HLA Antigen Profile Differences in Patients with SCC (Squamous Cell Carcinoma) In-Situ /Actinic Keratosis and Invasive SCC: Is There a Genetic Succeptibility for Invasive SCC Development?

İnvaziv Skuamöz Hücreli Karsinomunun Gelişiminde HLA Antijen Dağılımının Aktinik Keretozlu ve İnvaziv Olmayan Skuamöz Hücre Kanserli Hastalardaki Rolü

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Abstract

Objective: Actinic keratoses (AK) are proliferation of neoplastic keratinocytes confined to the epidermis induced by damaging solar ultraviolet radiation (UVR). When the neoplastic keratinocytes extend in to papillary -reticular dermis, then the lesion termed as squamous cell carcinoma (SCC). We have compared HLA class I and II antigen profiles in three patient groups namely: AK (n: 31) (patients without past or present invasive SCC), invasive SCC (n: 38), and SCC derived from / inconjuction with AK (n: 11).

Materials and Methods: Low-resolution typing for the HLA-A, B, C and HLA-DR/DQ was performed by means of the PCR-sequence specific primer (PCR-SSP) method using SSP HLA class I generic DNA Typing Tray.

Results: HLA results of these three groups were compared with the healthy control group (n: 100). There were not significant difference in HLA class I and II antigen profiles in AK group compared to the control. Whereas HLA-A2 allele (60.52%, p=0.016, OR=2.726, 95%CI=1.265-5.876), HLA-B60 (13.15%, p=0.025, OR=7.424, 95%CI=1.375-40.099) were higher in SCC group than the control. HLA-B51 allele (72.72%, p=0.008, OR=6.853, 95%CI=1.696-27.720) distribution were more common in SCC derived from AK than the control.

Conclusions: Historically, AKs have been characterized as premalignant. It has, however, been considered that AK and SCC represent the same disease process at the different stages of evaluation. Clinically, and histopathologically it is difficult to determine where AK ends and invasive SCC begins. From dermatopathological point of view AK is clearly SCC in-situ, however although AK is a common lesion in Caucasians, not all AKs develop in to invasive SCC, at least not with the same biological pace. We concluded that genetic differences such as HLA class I and II distribution between AK and SCC may not seem to play susceptibility role for invasive SCC development.

Keywords: Actinic keratoses, Squamous cell carcinoma, HLA

Özet

Amaç: *Aktinik Keratoz (AK) solar ultraviole radyasyon hasarıyla uyarılan epidermise özel neoplastik kerotinositlerin proliferasyonudur. Neoplastik kerotinositler papiller retiküler dermise yayıldığında oluşan lezyon SCC olarak adlandırılır Çalışmamızın hedefi HLA sınıf-I ve sınıf-II antijen dağılımını 3 hasta grubunda karşılaştırmaktır. Bunlar AK(n:31) (geçmişlerinde ve halihazırda invaziv SCC olmayanlar, invaziv SCC (n: 38) ve AK den kaynaklanan SCC li hastalar(n: 11).*

Gereç ve Yöntem: *PCR–SSP yöntemiyle düşük rezolusyonu (2 digit) HLA-A, B, C ve HLA-DR/DQ doku tiplendirilmesi yapıldı.*

Bulgular: *Bu 3 grubun HLA sınıf-I ve sınıf-II sonuçları sağlıklı kontrol grubu (n: 100) ile de karşılaştırıldı. AK grubunda HLA sınıf-I ve sınıf-II dağılımıyla kontrol grubu arasında fark bulunamadı. Fakat HLA-A2 alleli (% 60,52, p=0,016, OR=2,726, % 95 CI=1,265-5,876) ve HLA-B60 (% 13,15, p=0,025, OR=7,424, % 95 CI=1,375-40,099) allelleri SCC grubunda daha yüksekti. HLA-B51 aleli (% 72,72, p=0,008, OR=6,853, % 95 CI=1,696-27,720) kontrol grubuna göre AK den kaynaklanan SCC grubunda daha yaygındı.*

Sonuç: *AK premalignant olarak değerlendirilmektedir, bununla beraber AK ve SCC nin birlikte aynı hastalık prosesinin farklı basamaklarından geliştiği düşünülmektedir. Klinik ve histopatolojik olarak AK in nerede bittiğini ve invaziv SCC nin nerede başladığını saptamak zordur. Dermatolojik acıdan, AK açıkça SCC in-situ dur. Bununla birlikte Kafkaslarda yaygın lezyon olmasına rağmen, bütün AK ler invaziv SCC ye dönüşmez. Çünkü bir kısmı aynı biyolojik temelden oluşmamaktadır. Çalışmamızın sonunda, AK ve SCC lu hastaların genetic acıdan HLA sınıf I ve II dağılımlarının SCC gelişimine bir katıkısının olmadığı gösterilmiştir.*

Anahtar Kelimeler: *Aktinik keratoz, Squamöz hücreli karsinom, HLA*

Introduction

The human leukocyte antigens (HLA) locus in humans is found on the short arm of chromosome 6 and codes for a variety of cell-surface proteins. The best known functions of these cell surface glycoproteins involve immune activation and cell to cell recognition. The class I antigens are coded for in the HLA-A, HLA-B and HLA-C loci and class I compatibility between cytotoxic T cell and target cell is often necessary for effective killing. Class II antigens are coded for DR and DQ loci, and class II compatibility between macrophages and T cell may be necessary for effective T- and B-cell activation. HLA are considered to be essential when tumor cells are recognized and attacked by host immune cells. Therefore, the tumor growth may be affected by the states of HLA expression. In various neoplasms, the grade of HLA expression has been clinically reported to be associated with the degree of differentiation and the prognosis regarding both class I and II antigens [1,2]. Malignant transformation of cells is frequently associated with abnormalities in HLA expression. These abnormalities may play a significant role in the clinical course of the disease because the cellular immune response to tumors relies on concomitant recognition of tumor antigens with self-HLA molecules. Since HLA molecules mediate interactions of tumor cells with specific receptors on T and natural killer (NK) cells, tight control of expression of HLA molecules is critical for initiation and implementation of an effective cellular immune response. In SCC, as in most cancer, there are reports of association between HLA class I-II alleles and the disease [3-5].

Materials and Methods

Subjects:

We evaluated class I and II HLA antigens in patients with AK (n: 31), in patients with SCC (n: 38) and in patients with SCC derived from AK (n: 11). HLA results of these three groups were compared with control group who were healthy donors of dialyze and bone marrow patients (n: 100).

The study was conducted on paraffin section of 31 AK (11 male, mean age 65.72±4.09; 20 female, mean age 60.30±2.23), 38 SSC (26 male, mean age: 64.65±3.42; 12 female; mean age: 62.33±2.28), 11 SCC (7 male, mean age: 63.42±5.04; 4 female, mean age: 69.25±2.39) which derived from AK unrelated patients. Parraffin tissue embedded blocks of patients was provided from Ankara University Dermatology department (Ankara) and Ataturk University Dermatology department (Erzurum). The control group consisted of 100 (42 male, mean age 50.13±3.27; 58 female, mean age: 40.64±1.82) unrelated normal organ donors of bone marrow and kidney. Inclusion criteria included no known HLA-associated disease. Tissue typing for HLA antigens on patients and control subjects was performed in the same laboratory.

Table 1. The phenotype frequencies of HLA alleles.

HLA	Controls (n= 100)	Patients SCC (n=38)	Patients AK (n=31)	SCC which derived from AK (n=11)	χ^2_Y	P	OR	CI (95%)
A*2	36 (36%)	23 (60.52%)	--------	---------	5.803	0.016	2.726	1.265-5.876
B*51	28 (28%)	---------	---------	8 (72.72%)	7.121	0.008	6.853	1.696-27.720
B*60	2 (2%)	5 (13.15%)	---------	----------	4.991	0.025	7.424	1.375-40.099
B*65	0 ----	3 (7.89%)	----	---	4.785	0.029	----	---
Cw*7	38 (38%)	3 (7.89%)	-	-	10.553	0.001	0.140	0.040-0.486

Preparation of DNA from paraffin embedded sections:

10 sections of 8µm parrafilms was used to prepare DNA applying the Sigma Kit (St. Louis, MO, USA) according to the manufacturer's instructions.

HLA class I and II genotyping:

Low-resolution typing for the HLA-A, B, C and HLA-DR/DQ was performed by means of the PCR-sequence specific primer (PCR-SSP) method using SSP HLA class I generic DNA Typing Tray, Lot 002 and using SSP HLA class II generic DNA Typing Tray, Lot 004 (One Lambda, Canoga Park, CA, USA) according to the manufacturer's instructions.

Data Analysis:

Chi-square analysis was performed and then standard p value and the significance of an association were evaluated. The degree as association was calculated by relative risk (R.R). All statistical calculations were performed with the use of the SPSS 11.0 program for windows software.

Results

HLA allele frequencies:

The phenotype frequencies of HLA-A alleles defined by the PCR-SSP method in all groups are shown in Table 1. Distribution of HLA-A2 allele and HLA-B60 allele in group with SCC appeared to be significant when assessed by using P-value (60.52%, p=0.016, OR=2.726, 95%CI=1.265-5.876), (13.15%%, p=0.025, OR=7.424, 95%CI=1.375-40.099) respectively.

HLA-B51 allele frequency was, however, 35.3% in the other group with SCC that was derived from AK, which was statically significant compared to that in the control group (72.72%, p=0.008, OR=6.853, 95%CI=1.696-27.720).

HLA-B65 allele was only found in SSC group (7.89%, p= 0.029) (Table 1).

The frequencies of HLA-C alleles have been shown in Table. HLA-Cw*07 was encountered in a significantly higher frequency (38%, p=0.001, OR=0.140, 95%CI=0.040-0.486) in control group then in SSC.

In the genotyping of HLA class II alleles, we could not find significant HLA allele distribution among groups.

Discussion

AK is proliferations of transformed, neoplastic keratinocytes that confined to the epidermis and induced by exposure to UV in sunlight. In time, these neoplastic cells possibly proliferate in the epidermis and probably extend into the dermis where metastatic

spread can occur. If neoplastic keratinocytes remain confined to the epidermis, they stay as actinic keratoses [6-9,17]. Here the discussion is if AK is the initiator of the SCC or no association with SC. We studied this discussion with HLA approach. We compared HLA class I and II alleles distribution among three groups (AK group, SCC group, SCC derived from SC group) with control group. It was thought that AK is non-cancerous lesions in one hand, it is precancerous pathology in other hand.

In our study results, distribution of HLA-A2 and HLA-B60 alleles appeared to be common, and HLA-B65 was only seen in SCC group. HLA-Cw7 allele was more common allele in control group as compared to SSC group. HLA-B51 was the more frequent allele in other group who are with SCC derived from AK. The third group with AK did not present association with HLA alleles. Interestingly, we have not found out relation between disease association and HLA class II alleles. So, there has not been an apparent discrepancy as to the nature of susceptibility and/or protective alleles. This may have been partly because ethnic differences in the distribution of HLA alleles and the contribution of other genes that could display, in different populations (8). The aim of this study was to investigate the role of HLA in distinguishing if solar keratosis leads to SCC.

HLA genes in the evaluation of cancer, the allele specific association of HLA molecules in cancer patients have not been well understood. The relation between skin cancer and HLA antigens in renal transplants recipients offers support to the notion that genetic background, immunologic responses and environmental factors all contribute to the development of skin cancers in humans [10-12]. Skin cancers are found mainly on sun-exposed areas, with their incidence increasing the closer to areas where there is direct sunlight. Additionally, Renal-transplant recipients have a high risk of skin cancers. Some studies reported that mismatching HLA-B antigens in renal Tx was strongly associated with skin cancer [11-13]. One HLA class II antigen, HLA-DR1 has been reported to be associated with increased susceptibility to multiple basal cell carcinomas [14].

The pathogenetic mechanisms of skin cancer associated with HLA alleles and those associated with MHC related susceptibility are of different types. In HLA-B mismatching, the HLA-B alloantigens of the donor appear to be responsible, rather than those of host [11,12,15].

Both genetic and environmental factors should be considered when a disease etiology is investigated. Several reports from different regions of the world with high risk populations, including the northern provinces of Turkey, indicate a positive family history among patients with SCC [16-17].

The mechanism by which HLA genes determine susceptibility to SSC is not completely understood. It is well known that major histocompatibility complex genes regulate immune response to infections, bind and present antigens to T cells and has an important role in T cell repertoire selection. So molecular mimicry has been proposed as a potential mechanism for streptococcal sequel leading to SSC, individuals with susceptibility HLA alleles could present cross-reactive peptides and develop the disease, whereas individuals with protector HLA alleles failed to present this cross-reactive peptides. This concept argues that a microbial peptide with certain degree of homology to a self-peptide can stimulate pathogenic self-reactive specific T cells to cause an autoimmune disease in genetically susceptible individuals. This study provides analysis of HLA-A, -B, -C, -DR and –DQ polymorphisms in control and patient groups (AK group, SCC group, SCC derived from SC group). Allelic distributions or allele carrier frequency was compared between control and three groups.

As a conclusion, our study indicates that susceptibility to SSC due to AK in Turkish population is quite important HLA class I and no relation to class II.

Conflict interest statement The authors declare that they have no conflict of interest to the publication of this article.

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