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Pigmented Melanocytes Are Protected Against Ultraviolet-A-Induced Membrane Damage

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The dominant skin pigment melanin is believed to protect human skin against several harmful effects of ultraviolet radiation. It is not clear, however, how melanin located inside melanin-producing melanocytes modulates the effect of ultraviolet radiation on melanocytes themselves. We have determined membrane damage in pigmented and unpigmented albino mouse melanocytes after ultraviolet A radiation, which is suspected to induce melanoma. Unpigmented cells were much more susceptible to ultraviolet-A-induced membrane permeability than pigmented cells. Unpigmented cells were also more susceptible to ultraviolet-A-induced lipid peroxidation than strongly pigmented cells. Furthermore, unpigmented cells were much more susceptible to ultraviolet-A-induced depletion of glutathione than pigmented cells. Reduced glutathione is known to be a major antioxidant of unpigmented skin cells such as fibroblasts and keratinocytes. To examine whether or not glutathione is also a major antioxidant in melanocytes, melanocytes were depleted of glutathione by means of buthionine sulfoximine. We found that depletion of glutathione in pigmented melanocytes did not change lipid damage induced by ultraviolet A radiation. In unpigmented melanocytes, however, depletion of glutathione significantly increased lipid damage induced by ultraviolet A radiation. Thus, pigmented melanocytes apparently contain antioxidants more potent than glutathione, protecting them from ultraviolet-A-induced membrane damage. ***Key words: melanin/melanocytes/membrane damage/ultraviolet A radiation. J Invest Dermatol 121:564–569, 2003***

The incidence of cutaneous melanoma has been increasing steadily in Caucasian, fair-skinned populations for several decades (Wang *et al*, 2001). Melanoma shows a relatively high incidence among young people compared to most other cancer types. The etiology of melanoma remains unclear. There is a good inverse correlation, however, between latitude, and therefore sun exposure, and incidence. Although direct evidence is lacking, it is assumed that solar ultraviolet A (UVA) radiation (320–400 nm) may play a significant role relative to ultraviolet B (UVB) radiation (290–320 nm) in melanoma etiology (Wang *et al*, 2001).

UVB radiation has been established as the main cause of nonmelanoma skin cancer – particularly squamous cell carcinoma, where the types of mutations in the p53 gene are characteristic of UVB radiation (Brash *et al*, 1991). The causes of such mutations are the direct DNA damage induced by absorption of UVB radiation in DNA. In contrast, UVA radiation is very weakly absorbed by DNA. Most of the studied biologic effects of UVA radiation are dependent on the presence of oxygen. Reactive oxygen species (ROS), which damage a wide range of biomolecules, mediate the biologic effects of UVA radiation (Tyrrell, 1994). Hence, the presence of redox active species, particularly antioxidants, is important in modulating the biologic effects of UVA radiation. In the melanocytes, which may give rise to melanoma, the dominant skin pigment melanin and its precursors are important redox active compounds (Meyskens *et al*, 2001).

Melanin is a complex and only partly characterized polymer of tyrosine metabolites that are produced inside the membrane-bound melanosomes of melanocytes. The two types of melanin, eumelanin and pheomelanin, are synthesized via the precursors dopachrome and 5-S-cysteinyl dopa, respectively (Prota, 2000). There are conflicting reports on the role of melanin or melanin precursors in modulating biologic effects of UV radiation (Meyskens *et al*, 2001). This is probably due to incomplete knowledge of factors that affect melanin chemistry such as the redox state of the studied system (Rozanowska *et al*, 1999). Thus, the effect of melanin or melanin precursors in cells may be quite different from effects studied with isolated or synthesized melanin.

There are reports on UV-induced peroxidation of liposomes in the presence of melanin precursors or melanin (Schmitz *et al*, 1995). In the absence of melanin-bound ferric iron, melanin or melanin precursors protected liposomes from UV-induced peroxidation. Lipid peroxidation and membrane damage is a prominent effect of UVA radiation of skin cells and is probably a major contributor to UVA-induced cell death (Girotti, 2001). Hence, it is of importance to understand the role of melanin in modulating the lipid peroxidation and membrane damage induced by UVA radiation.

In normal skin cells such as keratinocytes, fibroblasts, and melanocytes, reduced glutathione (GSH) is present in millimolar concentrations, which makes it a major antioxidant (Tyrrell and Pidoux, 1988). Thus, when fibroblasts are selectively depleted of

Manuscript received November 22, 2002; revised February 23, 2003; accepted for publication April 2, 2003

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Abbreviations: BSO, buthionine sulfoximine; DHR, dihydrorhodamine 123; GSH, reduced glutathione; HPLC, high performance liquid chromatography; ROS, reactive oxygen species; TBARS, thiobarbituric reactive substances.

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glutathione, their susceptibility to inactivation by UVA radiation and other forms of stress is strongly increased (Tyrrell and Pidoux, 1986). There is little information about the role of glutathione in protecting pigmented melanocytes. It is conceivable, however, that melanin, which may be present in large concentration in melanocytes, may be even more important than glutathione as an antioxidant.

We have used tyrosine to selectively modulate the synthesis of melanin in three types of melanocytes from mice with different coat color. UVA-induced lipid peroxidation and membrane damage was measured as a function of melanin content of cells. Unpigmented melanocytes from albino mice were used for comparison. Finally, the contribution of glutathione to the antioxidant capacity of melanocytes was studied.

MATERIALS AND METHODS

Cell cultivation The authors' institution approved of the work with mouse cells described in the manuscript. Mouse melanocytes – melan A, melan B, and melan C – were cultured in F-10 (Ham) medium (Gibco, Paisley, UK) with HEPES buffer, 10% fetal bovine serum, streptomycin (100 μg per ml), penicillin (100 U per ml), and 200 nM 12-O-tetradecanoyl phorbol-13-acetate (TPA). The melanocytes, which were a kind gift from Dorothy Bennett, were established from C57/BL6 black (BB/CC), DBA2 brown (bb/CC), and albino (BB/cc) mouse embryos (Bennett *et al*, 1989). Melan A cells (from black mouse), melan B cells (from brown mouse), and melan C cells (from albino mouse) were used from passages 22, 35, and 18, respectively, and 10 passages forward. TPA was renewed weekly. The pH of the medium was adjusted to 6.9 by adding 9 mM HCl and the pH was maintained with 5% CO_2. Melanocytes were continuously examined for melanin content and morphology, two parameters that did not change during the experiments. Primary human male foreskin fibroblasts were obtained from Cytotech (Hellebaek, Denmark) and cultured in the same medium as used for melanocytes except that TPA was not added and the pH was not adjusted with HCl.

Melanin The melanin content of cells was determined as previously described by dissolving cells in 1 N NaOH (Kvam and Tyrrell, 1999). Melaninsynthesis of melanocytes was modulated by adding the melanin precursor L-tyrosine to the medium.

Glutathione The GSH content of cells was determined by high performance liquid chromatography (HPLC) and electrochemical detection as described by Smith *et al* (1995) with a few modifications. Briefly, cells were harvested with a soft rubber policeman. After centrifugation, the cells were resuspended in 250 μL phosphate-buffered saline (PBS) and a fixed volume (10 μL) of cell suspension was used for determining the protein content by the Bradford method (Biorad, Hercules, CA), using 2 mM NaOH to dissolve cells. GSH was released from cells by adding perchloric acid (5%) to the cell suspension. After 15 min at room temperature, the acid was neutralized by adding KOH and the sample was centrifuged at 13,000*g* for 5 min. The supernatant contained GSH.

For HPLC analysis we used an Alltech Adsorbosphere HS C18/5μ column and an ESA Coluochem II electrochemical detector (ESA, Chelmsford, MA) equipped with a 5010 analytical cell. The measuring electrode was set to 900 mV. A Spectraphysics SP8800 pump delivered a flow rate of 1 mL per min using the mobile phase 30 mM Na phosphate buffer (pH 2.7) with 6% methanol. Samples were diluted 1:3 in the HPLC mobile phase before freezing at −20°C or direct injection into the HPLC system. (Oslo, Norway) GSH standards were obtained from Sigma.

Irradiation Cells were seeded in 6 or 10 cm tissue culture dishes 1 wk prior to irradiation. After reaching confluency, the cell medium was carefully aspirated and replaced with PBS. Cell dishes were UVA irradiated from above with a Sellamed 3000 UVA lamp (Sellamed, Gevelsberg, Germany) filtered to emit broadband UVA1 (340–400 nm) and near visible light (400–420 nm). The main emission of the lamp was between 350 and 400 nm. The lamp spectrum was determined with a calibrated Avantes USB 2000 spectrometer (Avantes, Eerbeek, The Netherlands). The light source, DH-2000-CAL (Avantes), used for calibrating the spectrometer was calibrated by Avantes using a NIST traceable light source. The light intensity at the position of the irradiated cell plates was 400 W per m^2.

Lipid peroxides The intracellular content of thiobarbituric reactive substances (TBARS) was determined after irradiation. Cells were released from the culture dishes with a soft rubber policeman. After centrifugation (1400 g), 250 μL of PBS was added to the cells, which were resuspended. A fixed volume (10 μL) of cell suspension was used for determining the protein content by the Bradford method (Biorad), using 2 mM NaOH to dissolve cells. We added 250 μL 1% sodium dodecyl sulfate and 25 μL 2% butylated hydroxytoluol (in ethanol) to the cell suspension. After whirlmixing, 500 μL of 30% trichloroacetic acid and 0.5 N HCl and 500 μL freshly prepared 0.75% thiobarbituric acid (in 20 mM NaOH) were added. The samples were kept in a water bath at 80°C for 20 min before being transferred to ice water. After centrifugation (1000*g*), the optical absorbance spectrum of the supernatant was recorded from 400 to 600 nm. The optical density at 535 nm was used for determination of TBARS content using an extinction coefficient of 1.53×10^5 per M per cm. The validity of the assay was confirmed by adding known amounts of 1,1,3,3-tetraethoxypropane to samples before analysis. By spiking samples with 1,1,3,3-tetraethoxypropane it was also found that the recovery of TBARS was the same for all the cell types examined (data not shown). Data were analyzed by a standard Student's *t* test.

ROS and cell permeability After irradiation, PBS was removed from the cells and 50 μM of the H_2O_2 probe dihydrorhodamine 123 (DHR) (Molecular Probes Leiden, The Netherlands) in PBS was added to the cells, which were kept at 37°C for 15 min. ROS transform the nonfluorescent DHR into the fluorescent rhodamine 123. The cells were kept in the incubator with cell medium for 2 h. Cells were trypsinized and gently scraped off the culture dishes with soft rubber policemen. Cells were washed in PBS, resuspended in PBS with the DNA probe Hoechst 33258, filtered through a fine nylon mesh to remove cell aggregates, and examined by flow cytometry (FACStarPLUS, Becton Dickinson, CA). For rhodamine 123 detection, 488 nm excitation and a 515–545 nm emission filter were used. For Hoechst 33258 detection, 350 nm excitation and a 424 nm emission filter were used.

To test the validity of the assay, cells incubated with DHR were dissolved in 1% sodium dodecyl sulfate and 0.1% H_2O_2 was added to some samples, which were sonicated for 10 s. The addition of H_2O_2 increased the fluorescence of irradiated and control cell samples 5-fold (data not shown). Thus, we conclude that there was a surplus of intact DHR in the examined cells.

RESULTS

Melanin synthesis in melanocytes was modulated by tyrosine treatment Three types of immortalized mouse melanocytes were used in the reported experiments: black mouse melanocytes, melan A, brown mouse melanocytes, melan B, and unpigmented albino mouse melanocytes, melan C. The melanocytes were grown in medium containing a low concentration of the melanin precursor tyrosine, and melanin synthesis was stimulated by increasing the concentration of tyrosine. **Figure 1** shows that the melanin content per cell was increased from 18 pg by a factor of 10 in melan A cells and from 5 pg by a factor of 2 in melan B cells.

UVA radiation induced more membrane permeability in unpigmented than in pigmented melanocytes Melanocytes with varying concentrations of melanin were exposed to UVA radiation, and cells permeable to a DNA stain 2 h after radiation were counted (**Fig 2**). The strongly pigmented melan A cells did not become more permeable to DNA stain even at a dose of 525 kJ per m^2 UVA, corresponding to about 3 h of midday sun exposure in summer in southern Norway. The weakly pigmented melan B cells were slightly permeable after 525 kJ per m^2 UVA radiation. Cells with low and high levels of melanin showed equal membrane permeability. In contrast, the unpigmented melan C cells were significantly permeabilized by 525 kJ per m^2 UVA radiation. The cells appeared to become more permeable with tyrosine supplementation.

UVA radiation induced more lipid peroxides (TBARS) in unpigmented than in strongly pigmented melanocytes To test whether or not the cellular levels of melanin correlated with

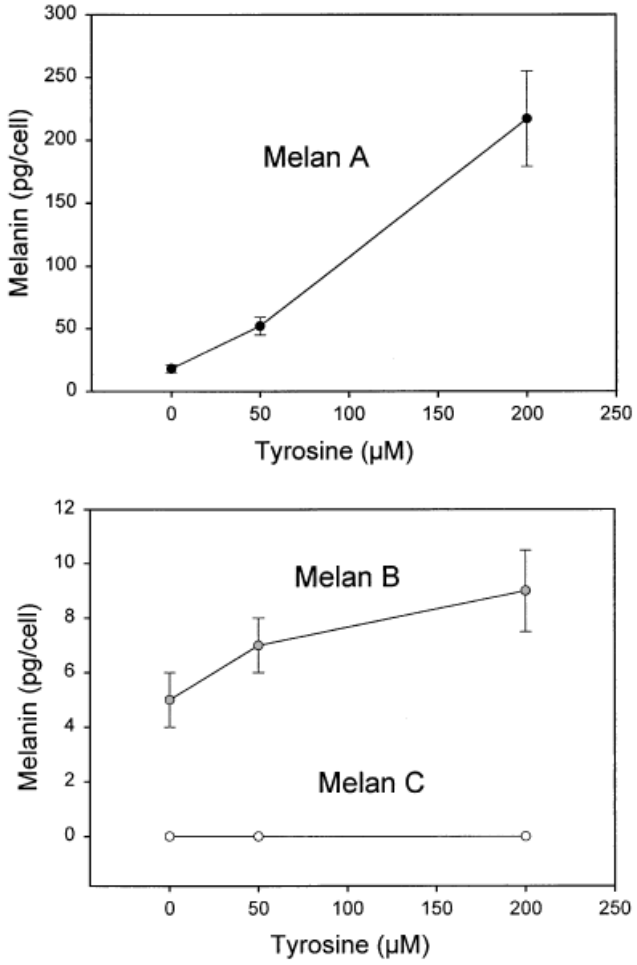

Figure 1. Induction of melanin synthesis in melanocytes. The melanocytes melan A, melan B, and melan C were treated with the melanin precursor tyrosine. The basal levels of melanin in melan A, melan B, and melan C cells were 18, 5, and 0 pg per cell, respectively. After 2 d, the total melanin content was determined. The error bars are SD for four to five samples.

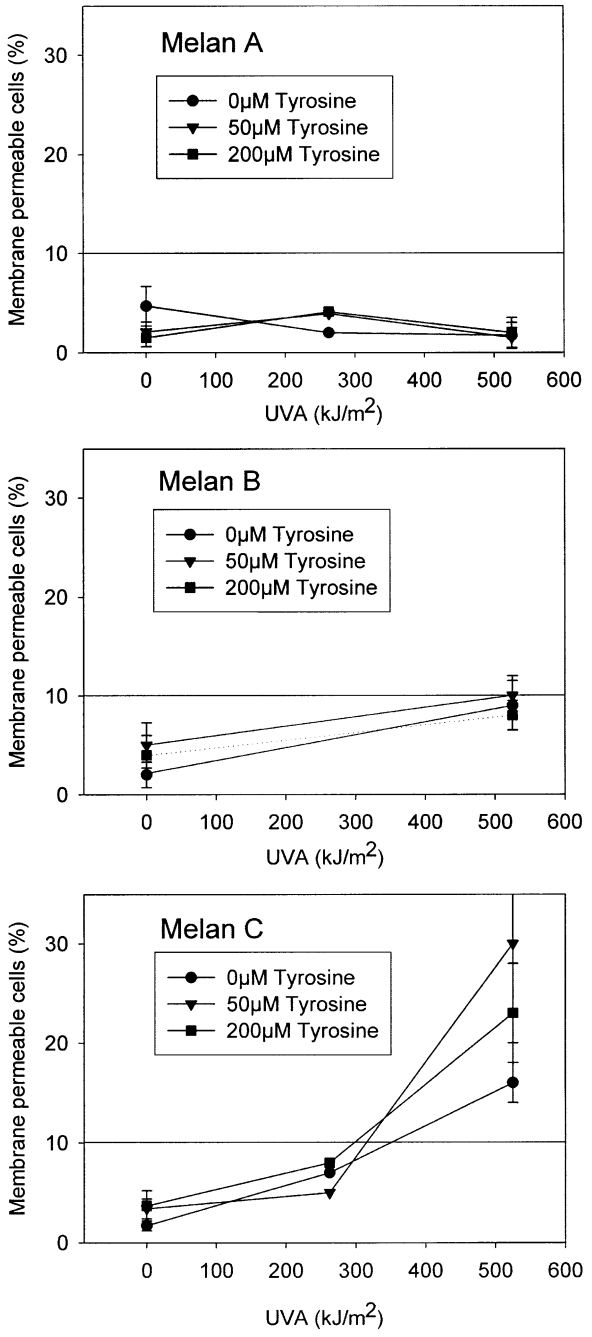

Figure 2. Induction of membrane-permeable cells by UVA radiation. Cells were treated with tyrosine to increase melanin synthesis (**Fig 1**) and exposed to UVA radiation. Two hours after UVA exposure, the fraction of cells permeable to the DNA stain Hoechst H33258 was determined by flow cytometry. The data represent the mean value of two to three counts of 10,000 cells. Error bars are SD of three samples.

UVA-induced lipid peroxidation, TBARS were determined in melanocytes with varying concentrations of melanin (**Fig 1**). **Figure 3** shows that the unpigmented melan C cells displayed more UVA-induced lipid peroxidation than the pigmented melanocytes. The strongly pigmented melan A cells had both a very low background level and a low UVA-induced level of lipid peroxides. The weakly pigmented melan B cells also showed a low UVA-induced level of lipid peroxides. **Figure 3** shows that melan A cells with high melanin content had higher levels of UVA-induced lipid peroxidation than melan A cells with low melanin content. The unpigmented melan C cells, however, also displayed significantly more UVA-induced lipid peroxidation in the presence of a high concentration of the melanin inducer tyrosine. Thus, the difference of melan A cells with high and low melanin content is probably due to an effect of tyrosine other than melanin production.

Low levels of UVA-induced ROS in melanocytes The induction of peroxides and membrane damage by UVA radiation is mediated by ROS. To determine the production of ROS, we used the probe DHR, which is an established probe for hydrogen peroxide (Royall and Ischiropoulos, 1993). **Figure 4** shows the production of ROS in the three types of melanocytes as well as in skin fibroblasts. Clearly, UVA radiation induced much more ROS in fibroblasts than in both pigmented and unpigmented melanocytes, which had similar levels of UVA-induced ROS.

UVA exposure reduced the intracellular level of GSH in unpigmented but not in pigmented melanocytes The UVA induction of peroxides and membrane damage in cells is inhibited by intracellular antioxidants. GSH is a prominent antioxidant in many types of skin cells (Niggli and Applegate, 1997). We measured the levels of GSH in the three types of melanocytes in the absence and presence of UVA radiation. **Figure 5** shows that the intracellular levels of GSH were similar

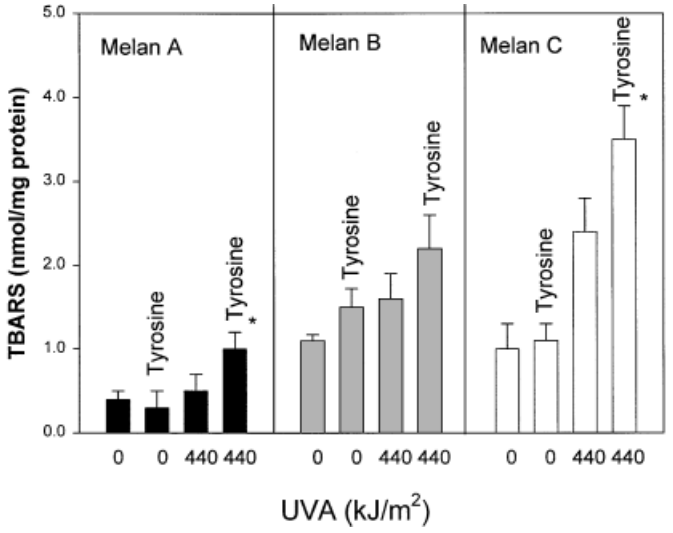

Figure 3. Induction of lipid peroxides (TBARS) in melanocytes after UVA radiation. Cells were treated with tyrosine (200 μM) to increase melanin synthesis (**Fig 1**). Immediately after UVA exposure, cells were harvested and the intracellular concentration of TBARS was determined. The error bars represent SD for three to five samples. The UVA-increased TBARS values are significantly different for the three types of melanocytes not treated with tyrosine (Student's *t* test, $p < 0.05$). The *asterisk* indicates that the UVA-increased TBARS values for tyrosine-treated cells are significantly different (Student's *t* test, $p < 0.02$) from those of cells not treated with tyrosine.

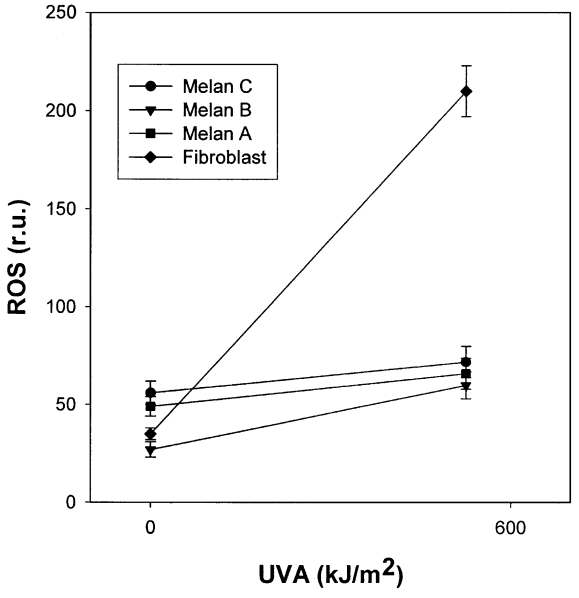

Figure 4. UVA induction of ROS in melanocytes. The ROS were assayed by the nonfluorescent probe DHR, which reacts readily with hydrogen peroxide to form fluorescent rhodamine 123. Data for primary human skin fibroblasts are included. Cells with intact plasma membrane were selected by flow cytometry by gating for background DNA staining with Hoechst H33258. The data represent the mean value in relative units (r.u.) of three independent counts of 10,000 cells. Error bars are SD.

in the three types of melanocytes. In the unpigmented melan C cells, however, 262 kJ per m^2 UVA radiation almost completely depleted the cells of GSH, whereas the GSH levels of pigmented melanocytes were unchanged.

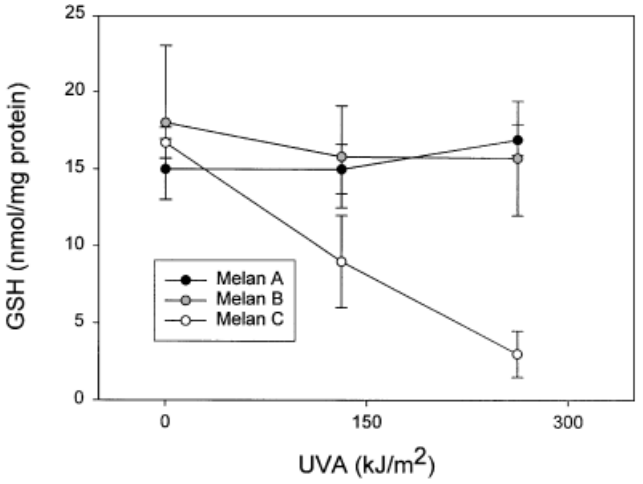

Figure 5. The intracellular level of GSH in melanocytes after exposure to UVA radiation. The level of GSH was determined immediately after UVA exposure by HPLC. The error bars are SD of three to five samples.

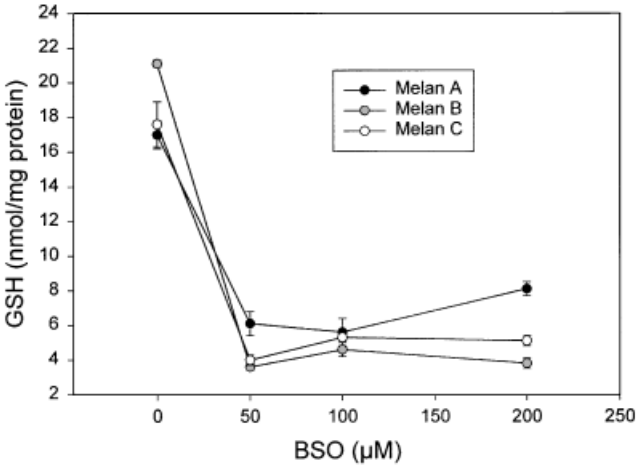

Figure 6. Depletion of GSH in melanocytes by an 18 h treatment with BSO. The error bars are SD of four to five samples.

UVA induction of lipid peroxides (TBARS) increased by depletion of GSH in unpigmented melanocytes The clear difference in UVA-induced GSH depletion in unpigmented and pigmented melanocytes might be related to the presence or absence of antioxidants other than GSH. We decided to selectively deplete all melanocytes of GSH by treatment with the specific inhibitor of γ-glutamylcysteine synthetase, buthionine sulfoximine (BSO), and study UVA-induced lipid peroxidation. **Figure 6** shows that BSO depleted most of the GSH in all three types of melanocytes. **Figure 7** shows UVA-induced lipid peroxidation in the melanocytes with high or low levels of GSH. Only in the unpigmented melan C cells was a low level of GSH associated with increased UVA-induced lipid peroxidation.

DISCUSSION

We have shown that pigmented melanocytes are more resistant to UVA-induced lipid peroxidation and membrane damage than unpigmented melanocytes (**Figs 2, 3**).

We have also shown that UVA radiation dramatically reduces the level of the antioxidant glutathione of unpigmented melanocytes

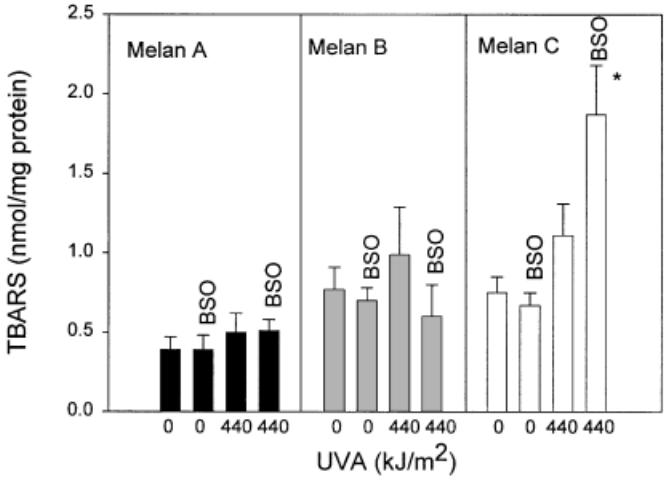

Figure 7. UVA induction of lipid peroxides (TBARS) in melanocytes depleted of GSH. Cells were treated with BSO (100 μM) to deplete cells of GSH (**Fig 6**). Immediately after UVA exposure, cells were harvested and the intracellular concentration of TBARS was determined. The error bars represent SD for three to five samples. The *asterisk* indicates that the UVA-increased TBARS values for BSO treated samples are significantly different (Student's *t* test, $p < 0.02$) from those of samples not treated with BSO.

but not of pigmented melanocytes (**Fig 5**). By selectively depleting melanocytes of glutathione, we have found that unpigmented, but not pigmented, melanocytes become more susceptible to UVA-induced lipid peroxidation (**Fig 7**). Taken together, these results point to factors, such as antioxidants, found to a higher degree in pigmented melanocytes than in unpigmented melanocytes – protecting pigmented melanocytes against lipid and membrane damage.

We found that increased melanin synthesis by tyrosine treatment only weakly changed UVA-induced lipid peroxidation in melanocytes (**Fig 3**). This could be interpreted in several ways: either the basal level of melanin is sufficient to inhibit damage, or melanin precursors generated during increased melanin synthesis enhance UVA-induced damage. It is also possible that compounds other than melanin are present in pigmented melanocytes and not in unpigmented melanocytes, such as unknown antioxidants, which protect them from UVA-induced damage. The similar yields of ROS assayed by DHR (**Fig 4**) do not support this possibility. The possibility could be further tested, however, by selectively depleting melanocytes of melanin by inhibitors of tyrosinase. But in our hands, tyrosinase inhibitors have not been able to deplete melan A or melan B cells of melanin (data not shown).

Our data are consistent with data from experiments where liposomes or arachidonic acid have been shown to be protected against peroxidation by melanin or melanin precursors (Napolitano *et al*, 1993; Schmitz *et al*, 1995). In contrast, other experiments with melanin or synthesized melanin precursors have shown that UV irradiation of melanin or melanin precursors, notably the pheomelanin precursor 5-S-cysteinyl dopa, produces ROS (Koch and Chedekel, 1986; Costantini *et al*, 1994). Thus, it is clear that melanin and melanin precursors may have antioxidant or prooxidant properties depending on the conditions under which they are studied, including their redox state (Rozanowska *et al*, 1999). It is therefore conceivable that pigmented melanocytes may enter states where melanin or melanin precursors enhance UVA-induced damage. This has previously been observed by us and others for UVA-induced oxidative DNA damage in melanoma cells and melanocytes (Wenczl *et al*, 1998; Kvam and Tyrrell, 1999).

The same types of cells as were used in our experiments have previously been used where their susceptibility to UVA- and UVB-induced cell inactivation (failure to form cell colonies) was measured (Hill *et al*, 1997). It was reported that the three types of melanocytes were almost equally susceptible to either UVA- or UVB-induced cell inactivation. We have not performed the same type of assay with our cells. If the results of Hill *et al* also apply to our cells, however, it appears that pigmented and unpigmented melanocytes die by different mechanisms as only unpigmented cells showed strong UVA-induced membrane permeability that indicates cell death (**Fig 2**).

Our results are relevant to a hypothesis about melanocyte carcinogenesis that has been proposed by Meyskens *et al* (2001). The hypothesis states that an essential part of melanocytes' malignant transformation is a change in the redox state of melanin from a mostly antioxidant state to a prooxidant state. The hypothesis is supported by data that show that melanoma cells as well as normal melanocytes of some, but not all, melanoma patients have a remarkably abnormal content of antioxidants, including vitamin E, polyunsaturated fatty acids, and catalase, which significantly reduces their resistance to oxidative stress (Picardo *et al*, 1999). Melanoma cells have also been shown to have a dramatically reduced capacity to neutralize hydrogen peroxide compared to normal melanocytes (Meyskens *et al*, 2001). It would therefore be interesting to examine the progeny of UV-irradiated normal melanocytes for the presence of cell clones with an altered redox state.

How does melanin in melanocytes modulate other effects of UV radiation on melanocytes? It has been reported that melanin may form supranuclear caps in epidermal cells (Kobayashi *et al*, 1998; Seiberg, 2001). Such supranuclear caps have been shown to correlate with reduced UVB-induced direct DNA damage such as pyrimidine dimers and 6–4 photoproducts. A recent report on melanocytes in culture has also demonstrated significant protection against UVB-induced direct DNA damage by increased melanin synthesis (Smit *et al*, 2001). Formation of supranuclear caps perhaps does not occur in melanocytes, however, or in all types of skin, and several experiments with melanocytes or melanoma cells have failed to demonstrate that melanin in melanocytes protects them significantly against UV-induced direct DNA damage (Schothorst *et al*, 1991; Li and Hill, 1997).

Our experiments support a role for melanin, or other factors selectively found in pigmented melanocytes, in protection against UVA-induced lipid and membrane damage. The few types of melanocytes examined do not allow us to draw general conclusions. In view of the potential role of UVA in melanoma carcinogenesis, however, the capacity of melanocytes to resist UVA-induced damage could be of importance. To better understand the importance, one would have to determine the role of various types of melanin and melanin precursors in modulating damage to lipids, DNA, as well as other cell components. Ultimately, one would have to understand how melanocyte mutagenesis and carcinogenesis is different from that of keratinocytes and how it is affected by the presence of melanin. Protection against UVA-induced membrane damage and cell inactivation by melanin might be harmful if premutagenic DNA damage such as 8-oxo-guanine accumulated in clonogenic cells (Kvam and Tyrrell, 1999). Thus, studies of mutagenesis and carcinogenesis of melanocytes should be intensified.

This work was supported by The Norwegian Cancer Society and The Norwegian Research Council. We are grateful to Kirsti Solberg Landsverk for performing flow cytometry and to Bjørn Høvik for operating the HPLC system.

REFERENCES

Bennett DC, Cooper PJ, Dexter TJ, *et al*: Cloned mouse melanocyte lines carrying the germline mutations albino and brown: Complementation in culture. *Development* 105:379–385, 1989

Brash DE, Rudolph JA, Simon JA, *et al*: A role for sunlight in skin cancer: UV-induced p53 mutations in squamous cell carcinoma. *Proc Natl Acad Sci USA* 88:10124–10128, 1991

Costantini C, d'Ischia M, Palumbo A, *et al*: Photochemistry of 5-S-cysteinyldopa. *Photochem Photobiol* 60:33–37, 1994

Girotti AW: Photosensitized oxidation of membrane lipids: Reaction pathways, cytotoxic effects, and cytoprotective mechanisms. *J Photochem Photobiol B* 63:103–113, 2001

Hill HZ, Hill GJ, Cieszka K, *et al*: Comparative action spectrum for ultraviolet light killing of mouse melanocytes from different genetic coat color backgrounds. *Photochem Photobiol* 65:983–989, 1997

Kobayashi N, Nakagawa A, Muramatsu T, *et al*: Supranuclear melanin caps reduce ultraviolet induced DNA photoproducts in human epidermis. *J Invest Dermatol* 110:806–810, 1998

Koch WH, Chedekel MR: Photoinitiated DNA damage by melanogenic intermediates *in vitro*. *Photochem Photobiol* 44:703–710, 1986

Kvam E, Tyrrell RM: The role of melanin in the induction of oxidative DNA base damage by ultraviolet A irradiation of DNA or melanoma cells. *J Invest Dermatol* 113:209–213, 1999

Li W, Hill HZ: Induced melanin reduces mutations and cell killing in mouse melanoma. *Photochem Photobiol* 65:480–485, 1997

Meyskens FL Jr, Farmer P, Fruehauf JP: Redox regulation in human melanocytes and melanoma. *Pigment Cell Res* 14:148–154, 2001

Napolitano A, Palumbo A, Misuraca G, *et al*: Inhibitory effect of melanin precursors on arachidonic acid peroxidation. *Biochim Biophys Acta* 1168:175–180, 1993

Niggli HJ, Applegate LA: Glutathione response after UVA irradiation in mitotic and postmitotic human skin fibroblasts and keratinocytes. *Photochem Photobiol* 65:680–684, 1997

Picardo M, Maresca V, Eibenschutz L, *et al*: Correlation between antioxidants and phototypes in melanocyte cultures. A possible link of physiologic and pathologic relevance. *J Invest Dermatol* 113:424–425, 1999

Prota G: Melanins, melanogenesis and melanocytes: Looking at their functional significance from the chemist's viewpoint. *Pigment Cell Res* 13:283–293, 2000

Royall JA, Ischiropoulos H: Evaluation of 2′,7′-dichlorofluorescein and dihydrorhodamine 123 as fluorescent probes for intracellular H_2O_2 in cultured endothelial cells. *Arch Biochem Biophys* 302:348–355, 1993

Rozanowska M, Sarna T, Land EJ, *et al*: Free radical scavenging properties of melanin interaction of eu- and pheo-melanin models with reducing and oxidising radicals. *Free Radic Biol Med* 26:518–525, 1999

Schmitz S, Thomas PD, Allen TM, *et al*: Dual role of melanins and melanin precursors as photoprotective and phototoxic agents: Inhibition of ultraviolet radiation-induced lipid peroxidation. *Photochem Photobiol* 61:650–655, 1995

Schothorst AA, Evers LM, Noz KC, *et al*: Pyrimidine dimer induction and repair in cultured human skin keratinocytes or melanocytes after irradiation with monochromatic ultraviolet radiation. *J Invest Dermatol* 96:916–920, 1991

Seiberg M: Keratinocyte–melanocyte interactions during melanosome transfer. *Pigment Cell Res* 14:236–242, 2001

Smit NP, Vink AA, Kolb RM, *et al*: Melanin offers protection against induction of cyclobutane pyrimidine dimers and 6–4 photoproducts by UVB in cultured human melanocytes. *Photochem Photobiol* 74:424–430, 2001

Smith NC, Dunnett M, Mills PC: Simultaneous quantitation of oxidised and reduced glutathione in equine biological fluids by reversed-phase high-performance liquid chromatography using electrochemical detection. *J Chromatogr B Biomed Appl* 673:35–41, 1995

Tyrrell RM: The molecular and cellular pathology of solar ultraviolet radiation. *Mol Aspects Med* 15:1–77, 1994

Tyrrell RM, Pidoux M: Endogenous glutathione protects human skin fibroblasts against the cytotoxic action of UVB, UVA and near-visible radiations. *Photochem Photobiol* 44:561–564, 1986

Tyrrell RM, Pidoux M: Correlation between endogenous glutathione content and sensitivity of cultured human skin cells to radiation at defined wavelengths in the solar ultraviolet range. *Photochem Photobiol* 47:405–412, 1988

Wang SQ, Setlow R, Berwick M, *et al*: Ultraviolet A and melanoma: A review. *J Am Acad Dermatol* 44:837–846, 2001

Wenczl E, Van der Schans GP, Roza L, *et al*: (Pheo) melanin photosensitizes UVA-induced DNA damage in cultured human melanocytes. *J Invest Dermatol* 111:678–682, 1998