Correlation Between Antioxidants and Phototypes in Melanocytes Cultures. A Possible Link of Physiologic and Pathologic Relevance

To the Editor

People with different skin color possess varied sensitivity to ultraviolet (UV) exposure, with darker skinned individuals being less susceptible to sun-induced skin alterations, including cancer, than fair skinned ones (Elwood and Diffey, 1993). Such a difference can be explained in terms of UV transmission of the epidermis because the skin color is also related to the type of melanin, the number, size, type, distribution and degradation of melanosomes, and the tyrosinase activity of melanocytes (Nordlund and Ortonne, 1998). Fair skinned individuals synthesize eumelanin and pheomelanin, in contrast to Negroid individuals who synthesize only eumelanin (Nordlund and Ortonne, 1998). Eumelanin is capable of scavenging the superoxide anion and hydrogen peroxide, whereas pheomelanin, due to its chemical properties, is capable of increasing the generation of free radicals following UV exposure (Prota, 1997). One of the first events following UV irradiation of the skin, in fact, is the generation of reactive oxygen species (ROS) associated with the peroxidation of the unsaturated lipidic component of the cell membranes. The administration of enzymatic or nonenzymatic antioxidants significantly reduce several adverse effects of UV light, including skin cancer (Shindo *et al*, 1993). Melanocytes, in particular, seem to be extremely susceptible to free radicals, either in the activation of their physiologic role or in deleterious effects (Picardo *et al*, 1991; Roméro-Graillet *et al*, 1996). Therefore, antioxidants are considered to be among the physiologic photoprotective compounds of the skin (Applegate and Frenk, 1995).

Recently, we have reported an alteration of the antioxidant system in cultured normal melanocytes from some patients with cutaneous melanoma, in comparison with cultures from normal individuals. Moreover, in the same cultures, an increase of the percentage of polyunsaturated fatty acids (PUFA) of cell membranes was observed (Picardo *et al*, 1996). Because the imbalance of the antioxidant system was correlated with an increased proliferation following the treatment with a peroxidative agent such as the cumene hydroperoxide, we have speculated that a constitutional alteration in the scavenger system can be present in some patients with melanoma, and that this could be the basis for an increased sensitivity to the effects of UV light (Grammatico *et al*, 1998). Subsequently, using a model of reconstructed epidermis, we evaluated the correlation between the phototype and the enzymatic antioxidant activities and the PUFA percentage demonstrating lower antioxidant enzyme activities and higher PUFA percentage in reconstructed skin with low phototype with respect to those with higher phototype (Bessou-Touya *et al*, 1998). Now, in order to correlate the antioxidant pattern of melanocyte cultures with the phototype, we have extended our previous studies performing analyses on seven new melanocyte cultures and re-evaluated or re-examined data on 29 cultures in relation to the phototype of the donor. The cell cultures were classified as "high phototype" (HPM n = 18) when deriving from patients with skin type III–IV–V (7, 8, and 3, respectively), and as "low phototype" (LPM n = 11) when from skin type I–II (3 and 7), according to the Fitzpatrick classification (Fitzpatrick, 1988). The enzymatic antioxidant superoxide dismutase (SOD) and catalase activities were evaluated by spectrophotometer on the cell lysates, and the intracellular concentration of vitamin E and the fatty acid pattern of cell membranes by gas chromatography mass spectrometry (Picardo *et al*, 1996). As shown in **Table I**, significant differences were observed in both the antioxidant values and the PUFA percentage of the two groups of cultures. The LPM catalase activity was significantly lower with respect to HPM (0.25 ± 0.09 *vs* 0.9 ± 0.18, U per 10^6 cells, $p < 0.001$) and vitamin E concentration significantly higher (4.1 ± 0.9 *vs* 2.55 ± 0.9 ng per 10^6 cells, $p < 0.001$), and the PUFA percentage with respect to the total fatty acid analyzed was $18.2 \pm 1.2\%$ *vs* 12.9 ± 1.5 ($p < 0.001$). On the contrary, SOD activity was not significantly different between the two groups (0.60 ± 0.21 *vs* 0.55 ± 0.12), so that the SOD/Cat ratio, considered as a parameter of the cells susceptibility to external oxidative stress, was 2.4 in LPM and 0.61 in HPM. The increased PUFA percentage makes these cells more susceptible to any external pro-oxidant stimulus and the high SOD/Cat ratio, considering the low glutathione peroxidase activity (GSH-Px) in melanocytes (Yohn *et al*, 1991), can lead to an increased intracellular production of hydrogen peroxide. Experiments performed with cumene hydroperoxide (CUH, 0.6–20 μM), in fact, confirmed that low phototype cultures underwent to a significant proliferation or to a cytotoxicity following the exposure to 0.6 or 20 μM CUH for 1 h, respectively, whether obtained from patients with melanoma or normal subjects (**Table II**). PUFA percentage and antioxidants pattern in melanocytes therefore seems to be correlated to the skin phototype, as proposed by Bessou-Touya *et al* (1998), rather than to the presence of melanoma, and may not be an "abnormal" condition but associated with the skin response to UV irradiation.

Physical perturbation of the plasma membrane or conformational changes in membrane proteins have been proposed as the basis for the UVB-induced receptor clustering and internalization (Rosette and Karin, 1996), and the membrane PUFA are likely involved in this process. Oxygen radicals, and in particular hydrogen peroxide, are considered as intracellular second messengers because their level is increased by extracellular ligands such as cytokines (Schreck and Baeuerle, 1991), and as biologic mediators of UV-induced phosphorylation of membrane receptors (Peus *et al*, 1998). The activation of growth factor receptors and in particular those of epidermal growth factor (EGFR) initiates multiple signaling responses associated with mitogenesis and cell growth regulation (Urlich and Schlessinger, 1990). The inducible transcription factors NF-kB, AP-1, and Egr-1 seem to be important physiologic targets of oxygen radicals and the subsequent gene activation can lead to pleiotropic responses, including the revival of cell cycle after repair or apoptosis of damaged cells (Huang *et al*, 1996; Ginn-Pease and Whisler, 1998). Therefore, being oxygen radicals involved in the signal pathways from the cell surface to the nucleus (Huang *et al*, 1996), they have to be normally generated inside the cells. In view of this, our results suggest that the different pattern of antioxidants and of membrane fatty acids of melanocytes is correlated with their physiologic response to UV light, and that can be considered as an adjunctive risk factor for people with low phototype when exposed to a level of UV light higher than in their original geographic region.

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Table I. Antioxidants and PUFA percentage in melanocytes cultures from 'low phototype' (LPM) and 'high phototype' (HPM) subjects[a]

	Vit. E, ng per 10^6 cells	SOD, U per 10^6 cells	Cat, U per 10^6 cells	Ratio, SOD per Cat	PUFA, % total fatty acids
LPM	4.10 ± 0.90	0.60 ± 0.21	0.25 ± 0.09	2.4	18.20 ± 1.20
HPM	2.55 ± 0.91*	0.55 ± 0.12	0.90 ± 0.18*	0.61*	12.90 ± 1.50*

[a]Each result represents the mean of two experiments in duplicate. *$p<0.001$.

Table II. Viability of melanocytes cultures from 'low phototype' (LPM) and 'high phototype' (HPM) subjects after treatment with CHU[a]

	0.66 μM	6.6 μM	20 μM
LPM	135 ± 10.0*	115 ± 4.5*	53 ± 4.1*
HPM	97.6 ± 4.2	96.2 ± 5.3	98.1 ± 6.6

[a]Cells were treated for 1 h in medium without fetal calf serum then washed and cultured in complete medium. Cell number was evaluated at 24 h by Trypan Blue test. Results are reported as percentage values compared with respective untreated controls and represent the mean ± SD of two experiments in triplicate. *$p<0.001$.

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Retardation of Hair Follicle Development by the Deletion of TrkC, High-Affinity Neurotrophin-3 Receptor

To the Editor:

Increasing evidence suggests that neurotrophins not only control neuronal development, plasticity, and maintenance (Lewin and Barde, 1996; Bothwell, 1997), but also are critically involved in the control of hair follicle (HF) development and growth (Holbrook *et al*, 1993; Crowley *et al*, 1994; Botchkarev *et al*, 1998a, 1999a).

Specifically, we have recently shown that neurotrophin-3 (NT-3) is functionally important for HF morphogenesis, as its overexpression or partial deletion in mice leads to a significant acceleration or retardation of HF development, respectively (Botchkarev *et al*, 1998a). As a member of the neurotrophin family, NT-3 shows multiple interactions with all types of neurotrophin receptors: NT-3 binds with high affinity to the tyrosine kinase C (TrkC) receptor, as well as with low affinity to the tyrosine kinase A (TrkA), tyrosine kinase B (TrkB), and p75 kDa neurotrophin receptor (p75NTR) (Lewin and Barde, 1996; Bothwell, 1997; Dechant and Barde, 1997).

Because all four receptors are expressed in the HF epithelium or mesenchyme during defined stages of HF morphogenesis (Botchkarev *et al*, 1998a),[1,2] the target receptor(s) that mediate the stimulatory effects of NT-3 on HF development are still unclear. TrkC, the high-affinity receptor for NT-3, is expressed by the hair placode epithelium during the initial steps of HF development, whereas in the fully developed HF TrkC expression appears in the dermal papilla, outer root sheath, and hair matrix (Botchkarev *et al*, 1998a). In order to explore the relative contribution of TrkC signaling in the control of HF, we have studied HF morphogenesis

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