Journal of Dermatological Science 74 (2014) 135–141

Contents lists available at ScienceDirect

Journal of Dermatological Science

journal homepage: www.jdsjournal.com

Association between genetic polymorphisms in apoptosis-related genes and risk of cutaneous melanoma in women and men

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ARTICLE INFO

Article history:
Received 20 August 2013
Received in revised form 20 December 2013
Accepted 25 December 2013

Keywords:
Melanoma
Apoptosis
Genetic polymorphisms
Risk

ABSTRACT

Background: The *P53* Arg72Pro, *MDM2* c.+309T > G, *BAX* c.–248G > A, and *BCL2* c.–717C > A polymorphisms have variable roles in the apoptosis pathways.
Objective: To clarify the roles of these polymorphisms in the risk for cutaneous melanoma (CM).
Methods: Genomic DNA of 200 CM patients and 215 controls was analyzed by PCR–RFLP.
Results: In women, the frequencies of *BAX* GG (83.0% vs. 71.0%, $P = 0.04$), *BCL2* AA (32.0% vs. 15.0%, $P = 0.003$), *P53* ArgArg plus *BAX* GG (84.9% vs. 63.2%, $P = 0.01$), *P53* ArgArg plus *BCL2* AA (37.0% vs. 13.1%, $P = 0.003$), *BAX* GG plus *BCL2* AA (70.3% vs. 33.3%, $P = 0.001$), *MDM2* GG plus *BAX* GG plus *BCL2* AA (27.3% vs. 3.7%, $P = 0.03$), and *P53* ArgArg plus *MDM2* GG plus *BAX* GG plus *BCL2* AA (33.3% vs. 5.6%, $P = 0.04$) genotypes were higher in patients than in controls. Female carriers of the respective genotypes were under 1.98 (95% CI: 1.01–3.91), 2.87 (95% CI: 1.43–5.77), 3.48 (95% CI: 1.34–9.04), 4.23 (95% CI: 1.63–10.96), 6.04 (95% CI: 2.10–17.37), 25.61 (95% CI: 1.29–507.24), and 25.69 (95% CI: 1.11–593.59)-fold increased risks for CM than others, respectively. In men, the frequencies of *BCL2* CA + AA (83.0% vs. 67.6%, $P = 0.01$) and *MDM2* TG + GG plus *BCL2* CA + AA (94.2% vs. 68.3%, $P = 0.003$) genotypes were higher in patients than in controls. Male carriers of the respective genotypes were under 2.43 (95% CI: 1.23–4.82) and 9.22 (95% CI: 2.16–39.31)-fold increased CM risks than others, respectively.
Conclusion: The data suggest for the first time that *P53* Arg72Pro, *MDM2* c.+309T > G, *BAX* c.–248G > A, and *BCL2* c.–717C > A polymorphisms, enrolled in apoptosis pathways, constitute distinct determinants of CM in women and men.

1. Introduction

Exposure to ultraviolet (UV) radiation from the sunlight, including UVA and UVB components, is considered the most important environmental risk factor for developing cutaneous melanoma (CM) [1].

UVA and UVB damage repair in epithelial cell DNA is required to maintain genome integrity and apoptosis failures may initiate the photo-carcinogenic process and originate CM [2,3]. The p53 protein promotes DNA repair and apoptosis [4]. The Mdm2 protein binds directly to and inhibits p53, regulating its location, stability, and activity as a transcriptional activator [5,6]. The Bax protein promotes cell death via apoptosis [7], whereas its homologous protein, Bcl2, inhibits cell death [8], under regulation of the p53 transcriptional factor [9].

It is already well established that abilities to induce apoptosis are variable in humans [10,11]. A *P53* single nucleotide polymorphism (SNP) is located at the 72nd amino acid residue, with an arginine (Arg) to proline (Pro) change because of a G→C transversion (Arg72Pro, rs1042522) [12], the protein encoded by Arg allele is more efficient in inducing apoptosis than that encoded by Pro allele [13]. A SNP located in promoter region of *MDM2* gene is characterized by a T→G substitution at the +309 nucleotide position (c.+309T > G, rs2279744) [14]. The protein encoded by G allele increases the affinity of the transcriptional activator specificity protein 1 (Sp1) for the *MDM2* promoter, resulting in higher expression of Mdm2 when compared with T allele, and subsequent attenuation of p53 pathway [14]. The *BAX* SNP with a G→A substitution at –248 nucleotide position (c.–248G > A, rs4645878) is located in the 5′-untranslated region [15]. The G

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0923-1811/$36.00
http://dx.doi.org/10.1016/j.jdermsci.2013.12.010

allele was associated with higher protein levels [16], and also with lower transcriptional activity [17] when compared with A allele. The *BCL2* SNP with a C→A substitution at –717 nucleotide position (c.–717C > A, rs2279115) is located on the *BCL2* gene promoter [18] and AA genotype was associated with increased Bcl2 expression in comparison with CC genotype [19,20].

The roles of *P53* Arg72Pro and *MDM2* c.+309T > G SNPs, analyzed predominantly in Caucasians from Europe and North America and Asians, in CM risk are controversial [21–30]. In addition, to the best of our knowledge, the roles of *BAX* c.–248G > A and *BCL2* c.–717C > A SNPs in CM risk are still unknown.

Conversely, melanin also protects skin from UV radiation, and is regulated by estrogen and androgen [31], which contributes to CM pathogenesis [32–34]. Sex hormones also alter *P53*, *MDM2*, *BAX*, and *BCL2* expression [35–39].

The Brazilian population is heterogeneous, mixed, and composed of Amerindians and European, Asian and African immigrants [40]. Furthermore, Brazilians have been highly exposed to UV rays, and the incidence of CM is rising rapidly in the country [41]. Since analyzes of various distinct populations are necessary to define the roles of genetic polymorphisms in the origin of a certain disease, the identification of *P53* Arg72Pro, *MDM2* c.+309T > G, *BAX* c.–248G > A and *BCL2* c.–717C > A SNPs in women and men highly exposed to UV rays was considered necessary to test their influences on CM risk.

2. Materials and methods

2.1. Study population

The case group comprised 200 consecutive CM patients at diagnosis (median age: 55 years, range: 20–89; 100 women, 100 men) followed at the Clinical Oncology and Dermatology Services of the University Hospital from June 2007 to March 2013. The control group comprised 215 healthy blood donors matched by gender and skin color (median age: 52 years, range: 23–60; 107 women and 108 men) followed at the same University Hospital during the same period of time in order to provide a representative group of the general population that seeks medical assistance in our hospital. Individuals with a personal or family history of CM and those who did not accept to participate of the study were excluded from the analyses. All procedures were carried out according to the Declaration of Helsinki.

Information obtained from a standardized questionnaire included self-reported host characteristics. Patients were classified according to light or non-light skin color, light (blue/green) or dark (brown/black) eye color and light (red/blond) or dark (brown/black) natural hair color. The numbers of nevi over the entire skin surface of patients were classified as none (0), few (1–20), moderate (21–50), and many (>50) [42]. Freckles were classified as none/few (limited to a single body part) and moderate/many (more than two body areas) [43]. The classification of skin phototypes (I to VI) was performed in accordance with the Fitzpatrick Classification Scale [44], considering constitutional skin color and the result of UV radiation exposure (I, white, very fair, red or blond hair, blue eyes, freckles, always burns and never tans; II, white, fair, red or blond hair, blue, hazel or green eyes, usually burns and tans with difficulty; III, white, fair with any eye or hair color, sometimes mild burn and gradually tans; IV, brown, typical Mediterranean Caucasian skin, rarely burns, tans with ease; V, dark brown, middle-eastern skin types, very rarely burns and tans very easily; VI, black, never burns and tans very easily). Sunburn episodes were defined as pain and erythema and/or blisters for more than 24 h in childhood [45]. Sun exposure was classified as intermittent or chronic [46]. Sun exposure time of patients was classified in less than, equal to, or greater than 20 years [45]. The tumor site was classified into axial (head, neck and trunk) and peripheral (upper and lower limbs), and the diagnosis of CM was histologically confirmed. The invasion depth and tumor stage were identified using Breslow (millimeters) and Clark (I–V) levels [47] and the American Joint Committee on Cancer "Melanoma Staging System" criteria [48], respectively.

2.2. Genetic polymorphism analysis

Genotyping was performed in genomic DNA of subjects' peripheral blood samples using polymerase chain reaction followed by enzymatic digestion, as previously reported for *P53* Arg72Pro (rs1042522) [49], *MDM2* c.+309T > G (rs2279744) [50], *BAX* c.–248G > A (rs4645878) [51], and *BCL2* c.–717C > A (rs2279115) [20] polymorphisms. The amount of 10–15% of genotype determinations was carried out twice in independent experiments with 100% of concordance.

2.3. Statistical analysis

The Hardy–Weinberg (HW) equilibrium was tested using the Chi-square (χ^2) statistics for the goodness-of-fit test. The differences between groups were analyzed by the χ^2 or Fisher's exact test. Multivariate analysis was performed using the logistic regression model and served to obtain age and skin color adjusted crude odds ratios (ORs) and assess the associations among genotypes and CM. Power of analysis (PA) was used to calculate the minimum effect size that is likely to be detected in a study using a given sample size. PA was calculated in analyses involving patients and controls, according to Pocock (1983) [52] and Hulley et al. (1988) [53], and using DSS Research Statistical Power Calculators (http://www.dssresearch.com/Knowledge Center/toolkitcalculators/statisticalpowercalculators.aspx) in analyses of groups of patients stratified by clinical aspects and tumor characteristics. Statistical significance was established at $P<0.05$ and all tests were done using the SPSS 15.0 software (SPSS Incorporation, Chicago, IL, USA). To evaluate genetic interaction among the polymorphisms and gender in our sample, we used the multifactor dimensionality reduction (MDR) model, which is a nonparametric and genetic model-free data mining for nonlinear interaction identification among genetic and environmental attributes [54–56]. To adjust results for multiple comparisons, we performed a MDR permutation test in our sample, totalizing 100,000 permutations. The MDR test was performed using MDR 2.0 and MDRPT 0.4.7 software.

3. Results

Similar clinical characteristics of patients and tumor biological aspects were seen in female and male patients. Only tumor location and distribution differed in patients stratified by gender: females presented tumor predominately in upper/lower limbs while tumors in head, neck, and trunk were more common in males (Table 1).

Samples of controls (women and men) were in HW equilibrium at all analyzed loci. Female patient samples were in HW equilibrium at *P53* Arg72Pro, *MDM2* c.+309T > G, and *BCL2* c.–717C > A loci but not at *BAX* c.–248G > A locus. Male patient samples confirmed HW expectations at *MDM2* c.+309T > G, *BAX* c.–248G > A, and *BCL2* c.–717C > A loci, but not at *P53* Arg72Pro locus (Table 2).

3.1. Association between genotypes and cutaneous melanoma risk

The frequencies of the genotypes and alleles of the *P53* Arg72Pro, *MDM2* c.+309T > G, *BCL2* c.–717C > A, and *BAX* c.–