ARE HLA DQB1 ALLELES CORRELATED WITH BREAST CANCER HISTOPRONOSTIC PARAMETERS IN TUNISIA?

Amal Baccar*, Besma Yacoubi Loueslati*, Wafa Troudi*, Slama Hmida**, Amel Dridi**, Afef Jridi**, Karima Mrad***, Khaled Ben Romdhane***, Amel Ben Ammar Elgaaied*

*- *Laboratoire de Génétique, Immunologie et Pathologies Humaines. Faculté des sciences de Tunis. -*
** *Service d'hémato-immunologie du centre national de transfusion sanguine de Tunis.****- Institut de Carcinologie Salah AZAIZ*

A. Baccar, B. Yacoubi Loueslati, W. Troudi, S. Hmida, A. Dridi, A. Jridi, K. Mrad, K. Ben Romdhane, A. Ben Ammar Elgaaied

LES ALLELES DQB1 SONT-ILS CORRELES AUX PARAMETRES HISTOPRONOSTIQUES DU CANCER DU SEIN EN TUNISIE?

LA TUNISIE MEDICALE - 2009 ; Vol 87 (n°06) : 372 - 374

A. Baccar, B. Yacoubi Loueslati, W. Troudi, S. Hmida, A. Dridi, A. Jridi, K. Mrad, K. Ben Romdhane, A. Ben Ammar Elgaaied

ARE HLA DQB1 ALLELES CORRELATED WITH BREAST CANCER HISTOPRONOSTIC PARAMETERS IN TUNISIA?

LA TUNISIE MEDICALE - 2009 ; Vol 87 (n°06) : 372 - 374

RÉSUMÉ

Prérequis : Les tumeurs expriment à leur surface des structures différentes de celles des cellules normales. Ces structures pourraient donc être reconnues par le système immunitaire qui assure la surveillance anti-tumorale. La présentation de ces antigènes fait intervenir les molécules HLA. Ces molécules étant codées par un système très polymorphe, les réponses immunes seraient modulées selon le patrimoine HLA disponible dans l'organisme. Le polymorphisme HLA pourrait donc être corrélé à l'échappement des cellules tumorales à la surveillance anti-tumorale.

But: Notre but est de rechercher d'éventuelles corrélations entre les allèles DQB1 et les parmètres histopronostiques des cancers mammaires dans la population tunisienne.

Méthodes : Les allèles DQB1 sont déduits par typage moléculaire PCR-SSO chez 100 femmes tunisiennes saines et non apparentées et 87 patientes.

Résultats : La distribution allélique chez la population malade et la population témoin a démontré l'absence de corrélations du locus DQB1 à la susceptibilité, le grade EE et l'envahissement ganglionnaire du le cancer du sein dans la population tunisienne.

Conclusion : Ces résultats pourraient être expliqués par le fait que le cancer du sein est une maladie multifactorielle qui résulte de l'interaction de plusieurs facteurs variables d'une population à l'autre.

MOTS-CLÉS

DQB1, cancer du sein, paramètres histopronostiques.

SUMMARY

Background: Tumor cells express surface structures different from normal cells. These structures may be recognized by the immune system, which ensure anti-tumoral surveillance. Antigenic presentation requires HLA molecules role. Since, these molecules are encoded by a high polymorphic system, immune response can be modulated according to HLA genotype. So, HLA polymorphism could be correlated with tumor escape from anti-tumor immunosurveillance.

Aim : We have aimed to search for possible associations between HLA DQB1 alleles and the histoprognostical parameters in breast cancer in the Tunisian population.

Methods: DQB1 alleles were determined by PCR-SSO molecular typing in 100 healthy matched and unrelated Tunisian female and 87 Tunisian women with breast cancer.

Results: Allelic distribution between the two studied groups showed no significant associations between this locus and the occurrence, the EE grade and the lymph node invasion of breast cancer in the Tunisian population.

Conclusion: This result may be explained by the fact that cancer is a multifactoral disease due to several interacting factors that might change from one population to another.

KEY-WORDS

DQB1, breast cancer, histoprognostical parameters

بالمعطيات المرضية و النسيجية لسرطان الثدي بتونس - 1صأط ماهي علاقة الجين

الباحثون : بكار .أ - يعقوبي الوسلاتي . ب - الطرودي . و - حميدة . س - دريدي . أ - الجريدي . أ - مراد . ك - بن رمضان . ك - بن عمار القايد . أ.

خلال هده الدراسة قمنا بالزمر النسيجي للجين 1صأط ومقارنة الصيغ بين مجموعة من النساء تعانين من درجات مختلفة من سرطان الثدي ومجموعة ثانية سليمة من أي مرض.

الاختلافات المسجلة للصيغات بين الفئات المدروسة لم تبين أي علاقة حماية أو مضاعفة لإمكانية تطور سرطان الثدي عند المرأة التونسية.

Breast cancer is known to be the main cause of women's death all over the world. Many types of diagnoses concerning this disease are made. In this work, we will focus on the histo-prognostical and anatomo-clinical parameters.

The histoprognostical diagnosis evaluates the seriousness of the disease. This analysis defines the histological grade by combining the following components: Tumor differenciation importance (presence or absence of glanduliform tubes), Tumor cells nucleus irregularity and Tumor cells mitotic activity. The most common grading is SBR (Scraff, Bloom and Richardson) [1] modified by Elston and Ellis (EE) [2]. According to this grading type, there are three EE grades: I, II and III meaning respectively, favorable, intermediate and unfavorable. However, Lymph node invasion is an anatomo-clinical criterion allowing appreciating the evolutionary stage of the cancer. This parameter defines the evolution of the tumor; the more the number of metastatic lymph node is high, the more the prognosis is worst.The immune system has the role of protection of the body against alterations that could disturb its integrity. So, it ensures the body anti-tumoral surveillance. Since, tumor cells express particular structures different from normal cells, the immune system recognizes them as antigens and react in order to destroy them. But, escape phenomena can take place in particular circumstances (Low-antigenicity, immune deficits), and a cancer occurs [3]. Antigen presentation is mediated by HLA class I and II molecul

es. Since this genetic system is highly polymorphic, individuals don't have the same ability to react against an organism alteration. Therefore, HLA alleles may prove different implications in tumors susceptibility. This was reported in many studies in different cancer types especially breast cancer within various populations [4 ; 5].In a previous report [6], we have demonstrated that no allele DQB1 was associated to the susceptibility to breast cancer in Tunisia. However, DQB1*03 was considered as a factor leading to protection against mammary tumors occurrence in the Caucasian population. Since, Tunisian population has an European affinity [7-9] and we have found the same DQB1 allelic distribution as that seen in the Caucasian population [10], we have supposed finding the same type of relationships between DQB1*03 and the occurrence of breast cancer in Tunisia as that defined by Chaudhuri and collaborators [4].

But, we found no significant associations between the considered marker and the susceptibility to mammary tumors in our sample. We reasoned then that associations may be detected when analyzing a greater sample and considering the histoprognostical parameters (EE grade, Lymph node invasion) of the cancer. So, in the present work, we focused on DQB1 alleles frequencies in female patients with different EE grades and with presence or absence of a nodal invasion in order to provide informations about the potential existence of significant correlations between DQB1 and breast cancer in Tunisia.

MATERIALS AND METHODS

1- Samples

The patients are 87 Tunisian female with breast cancer seen at Salah Azaiz Oncology institute from 2001 to 2003. The age of these patients is from 25 to 70. The EE grade was defined only for 83 patients (43 EE III Patients, 40 EE I or II (EE I/II) patients). Data concerning nodal invasion are mentioned in 73 subjects (52 patients with Lymph node envadement (N+), 21 patients with no lymph node invasion (N-)).

Control group includes 100 healthy and non-related blood female donors having the same range of age and geographical origin as patients.

2- Genomic DNA extraction and HLA genotyping

Genomic DNA was extracted from the whole blood by phenol-chloroforme procedure. The exons 2 and 3 of DQB1 gene were amplified by PCR using specific primers provided by INNO-LiPA amplifications kits from Immunogenetics (Réf : 80337). PCR product was then hybridized to probes fixed on strips. One strip is used by subject and contains 37 probes corresponding to the amplified exons (exons 2and 3). Hybridization strips and reagents are provided by INNO-LiPA hybridization kits from Immunogenetics (Réf: 80335). Controls typing results were given by the Tunisian Center of Blood Transfusion.

3- Statistics

Allelic frequencies were calculated and comparison of alleles distribution was performed by p value and fisher exact test where appropriate (Subject number <5 in a table cell). The level of significance was fixed at p=0.05. When p is significant (p<5) Odds Ratio are given with 95 % confidence intervals. Statistics were perfomed by Epi-Info 6 program (http://www.ensp.fr/services/logiciels/epiinfo_604d_fr.htm).

RESULTS

1- DQB1 polymorphism and susceptibility to breast cancer

In order to deduce possible correlations between DQB1 alleles and the occurrence of breast cancer, we have made comparison as far as allelic distribution is concerned. This comparison is between 87 patients and 100 healthy controls (Table 1). Both, in patients and controls, we defined five DQB1 alleles in which DQB1*03 was the most frequent (32.75 vs 31 %). The allelic distribution observed is not different from that already reported for the Tunisian population [10]. No significant difference was detected between Tunisian controls and patients. This result confirms the absence of correlation between DQB1 locus and the occurrence of breast cancer in Tunisia [6].

Table 1 : HLA-DRB1* alleles in breast cancer patients and healthy controls

DQB1 Alleles	Patients N= 87 %	Controls N=100 %	p value
DQB1*02	29.31	28.5	NS
DQB1*03	32.75	31	NS
DQB1*04	4.02	5.5	NS
DQB1*05	12.06	14	NS
DQB1*06	21.83	21	NS

Nominal value for comparison, P≤ 0.05; degree of freedom= 1

2- DQB1 polymorphism and the histoprognostical and anatomoclinical parameters of breast cancer

To search possible correlations between DQB1 and the gravity of breast cancer in the Tunisian population, we focused on SBR grade. Allellic distribution was compared between patients having different EE grades, on the one hand and between each group and controls on the other hand (Table2). This demonstrates no significant correlations between DQB1 alleles and the seriousness of breast cancer in Tunisia.

In addition, the absence of association was deduced when analyzing the relationships between the same molecular markers and the lymph node invasion. In fact, we compared to healthy controls patients with lymph node invasion (N+) and those with no lymph node invasion(N-). Allelic distribution differences between patients (N+ vs N-) and between patients and controls were not significant (Table3).

Table 2 : HLA-DRB1* alleles in healthy controls and breast cancer patients with different EE grades

DQB1 Alleles	Patients EE III N= 43 %	Patients EE I/II N=40 %	Controls N=100 %	p value EE III vs EE I/II	EE III vs Controls	EE I/II vs Controls
DQB1*02	33.72	23.75	28.5	NS	NS	NS
DQB1*03	27.90	38.75	31	NS	NS	NS
DQB1*04	1.16	6.25	5.5	NS	NS	NS
DQB1*.5	13.95	8.75	14	NS	NS	NS
DQB1*06	23.25	22.5	21	NS	NS	NS

Nominal value for comparison, P≤ 0.05; degree of freedom= 1

Table 3 : HLA-DRB1* alleles in healthy controls and breast cancer patients with presence or absence of Lymph node envadement

DQB1 Alleles	Patients N+ N=52 %	Patients N- N= 21 %	Controls N=100 %	p value N+ vs N-	EN+ vs Controls	N- vs Controls
DQB1*02	33.65	26.19	28.5	NS	NS	NS
DQB1*03	29.8	33.33	31	NS	NS	NS
DQB1*04	3.84	2.38	5.5	NS	NS	NS
DQB1*.5	11.52	9.52	14	NS	NS	NS
DQB1*06	21.53	28.57	21	NS	NS	NS

Nominal value for comparison, P≤ 0.05; degree of freedom= 1
N+/-: presence / absence of Lymph node envadement

DISCUSSION

The present study suggests that DQB1 alleles may be considered as neutral markers in Tunisian women with breast cancer. First, we confirmed the absence of DQB1 alleles that may confer resistance or susceptibility to breast cancer [6]. Then, we demonstrated the absence of correlations between HLADQB1 locus and the histoprognostical parameters of breast cancer in Tunisia. In our report, we have aimed to find an explanation for the differences shown between our population and the Caucasian one concerning the protective role of DQB1*03 [4]. We have reasoned that the problem might be resolved by the disease profile since our sample is mostly belonging to a European cluster [7 - 9]. Nevertheless, the present report suggests that the Tunisian population has a different genetic structure from other European populations when considering especially the DQB1 marker. These results confirm the diversity of genetic and environmental factors that might be involved in cancer and which may be different from one population to another and from one ethnic group to another.

RÉFÉRENCES

1. Bloom Hjg, Richardson Ww. Histological grading and prognosis in breast cancer: study of 1409 cases of which 539 have been followed for 15 years. British Journal of Cancer 1957; 11: 359-377.
2. Elston Cw, Ellis Io. 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 1991; 19: 403-410.
3. Dunn Gp, Old Lj, Schreiber RD.The Immunobiology of Cancer Immunosurveillance and Immunoediting. Immunity 2004; 21: 137-148.
4. Chaudhuri S, Cariappa A, Tang M, et al. Genetic susceptibility to breast cancer: HLA DQB*03032 and HLA DRB1*11 may represent protective alleles. P.N.A.S. 2000; 97 : 11451-11454.
5. Ghaderi A, Talei A, Gharesi-Fard B, Farjadian Sh, Amirzagar A , Vasei M. HLA-DRB1 alleles and the susceptibility of Iranian patients with Breast cancer. Pathology Oncology Research 2001;1: 39-4.
6. Baccar H A, Yacoubi Lb, Troudi W et al. HLA Class II Polymorphism: Protective or risk factors to breast cancer in Tunisia? Pathology and Oncology Research 2006; 12:79–81.
7. Khodjet El Khil H, Triki Mr, Yacoubi Lb, Langaney A, Benammar E A.Y chromosome microsatellite variation in three populations of Jerba Island (Tunisia). Annals of Human Genetics 2001; 65: 263-270.
8. Yacoubi Lb, Ennafaa H, Khodjet El Khil H et al. Human mitochondrial DNA types in two different ethnic groups of Jerba. Biologia Tunisie 2004; 2: 40-48.
9. Yacoubi Lb, Cherni L, Khodjet El Khil H et al. Islands inside an Island: Reproductive Isolates on Jerba Island. American Journal of Human Biology 2006; 18: 149-153.
10. Hmida S, Gauthier A, Dridi A, et al. HLA class II polymorphism in Tunisians. Tissue Antigens 19995; 45: 63-68.