 36: 156-159, 1997

6.Ozdemir E, Kakehi Y, Mishina M, et al:
High-resolution HLA-DRB1 and DQB1 genotyping in Japanese patients with testicular germ cell carcinoma. Br J Cancer 76 (10): 1348-1352, 1997

7. Biswal BM, Kumar R, Julka PK,et al: Human leucocytic antigens (HLA) in breast cancer. Indian J Med Sci 52 : 177-183, 1998

8. Tokumoto H. Analysis of HLA-DRB1-related alleles in Japanese patients with lung cancer--relationship to genetic susceptibility and resistance to lung cancer. J Cancer Res Clin Oncol 124 (9): 511-516, 1998

9. Ferrera A, Olivo A, Alaez C,et al :. HLA DOA1 and DOB1 loci in Honduran women with cervical dysplasia and invasive cervical carcinoma and their relationship to human papillomavirus infection. Hum Biol 71 (3): 367-379, 1999

10-Mokni-Baizig N, Ayed K, Ayed FB,et al : Association between HLA-A/-B antigens and –DRB1 alleles and nasopharyngeal carcinoma in Tunisia. Oncology 61 (1): 55-58, 2001

*11. Chaudhuri S, Cariappa A, Tang M,et al: Genetic susceptibility to breast cancer: HLA DQB*03032 and HLA DRB1*11 may represent protective alleles. Proc Nati Acad Sci 97/21: 11451-11454, 2000*

12. Ghaderi A, Talei A, Gharesi-Fard B, et al: DRB1 alleles and the susceptibility of Iranian patients with Breast cancer. Pathol Oncol Res 1:39-44, 2001

13. Beskow AH, Gyllensten UB. Host genetic control of HPV 16 titer in carcinoma in situ of the cervix uteri. Int J Cancer 101 (6): 526-531, 2002

14.Baccar HA, Yacoubi LB, Troudi W, et al: HLA Class II Polymorphism: Protective or risk factors to breast cancer in Tunisia? Pathol and Oncol Res 12:79-81, 2006

15. Gopalkrishnan S, Patil, S, Badwe CR, et al: HLA alleles in pre-menopausal breast cancer patients from western India. L. Indian J Med Res 124: 305-312, 2006

16. Holler PD, Kranz DM: Quantitative analysis of the contribution of TCR/pepMHC affinity and CD8 to T cell activation. Immunity 18: 255–264, 2003

17. Geopel JR, Rees RC, Rogers K,et al: Loss of monomorphic and polymorphic HLA antigens in metastatic breast and colon carcinoma. Br J Cancer 64 : 880-883, 1991

18. Moller P, Hammerling GJ. The role of surface HLA-A,B,C molecules in tumour immunity. Cancer Surv 13: 101-127, 1992

19. Saio M, Teicher M, Campbell G, et al: Immunocytochemical demonstration of down regulation of HLA class-I molecule expression in human metastatic breast carcinoma. Clin Exp Metastasis 21: 243-249, 2004

20. Dalva K, Beksac M : HLA typing with sequence-specific oligonucleotide primed PCR (PCR-SSO)and use of the Luminex technology. Methods Mol Med 134:61-69, 2007

21. Elston CW, Ellis IO : 1991. Pathological prognosis factors in breast cancer.I. The value of histological grade in breast cancer : experience from a large study with long-term follow-up. Histopathology 19: 403-410, 1991

Tables

Table 1. HLA alleles and breast cancer occurrence in Tunisia

HLA A ALLELES	Patients N=64 n(%)		Controls N=74 n(%)		p value	OR	OR 95% Confidence interval (CI)
A*01	18	(14,06)	18	(12,16)	0,64		
A*02	29	(22,66)	39	(26,35)	0,477		
A*03	15	(11,72)	13	(8,78)	0,42		
A*08	1	(0,78)	0	(0)	0,463		
A*11	5	(3,90)	4	(2,70)	0,410		
A*23	8	(6,25)	11	(7,43)	0,698		
A*24	8	(6,25)	14	(9,46)	0,326		
A*25	1	(0,78)	0	(0)	0,46		
A*26	1	(0,78)	5	(3,38)	0,144		
A*29	3	(2,34)	3	(2,02)	0,58		
A*30	**14**	**(10,94)**	**6**	**(4,05)**	**0,027**	**2,91**	**] 1 ; 8,79[**
A*31	0	(0)	2	(1,35)	0,28		
A*32	5	(3,90)	6	(4,05)	0,95		
A*33	3	(2,34)	7	(4,72)	0,233		
A*34	1	(0,78)	3	(2,02)	0,367		
A*66	2	(1,66)	2	(1,35)	0,632		
A*68	9	(7,03)	12	(8,11)	0,736		
A*74	1	(0,78)	2	(1,35)	0,554		
A*80	4	(3,12)	1	(0,68)	0,143		

Table 2. HLA A associations to Elston and Ellis grading of breast tumours in Tunisia

HLA A alleles	Patients EE III N=30 n(%)	Patients EE I/II N=25 n(%)	Controls N=74 n(%)	p value		
				Patients EE III vs EEI/II	Patients EE III vs Controls	Patients EEI/II vs Controls
A*01	5 (8,33)	12 (24)	18 (12,16)	**0,023*1**	NS	**0,043*3**
A*02	16 (26,66)	10 (20)	39 (26,35)	NS	NS	NS
A*03	5 (8,33)	8 (16)	13 (8,78)	NS	NS	NS
A*08	1 (1,66)	0 (0)	0 (0)	NS	NS	NS
A*11	2 (3,33)	1 (2)	4 (2,70)	NS	NS	NS
A*23	6 (10)	2 (4)	11(7,43)	NS	NS	NS
A*24	3 (5)	3 (6)	14 (9,46)	NS	NS	NS
A*25	1 (1,66)	0 (0)	0 (0)	NS	NS	NS
A*26	0 (0)	1 (2)	5 (3,38)	NS	NS	NS
A*29	2 (3,33)	1 (2)	3 (2,02)	NS	NS	NS
A*30	8 (13,33)	2 (4)	6 (4,05)	NS	**0,01*2**	NS
A*31	0 (0)	0 (0)	2 (1,35)	NS	NS	NS
A*32	4 (6,66)	1 (2)	6 (4,05)	NS	NS	NS
A*33	1 (1,66)	1 (2)	7 (4,72)	NS	NS	NS
A*34	1 (1,66)	0 (0)	3 (2,02)	NS	NS	NS
A*66	0 (0)	1 (2)	2 (1,35)	NS	NS	NS
A*68	4 (6,66)	4 (2)	12 (8,11)	NS	NS	NS
A*74	1 (1,66)	0 (0)	2 (1,35)	NS	NS	NS
A*80	0 (0)	3 (6)	1 (0,68)	NS	NS	NS

*1 OR= 0,29 ; OR 95% confidenc Intervall (CI):]0,08-0,98[
*2 OR= 3,64 ; OR 95% confidenc Intervall (CI):]1,08-12,53[
*3 OR= 2,28; OR 95% confidenc Intervall (CI):]0,93-5,53[

Table 3. Correlations between HLA A polymorphism and the ganglionic invasion in Tunisian women with breast cancer

HLA A alleles	Patients N+ N=31 n(%)	Patients N- N=16 n(%)	Controls N=74 n(%)	p value		
				Patients N+ vs N-	Patients N+ vs Controls	Patients N- vs Controls
A*01	9 (14,51)	5 (15,62)	18 (12,16)	NS	NS	NS
A*02	13 (20,96)	11 (34,37)	39 (26,35)	NS	NS	NS
A*03	9 (14,51)	2 (6,25)	13 (8,78)	NS	NS	NS
A*08	1 (1,61)	0 (0)	0 (0)	NS	NS	-
A*11	2 (3,22)	0 (0)	4 (2,70)	NS	NS	NS
A*23	4 (6,45)	1 (3,12)	11 (7,43)	NS	NS	NS
A*24	2 (3,22)	2 (6,25)	14 (9,46)	NS	NS	NS
A*25	1 (1,61)	0 (0)	0 (0)	NS	NS	-
A*26	0 (0)	0 (0)	5 (3,38)	-	NS	NS
A*29	3 (4,83)	0 (0)	3 (2,02)	NS	NS	NS
A*30	5 (8,06)	5 (15,62)	6 (4,05)	NS	NS	0,01*
A*31	0 (0)	0 (0)	2 (1,35)	-	NS	NS
A*32	3 (4,83)	2 (6,25)	6 (4,05)	NS	NS	NS
A*33	1 (1,61)	1 (3,12)	7 (4,72)	NS	NS	NS
A*34	1 (1,61)	0 (0)	3 (2,02)	NS	NS	NS
A*66	0 (0)	0 (0)	2 (1,35)	-	NS	NS
A*68	5 (8,06)	3 (9,37)	12 (8,11)	NS	NS	NS
A*74	1 (1,61)	0 (0)	2 (1,35)	NS	NS	NS
A*80	2 (3,22)	0 (0)	1 (0,68)	NS	NS	NS

* OR= 4,38 ; OR 95% confidenc Intervall (CI):]1,06-17,84[