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Ultraviolet B sensitivity of peripheral lymphocytes as an independent risk factor for cutaneous melanoma

Rémy Pedeux[a,b,c,d,*], *François Sales*[e], *Julie Pourchet*[a,b], *Mireille Kallassy*[a,b,1], *Caroline Fayolle*[a,b], *Mathieu Boniol*[a,b], *Gianluca Severi*[f,2], *Ghanem Ghanem*[e], *Hisayoshi (Nym) Nakazawa*[g], *Philippe Autier*[e], *Jean-François Doré*[a,b]

[a]*INSERM U 590, Lyon, France*
[b]*Université Lyon 1 Claude Bernard, Lyon, France*
[c]*INSERM U 578, 38706 La Tronche Cedex, France*
[d]*Universite de Grenoble 1 Joseph Fourier, Institut Albert Bonniot, 38706 La Tronche Cedex, France*
[e]*Institut Jules Bordet, Brussels, Belgium*
[f]*Division of Epidemiology and Biostatistics, European Institute of Oncology, Milan, Italy*
[g]*National Cancer Center, Tokyo, Japan*

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ABSTRACT

Susceptibility to solar ultraviolet is an important melanoma risk factor. We investigated the relationship between individual susceptibility to ultraviolet and risk of melanoma by measuring the apoptosis triggered in peripheral lymphocytes by a low-dose ultraviolet B irradiation (50 J/m^2) in young and older melanoma patients and controls. Melanoma patients below the age of 40 are more sensitive to UVB-induced apoptosis than older melanoma patients and healthy controls. Analysis of data (adjusted for age and phototype) shows that UVB-induced apoptosis is an important risk factor for melanoma (OR 9.1, 95% CI [3–28], $P = 0.0001$). UVB-induced apoptosis is independent of phototype ($P = 0.11$, Wald test) and tumour thickness ($P = 0.88$, Spearman correlation, for all cases and 0.26 for patients younger than 40 years), and may be used as a functional laboratory test for studying the genetic-environment interactions involved in melanoma occurrence.

1. Introduction

Exposure to solar ultraviolet (UV) irradiation and host susceptibility factors are important risk factors for cutaneous melanoma [1,2]. Little is known of the biological mechanisms and/or genetic factors that mediate susceptibility to UV in sporadic melanoma. However, there are some indications that individual susceptibility to mutagens [3]; or reduced DNA repair capacity [4,5] may be risk factors for melanoma. Melanoma is a heterogeneous disease, 40–50% of melanomas occur in patients below the age of 50. Melanomas occurring before and after the age of 50 differ in their anatomical localisation, which may reflect differences in their relation to sun exposure [6].

Since inherited susceptibility factors are likely to favour melanoma development at an earlier age [7], we hypothesized that people with melanoma at a young age could be more

* *Corresponding author*: Tel.: +33 476 54 95 58; fax: +33 476 54 94 13.
E-mail address: remy.pedeux@ujf-grenoble.fr (R. Pedeux).
[1] Present address: Campus des Sciences et des Technologies, Université Saint Joseph, Beirut, Lebanon.
[2] Present address: Cancer Epidemiology Centre, The Cancer Council Victoria, Melbourne, Australia.
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susceptible to UV irradiation than people with melanoma at an older age. To test our hypothesis, we measured the apoptosis induced by ultraviolet B irradiation in peripheral blood lymphocytes from patients with cutaneous melanoma of the superficial spreading type and healthy controls. Only young and older patients were considered and the inclusion of patients and controls was stratified according to three age groups: 18–29, 30–39 and 60–69 years old.

2. Patients and methods

2.1. Study subjects

Fifty-six melanoma patients of either gender, with histologically proven cutaneous melanoma of the superficial spreading type of the trunk or limbs exclusively and untreated previously with chemotherapy or interferon, were recruited from the Institut Jules Bordet, Brussels, from March 1999 to June 2001. Patients with head and neck melanoma were not eligible. The study was approved by the Institut Jules Bordet ethical review committee and patients signed an informed consent. For practical reasons, 86 controls were obtained from the Centre de Transfusion Sanguine, Lyon. Controls sampled from a population with similar host factors are acceptable when exposure of interest is not an environmental exposure and is related to genetic baggage. Patient and control inclusion was stratified according to three age groups: 18–29, 30–39, 60–69 years old. Age, skin phototype and hair colour were documented for all patients and controls, and results were adjusted for these characteristics.

2.2. Apotosis assay

Heparinized blood samples were collected and within 24 h, peripheral blood lymphocytes (PBL) were isolated by Ficoll gradient centrifugation at approximately 1200*g* for 30 min at room temperature. Isolated cells were resuspended in RPMI-1640 culture medium without phenol red (supplemented with 20% foetal calf serum and antibiotics) at a concentration of 2×10^6 cells/ml, and were irradiated with 50 J/m^2 UVB (Spectrolinker XL 1000, Spectronics Corporation) as described previously [8]. The UV dose delivered was monitored by a sensor calibrated by the manufacturer and placed at the same level as the cells in the irradiator. Cells were incubated for 16 h at 37 °C, recovered, fixed with 4% paraformaldehyde and kept at 4 °C until they were processed. Cells were tested for apoptosis using the TUNEL procedure with the kit "In Situ Cell Death Detection Kit" from Boehringer Mannheim. Nuclei were counterstained with Hoechst. For each sample, at least 300 nuclei were counted and green nuclei were scored as apoptotic. Apoptosis was expressed as a percentage of apoptotic cells. Ap_{CT} is the percentage of apoptosis in non-irradiated samples and Ap_{UV} is the percentage of apoptosis in irradiated samples. Readings were performed by a technician without knowledge of the goal of the study and the status of the samples.

2.3. Statistical analysis

To overcome the variability in the basal level of spontaneous apoptosis in non-irradiated blood samples (Ap_{CT}), we defined the specific apoptosis (Ap_{SP}) triggered by UVB with the following formula: $Ap_{SP} = (Ap_{UV} - Ap_{CT})/Ap_{CT}$ where Ap_{UV} is the apoptosis induced by UVB. Specific apoptosis is not normally distributed; therefore, the median and interquartile range were used to characterize UVB-induced apoptosis in specific age groups. Comparison was performed using Kruskal–Wallis non-parametric test. In all statistical calculation using Ap_{SP} as a continuous variable, Ap_{SP} was normalized as log (Ap_{SP} + 1). To examine the correlation between normalized Ap_{SP} and age, among controls, Spearman correlation test was used. Interaction between normalized UVB-induced apoptosis and phototype was tested with a logistic regression model including skin phototype (categorized as I and II vs. III and IV) and age group (below 40 and above 60). UVB-induced apoptosis was further analyzed in subjects below the age of 40 dichotomized at the level of the 75th percentile of apoptosis in controls. Risk estimates are expressed as odds ratio adjusted on phototype. Significance was assessed using the Wald test that follows a Student *t*-test distribution.

3. Results

Specific apoptosis (Ap_{SP}) triggered by UVB in peripheral blood lymphocytes was significantly higher in melanoma patients than in controls (median 2.4 vs. 1.3, $P = 0.0002$, Table 1). In controls, UVB induced a similar apoptosis in all classes of age. A moderate increase was observed between the three different age groups with a trend going from young to old but, it was not significant ($P = 0.2$, Spearman correlation). By contrast, among melanoma patients a strong and significant difference was observed in the Ap_{SP} between the different age groups (Table 1). The Ap_{SP} was significantly higher among young patients (age groups: 18–29 and 30–39) than in older patients (age group 60–69) ($P = 0.03$ and 0.01, respectively). There was no difference between the two age groups 18–29 and 30–39 ($P = 0.9$). Interestingly, when we compared the Ap_{SP} between melanoma patients and controls, within an age group, we observed a highly significant difference in the younger groups but no difference in the older group (Table 1). Thus, in the youngest age group (18–29), the median Ap_{SP} was approximately three times higher in melanoma patients than in controls. Therefore, melanoma patients below the age of 40 are more sensitive to UVB irradiation than older patients, and young melanoma patients are more susceptible to UVB irradiation than controls.

UVB susceptibility was further analyzed in subjects below the age of 40. Ap_{SP} was dichotomized at the level of 2.14 (the level of the 75th percentile of apoptosis in controls) (Table 2). The risk estimate associated with apoptosis was 9.1 (95% CI [3–28], $P < 0.0001$). To determine whether the susceptibility to UVB evidenced by apoptosis was independent of a known susceptibility factor to melanoma such as skin phototype, we tested the interaction between UVB-induced apoptosis and skin phototype (categorized as I and II vs. III and IV). No statistical interaction was found between skin phototype and apoptosis ($P = 0.11$, Wald test). Analysis of data, adjusted for age and phototype, showed that UVB-induced apoptosis normalized as log (Ap_{SP} + 1) is an important linear risk factor for melanoma: the risk increased by 8.6-fold for 1 point increase in normalized specific apoptosis (OR 8.6, 95% CI [1.9–36.5], $P = 0.005$). Furthermore, among patients, UVB-induced

Table 1 – Specific apoptosis triggered by low-dose UVB radiation in peripheral blood lymphocytes from controls and melanoma patients, by age category

Age group	Cases $n = 56$		Controls $n = 86$		P (Kruskal–Wallis test)
18–29	3.5[a] 15[b]	(1.8–7.1)	1.00 30	(0.6–2.2)	0.0015
30–39	3.1 20	(1.5–4.6)	1.2 30	(0.60–2)	0.0002
60–69	1.8 21	(0.7–2.6)	1.6 26	(1–2.6)	0.50
All ages	2.4	(1.1–4.6)	1.3	(0.7–2.5)	0.0002

a Median (interquartile range). To overcome variability in the basal level of spontaneous apoptosis in non-irradiated blood samples (Ap_{CT}), specific apoptosis (Ap_{SP}) triggered by UVB was calculated according to the formula: $Ap_{SP} = (Ap_{UV} - Ap_{CT})/Ap_{CT}$, where Ap_{UV} is the apoptosis induced by UVB in irradiated samples. Specific apoptosis is not normally distributed; therefore, median and interquartile range were used and comparisons were performed using Kruskal–Wallis non-parametric test.
b number of cases.

Table 2 – Analysis of specific apoptosis triggered by low-dose UVB radiation in peripheral blood lymphocytes from controls and melanoma patients younger than 40 years old

	Cases ($n = 35$)		Controls ($n = 60$)		Adj. OR[a]	IC 95%	P (Wald test)
Specific apoptosis							
<2.14[b]	11	(31.4)	45	(75.0)	1.00		
⩾2.14	24	(68.6)	15	(25.0)	9.1	[3–28]	<0.0001

a Risk estimates are expressed as odds ratio estimated from an unconditional logistic regression adjusted for phototype. Statistical significance was assessed using the Wald test that follows a Student t-test distribution.
b 75th percentile of apoptosis in controls.

apoptosis is independent of tumour Breslow's thickness ($P = 0.88$, Spearman correlation, for all cases and $P = 0.26$ for patients younger than 40 years).

4. Discussion

Although it is widely accepted that melanoma is influenced by exposure to solar ultraviolet radiation [1], the spectrum of solar ultraviolet involved in melanocyte transformation is not known. However, recent epidemiological and experimental data tend to document a role for exposure to UVB at early ages in the occurrence of melanoma [9–11].

To measure the susceptibility of melanoma patients to UVB irradiation, we chose to detect apoptosis triggered by a small dose of UVB in lymphocytes. This is a quick and direct method which can easily be set up on a large scale to screen populations; and host cell reactivation assays, measuring DNA repair capacity have been conducted using lymphocytes [4,12]. In our experiments 50 J/m^2 UVB was used and is considered to be a small dose. This dose was selected because it does not result in massive apoptosis and is compatible with the amount of UVB that reaches the dermis under natural exposure of the skin to sunlight. It is currently accepted that about 10% of UVB would reach the dermis, so that 50 J/m^2 in vitro is comparable to an irradiation of the skin with two minimum erythemal doses.

Peripheral blood lymphocytes of young melanoma patients are more sensitive to UVB-induced apoptosis than cells of older melanoma patients and controls. This hypersensitivity could result from a reduced ability to properly repair UVB-induced DNA damages. Indeed, defects in DNA repair mechanisms are associated with melanocyte malignant transformation and a significant proportion of patients with Xeroderma pigmentosum, a rare condition associated with a defect in DNA repair and hypersensitivity to UV, develop melanomas [12]. Furthermore, a deficit in DNA repair mechanisms has been documented in normal individuals belonging to melanoma prone families [13]. Mechanistically, DNA repair deficiency in lymphocytes would trigger apoptosis since these cells are sensitive to UV; whereas in melanocytes which are known to be resistant to UV irradiation, it would result in the accumulation of lesions and presumably of mutations favouring transformation of these cells.

So far, only few studies have shown a strong risk of sporadic melanoma associated with DNA repair deficiency [2–4]. No deficiency in repair of DNA cyclobutane pyrimidine dimers or 6–4 photoproducts has been detected in skin of melanoma patients [14,15]. Host cell reactivation assays used to measure DNA repair capacity in some studies [4,5,12] do involve peripheral blood lymphocytes that have not been submitted to UV irradiation, and may therefore lack sensitivity to subtle defects such as those associated with polymorphism in DNA repair genes which may modulate the apoptotic response to UV in lymphocytes [16]. Polymorphisms in DNA repair genes *XPD* (also known as *ERCC2*) and *XRCC3* are associated with susceptibility to melanoma [16,17], at least in subjects with specific host characteristics such as older age, lack of dysplastic nevi or low tanning ability [18]. However, further studies

failed to confirm earlier results for *XRCC3* variants [19,20]; therefore, there is currently little evidence for a significant role for polymorphisms in DNA repair genes in susceptibility to melanoma.

Since the association between UVB-induced apoptosis and melanoma does not seem to be influenced or to interact with pigmentary traits such as phototype, UVB-induced apoptosis appears to be a new independent risk factor for melanoma. Further studies are needed to evaluate the eventual interactions with other genetically determined host factors such as naevi or freckles which could not be determined in controls. Nevertheless, our study is the first clear indication that patients developing melanoma may demonstrate hypersensitivity in their functional response to UV irradiation, and that such hypersensitivity is only observed in younger melanoma patients. It may explain why differences in the epidemiology of melanoma in young people vs. older people have been described [6,11]. It also suggests that aetiology of melanoma in young and older people could result from different mechanisms. The genetic background of the individual who is hypersensitive to ultraviolet light in young people is not currently known and remains to be determined. Studies reporting on more patients and controls and looking at various DNA repair pathways are needed in order to improve our knowledge on the relationship between DNA repair, UV and melanoma risk. Apoptosis induction in PBL following low UVB irradiation brings a new functional insight, which has potential implications on the aetiology and prevention of melanoma especially for those below 40, and could enable the identification of populations at risk and target subgroups for cancer prevention strategies.

Conflict of interest statement

None declared.

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