α-Melanocyte-stimulating Hormone Protects from Ultraviolet Radiation-induced Apoptosis and DNA Damage*

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Ultraviolet radiation is a well established epidemiologic risk factor for malignant melanoma. This observation has been linked to the relative resistance of normal melanocytes to ultraviolet B (UVB) radiation-induced apoptosis, which consequently leads to accumulation of UVB radiation-induced DNA lesions in melanocytes. Therefore, identification of physiologic factors regulating UVB radiation-induced apoptosis and DNA damage of melanocytes is of utmost biological importance. We show that the neuropeptide α-melanocyte-stimulating hormone (α-MSH) blocks UVB radiation-induced apoptosis of normal human melanocytes in vitro. The anti-apoptotic activity of α-MSH is not mediated by filtering or by induction of melanin synthesis in melanocytes. α-MSH neither leads to changes in the cell cycle distribution nor induces alterations in the expression of the apoptosis-related proteins Bcl2, Bcl-x, Bax, p53, CD95 (Fas/APO-1), and CD95L (FasL). In contrast, α-MSH markedly reduces the formation of UVB radiation-induced DNA damage as demonstrated by reduced amounts of cyclobutane pyrimidine dimers, ultimately leading to reduced apoptosis. The reduction of UV radiation-induced DNA damage by α-MSH appears to be related to induction of nucleotide excision repair, because UV radiation-mediated apoptosis was not blocked by α-MSH in nucleotide excision repair-deficient fibroblasts. These data, for the first time, demonstrate regulation of UVB radiation-induced apoptosis of human melanocytes by a neuropeptide that is physiologically expressed within the epidermis. Apart from its ability to induce photoprotective melanin synthesis, α-MSH appears to exert the capacity to reduce UV radiation-induced DNA damage and, thus, may act as a potent protection factor against the harmful effects of UV radiation on the genomic stability of epidermal cells.

Apoptosis of epidermal cells by ultraviolet B (UVB; 290–320 nm) radiation is a well described phenomenon in vitro and in vivo and has been extensively studied in keratinocytes, the major target cells of solar UV radiation. It is considered a protective mechanism for minimizing the survival of cells with irreparable DNA damage (1), thereby preventing malignant transformation. The molecular pathways leading to UVB radiation-induced apoptosis include the formation of cyclobutane pyrimidine dimers (CPDs) and (6–4) photoproducts (2, 3), the activation of death receptors including CD95 (Fas/APO-1) (2, 4), the release of death ligands, e.g. tumor necrosis factor-α (5, 6), and the formation of reactive oxygen species (7). These pathways are orchestrated by positive and negative factors that act within the epidermis in an autocrine and/or paracrine fashion. For example, hepatocyte growth factor/scatter factor produced by dermal fibroblasts inhibits UVB radiation-induced apoptosis of human keratinocytes via the phosphatidylinositol 3-kinase/AKT pathway (8). Insulin-like growth factor-1, which is expressed by melanocytes and fibroblasts (9), delays UVB radiation-induced apoptosis in human keratinocytes via the same mechanism (10). Recently, we reported that the immunomodulatory cytokine interleukin (IL)-12 significantly reduces DNA lesions induced by UVB irradiation in vitro and in vivo. IL-12 did not reduce the number of sunburn cells in mice deficient in nucleotide excision repair (NER), indicating that the DNA damage-reducing effect of IL-12 may be linked to NER (11).

In the epidermis, one of the most biologically relevant protective mechanisms against UVB radiation-induced DNA damage is provided by melanocytes. These neural, crest-derived cells deliver melanin in melanosomes via dendrites to adjacent keratinocytes. Failure to produce melanin, for example due to the loss of function of tyrosinase as in ocular cutaneous albinism type 1, leads to accumulation of UV radiation-induced DNA damage that finally results in a dramatically increased risk for squamous and basal cell carcinoma as well as malignant melanoma.

Little is known about the natural paracrine or autocrine factors that regulate melanocyte apoptosis upon UVB radiation exposure. Only a limited number of peptide growth factors induce proliferation and melanogenesis in vitro. These mitogens include basic fibroblast growth factor, hepatocyte growth factor/scatter factor, mast cell growth factor, and endothelin-1.
(12, 13). Another group of melanocyte growth factors belongs to the evolutionary conserved family of melanocortins, which includes α-, β-, and γ-melanocyte-stimulating hormone (MSH) and adrenocorticotropin (ACTH) (14). These bioactive peptides were initially characterized as pituitary gland-derived peptides that are structurally derived from a common precursor, proopiomelanocortin (POMC) (15). Both α-MSH and ACTH bind with similar affinity to the human melanocortin-1 receptor (MC-1R) expressed by human melanocytes (16). MC-1R belongs to the superfamily of G protein-coupled receptors with seven transmembrane domains that activate adenylate cyclase (17). In recent years, however, it has become apparent that the skin as well as the majority of skin cell types in vitro express POMC and are capable of generating POMC peptides including α-MSH and ACTH (18, 19). Pro-inflammatory cytokines and UV radiation exposure were identified as physiological inducers of the skin POMC system, leading to the concept of a cutaneous analogon of the hypothalamic-pituitary-adrenal axis (19).

Moreover, MC-1R has been demonstrated as being expressed by a variety of extra-pigmentary cell types of the skin, including dermal microvascular endothelial cells (20), epidermal keratinocytes (21), secretory epithelial cell types (22–24), and dermal fibroblasts (25).

In light of the broad expression of MC-1R by various cutaneous cell types and the fact that UVB radiation of human skin cells in vitro and in vivo induces POMC expression and α-MSH release (26–28), we hypothesized that α-MSH could serve as a protection factor against UV radiation-induced cell damage. In this study we explored the effect of α-MSH on UVB radiation-induced cell damage of normal human melanocytes (NHMs).

We have identified a new physiologic pathway that reduces UVB radiation-induced DNA damage in NHMs and thus may minimize the risk of developing malignant melanoma.

MATERIALS AND METHODS

Cell Culture—NHMs and human dermal fibroblasts (HDFs) were established from newborn foreskin and purchased from CellSystems (St. Katharinen, Germany). HDF cultures were derived from three individual donors, and HDF cultures were derived from two individual donors. For experiments on NHKs, pooled cultures from several donors (CellSystems, St. Katharinen, Germany) instructions. NHMs were routinely grown in melanocyte growth medium M2 (PromoCell, Heidelberg, Germany) or as previously cultured in RPMI 1640 (PAA, Co¨lbe, Germany) and centrifuged, and cell pellets were dissolved in 1N NaOH. Melanin concentration was determined by measurement of the optical density at 475 nm in relation to a standard curve generated by synthetic melanin (Sigma).

MC-1R Genotyping and Sequencing—Genomic DNA extraction from NHMs, HDFs, and XPA fibroblasts was performed using a protocol from Gentra Systems (Minneapolis, MN). The MC-1R gene was amplified by PCR using established protocols (30) with forward (5′-ATGCGTG-CTCAACCATCC-3′) and reverse (5′-TCACAGGCA-CAC-3′) primers that were specific for MC1R. The reactions (primers, 2 mM MgCl2, 200 μM dNTPs, 1× OptiBuffer (Bioline, London, United Kingdom), and BioTaq Red (Bioline)) were carried out in a PerkinElmer Life Sciences Cetus 9700 thermal cycler and included an initial denaturation phase at 94 °C for 5 min followed by 35 cycles of 94 °C for 1 min, 62 °C for 1 min, and 72 °C for 2 min with a final extension phase of 7 min. The products (954 bp) were purified using a Qiagen PCR prep column (Qiagen), and the MC-1R coding region was sequenced on a model ABI 377 automated DNA sequencer (Applied Biosystems) using a dye terminator cycle sequencing kit (Amersham Biosciences) and the primers 5′-GCCGTGCTGCGACGCGCTGG-3′, 5′-TGCTGCAAGCAGCACCCGAGCC-3′, 5′-ACCAAGGGCCACAGGCA-3′, and 5′-GGCCTGTGCACGAGC-3′.

Colonies Formation Assays—NHMs were seeded into 6-cm-diameter tissue culture plates at a density of 2 × 104 cells per milliliter. Cells were treated with α-MSH at 10−6 M as outlined above. Following irradiation with UVB doses ranging from 150 to 400 J/m2, cells were re-stimulated with α-MSH in chemically defined medium for an additional 18 h. Culture medium was subsequently switched to routine melanocyte growth medium M2. Cells were kept in culture for 2–3 weeks before renewal of medium. Cultures were monitored daily. When colonies were easily visible, cells were stained with 0.1% crystal violet. The number and size (actually the area being covered) of the colonies were analyzed using the Image Analysis software Image J (rsbweb.nih/ij/docs/index.html). Three adjacent fields (3 × 3 cm each) per 10-cm-diameter tissue culture dish per experiment were used. Data were analyzed by one-way ANOVA with a post-hoc Tukey test.

Data Content Analysis—NHMs were seeded at a density of 1 × 105 cells per milliliter into 6-cm-diameter tissue culture plates in chemically defined medium without BPE. Cells were preincubated with α-MSH and irradiated with 600 J/m2 as described above. After 18 h cells were harvested by trypsinization, and DNA content analysis was determined by flow cytometry (31). Cell cycle analysis was performed using the ModFit LT software (Verity, Topsham, ME).

Western Immunoblotting—NHMs were collected by trypsinization and centrifugation. Pellets were washed with PBS and then suspended in 1× SDS sample buffer and boiled for 5 min. Liquid samples were analyzed by one-dimensional 10% SDS-PAGE and stained with 0.1% Coomassie blue. Membranes were reacted with a horseradish peroxidase-conju-
gated secondary antibody (Amersham Biosciences) followed by enhanced chemiluminescence kit (Amersham Biosciences). Membrane stripping was performed as described previously (13).

**Southwestern Dot Blot—**Genomic DNA was prepared from 1 × 10⁸ NHM immediately or 8 h after UV irradiation (150 J/m²). Genomic DNA was transferred to a nylon membrane by vacuum dot blot and fixed by baking the membrane for 15 min at 80 °C. CPD were detected by probing the membrane with a monoclonal antibody directed against thymine dimers (1:500; Kamiya Biomedical Company, Seattle, WA). Bound antibodies were visualized using a horseradish peroxidase-conjugated anti-mouse antibody (Amersham Biosciences).

**Immunofluorescence Studies—**Visualization of CPDs in NHMs by immunofluorescence was performed using a modified protocol from Roza et al. (32). 8 h after UV irradiation (150 J/m²), cells were trypsinized and centrifuged. Cell pellets were resuspended in PBS and processed for cytospin preparation (60,000–70,000 cells/slide). Cells were rinsed in PBS and fixed with 45% ethanol for 5 min followed by 70% ethanol at 20 °C for 10 min. Cells were subsequently permeabilized with 0.3% Triton X for 30 min. DNA denaturation was performed by treating the cells with 0.5 M HCl and 0.05% pepsin (Sigma) at 37 °C for 30 min. Slides were incubated with the antibody against CPD (1:2000) in 5% fetal calf serum and 0.05% Tween 20 for 1 h at 37 °C. Bound antibodies were visualized with a rabbit anti-mouse antibody coupled to Texas Red (Dianova, Hamburg, Germany). Cells were imaged with a fluorescence microscope (Leica DMRD/XXA, Wetzlar, Germany). Red fluorescence was excited using the TX2 filter (bandpass 560/40 nm). Emission was measured at 645 ± 75 nm for Texas Red. For DAPI staining an excitation wavelength of 360/40 nm was used. The number of CPD immunoreactive cells per 100 DAPI positive cells was determined in three individual high power viewing fields per experiment. Data were subsequently analyzed by the Student’s t test.

**RESULTS**

**α-MSH Protects from UVB Radiation-induced Apoptosis—**To determine the effect of α-MSH on UVB-induced apoptosis of NHMs, we treated cells with α-MSH (10⁻⁶ M) 6 h prior to exposure to UVB radiation (600 J/m²). After irradiation, cells were incubated for additional 16 h in the presence or absence of α-MSH followed by determination of mononucleosomes and oligonucleosomes using a death detection ELISA. In another set of experiments, NHMs were treated as described for panel A but by using the COOH-terminal tripeptide of α-MSH, KPV, at 10⁻⁶ M. NHKs were likewise deprived from BPE, preincubated with α-MSH (10⁻⁶ M) for 6 h, and irradiated with 250 J/m² UVB. After 18 h in the presence or absence of α-MSH, apoptosis was measured using the death detection ELISA. D and E, suppression of UVB radiation-induced apoptosis of NHMs (D) and NHKs (E) by α-MSH as shown by annexin V staining. Cells were treated exactly as described above followed by fluorescence-activated cell sorter analysis using an antibody against annexin V. Data are representative sets from three independent experiments with similar results. *p < 0.001 from triplicate analysis; N/A, non-treated cells.
despite the fact that none of the utilized NHM cultures established from the three individual donors were homozygous for a signaling-deficient MC-1R variant (two NHM cultures with both wild type MC-1R alleles and one NHM culture with a heterozygous R151C variant), the anti-apoptotic effect of α-MSH was not due to an increment in melanin content during the incubation period with α-MSH prior to UVB radiation exposure (data not shown). Moreover, spectrophotometric analysis did not demonstrate any filtering effect of α-MSH (data not shown). In accordance with an anti-apoptotic effect independent of melanogenesis, α-MSH blocked apoptosis of NHKs exposed to UVB radiation (250 J/m²) as shown by death detection ELISA (Fig. 1C). To confirm the protective effect of α-MSH on UVB radiation-induced apoptosis in NHMs and NHKs by another readout system, we performed annexin V surface staining and flow cytometry. Again, α-MSH suppressed UVB-induced apoptosis in NHMs (mean fluorescence intensity of non-irradiated cells was 10.6 versus 26.2 for UVB-treated cells and 17.4 for cells treated with α-MSH plus UVB) (Fig. 1D) as well as in NHKs (mean fluorescence intensity of non-irradiated cells was 7.4 versus 18.3 for UVB radiation-treated cells versus 9.7 for cells treated with α-MSH plus UVB) (Fig. 1E).

α-MSH Promotes Long Term Survival after UVB Irradiation—To exclude the possibility that α-MSH only delays apoptosis but indeed enhances the survival of NHMs, colony forming assays were performed. To this end, cells were preincubated with α-MSH, exposed to UVB radiation, and maintained in a routine culture medium for several weeks. To allow the survival of a sufficient number of cells suitable for colony formation, a lower UVB dose (150 J/m²) was applied than that used in the previous experiments. A single exposure to this dose drastically reduced the number of colonies detectable after 3 weeks (Fig. 2A). α-MSH (10⁻⁶ M) significantly increased the number and size of the visible colonies as compared with UVB radiation-exposed NHMs (Fig. 2, B and C). In contrast, α-MSH alone did not have any robust effect on the number of colonies in sham-irradiated cells. These data demonstrate that α-MSH indeed promotes long term survival of NHMs exposed to a single dose of UVB radiation.

α-MSH-mediated Protection of UVB-induced Apoptosis Is Not Due to Cell Cycle Changes—The protective effect of α-MSH on UVB radiation-induced apoptosis of NHMs could be caused by changes in the cell cycle, giving the cells more time to repair UVB radiation-induced DNA damage. It has been reported that in asynchronously growing NHKs and HaCaT cells, sublethal doses of UVB irradiation induce G₂ cell cycle arrest (31). On the other hand, NHMs were shown to arrest in G₁ after UVB irradiation in vitro (37). However, the experimental conditions as presented in this work suggested a priori a G₁ arrest in untreated NHMs and NHKs, as these cells had been deprived of BPE prior to treatment with α-MSH and/or UVB radiation (see “Materials and Methods”). DNA content analysis of untreated NHM revealed 88.6 ± 1.8% of cells were arrested in G₁ and 10.7 ± 1.3% were arrested in G₂/M (Table I). Although α-MSH, UVB radiation, and α-MSH plus UVB radiation lead to a small but statistically significant increase in the percentage of cells in S phase as compared with non-treated cells, α-MSH failed to increase the percentage of cells in G₁ or G₂/M as compared with UVB radiation alone (Table I). Therefore, it can be concluded that the protective effect of α-MSH from UVB radiation-induced apoptosis is not mediated via the accumulation of NHMs in the G₁ or G₂ phase.

α-MSH Does Not Alter Protein Expression of Bcl₂, Bclₓ, Bax, p53, CD95, or CD95L—To further elucidate the mechanisms by which α-MSH protects NHMs from UVB radiation-induced apoptosis, we analyzed the protein expression of a number of molecules crucially involved in the regulation of apoptosis. We first examined the expression of Bcl₂, a well known anti-apoptotic protein. As determined by Western immunoblotting of total cell lysates obtained 18 h after UVB irradiation (600 J/m²), UV radiation exposure resulted in a clear-cut reduction
in protein expression of Bcl2 as compared with sham-irradiated NHMs. α-MSH (10⁻⁶ M) only marginally affected this UVB radiation-mediated reduction in Bcl2 expression (Fig. 3). Neither UVB irradiation nor α-MSH treatment altered the amounts of Bclx protein, suggesting that this molecule is not involved in the protection of UVB radiation-induced apoptosis by α-MSH (Fig. 3). In another set of experiments we determined the protein expression of CD95 (Fas/APO-1) and its ligand CD95L (FasL) in NHMs treated with UVB radiation and/or α-MSH. CD95 was constitutively expressed in NHMs and not induced either by UVB radiation or α-MSH (Fig. 3). On the other hand, the CD95L protein was undetectable in NHM (Fig. 3), and treatment with UVB radiation or α-MSH did not induce its expression (data not shown). UVB irradiation slightly increased the amount of the p53 protein as compared with non-irradiated NHMs. However, co-treatment with α-MSH did not lead to any significant change in p53 protein expression (Fig. 3). In addition, although α-MSH alone appeared to increase Bax protein expression, it did not alter the relative expression levels of this proapoptotic regulator in UVB radiation-treated NHM as compared with cells irradiated with UVB light alone (Fig. 3).

**α-MSH Reduces the Amount of UVB Radiation-induced CPDs**—Because UVB radiation-induced DNA damage has been identified as the major trigger for UV radiation-induced apoptosis (2, 3), the effect of α-MSH on CPD formation was determined. 8 h after irradiation, genomic DNA was extracted and subjected to Southwestern dot blot analysis using an antibody against CPD. CPDs were undetectable in non-irradiated NHMs or in cells stimulated with α-MSH alone, respectively (Fig. 4A). UVB irradiation strongly induced CPDs in NHM. This effect was markedly suppressed by α-MSH (Fig. 4A). Induction of CPDs by UV radiation is an immediate event and, thus, is detectable shortly after UV exposure. When NHMs were analyzed for CPDs immediately after UVB radiation exposure, no differences were observed in cells treated with α-MSH (Fig. 4B). This finding implies that α-MSH does not prevent induction of CPDs, thereby excluding a filtering effect. To further confirm the effect of α-MSH on the CPDs induced by UVB radiation, we performed immunocytochemical analysis of NHMs after in situ DNA denaturation and CPD epitope retrieval. The extent of CPDs visible as immunoactive nuclei was related to the total number of cells as demonstrated by double staining with the nuclear marker DAPI (Fig. 5, A and B). As expected, CPDs were absent in non-irradiated NHMs. Irradiation with UVB light (150 J/m²) resulted in dramatic induction of CPD immunoreactive cells 8 h after irradiation with virtually every nucleus immunoreactive for CPD. α-MSH treatment significantly reduced the number of nuclei immunoreactive for CPD compared with NHMs exposed to UVB radiation alone (Fig. 5, A and B).

The fact that the amounts of CPDs were the same in the

**Table 1**

| Cell cycle phase | N/A | M | UVB | M + UVB |
|------------------|-----|---|-----|---------|
| G<sub>1</sub>     | 86.6 ± 1.8 | 85.1 ± 0.2 | 86.0 ± 3.3 | 87.4 ± 1.4 |
| G<sub>2</sub>/M   | 10.7 ± 1.3 | 10.5 ± 0.7 | 8.5 ± 4.5 | 9.2 ± 1.7 |
| S                 | 0.8 ± 0.5 | 4.3 ± 1.0<sup>a</sup> | 4.6 ± 1.2<sup>a</sup> | 3.4 ± 0.4<sup>a</sup> |

<sup>a</sup> p < 0.05 versus N/A.

**Fig. 3.** α-MSH does not alter the protein expression of Bcl<sub>2</sub>, Bcl<sub>x</sub>, Bax, p53, CD95, and CD95L in NHMs irradiated with UVB light. NHMs were deprived from BPE, preincubated with α-MSH (10⁻⁶ M), and irradiated with UVB light (600 J/m²). After 18 h in the presence or absence of α-MSH, total cell lysates were prepared. Identical amounts of proteins (top panel, 10 μg/lane; middle panel, 20 μg/lane) were subjected to Western immunoblotting. Membranes were sequentially probed with antibodies against Bcl<sub>2</sub>, CD95, and α-tubulin (top panel) and Bcl<sub>x</sub>, p53, and α-tubulin (middle panel). As a positive control for the expression of CD95L, HL60 leukemia cells (bottom panel) were used. N/A, non-treated cells. Shown are representative panels of at least three independent experiments with identical results.

**Fig. 4.** α-MSH reduces the amounts of UVB radiation-induced CPDs in NHMs. A and B, cells were deprived of BPE and preincubated with α-MSH (10⁻⁶ M) for 6 h followed by irradiation with 150 J/m² UVB light. Cells were kept in the presence or absence of α-MSH for additional 8 h (A) or harvested immediately after irradiation (B). 2 μg of genomic DNA per sample was subjected to Southwestern dot blot using a monoclonal antibody against CPDs. The set is one of three independent experiments with identical results. N/A, non-treated cells.
radiation-induced DNA lesions. Because NER is the major mechanism by which CPDs are removed in mammalian cells, the effect of α-MSH on UVB radiation-induced apoptosis of NER-deficient cells was determined. The XPA gene is an essential component of the NER; thus, cells with a mutated XPA gene completely lack a functional NER. Therefore, dermal fibroblasts established from a patient with XPA and normal HDFs were examined for the effect of α-MSH on UVB radiation-induced apoptosis. It had been previously established that HDFs express functional receptors for α-MSH that have been identified by reverse transcription PCR, immunofluorescence analysis, and radioligand binding studies as MC-1R (25). α-MSH again significantly blocked UVB radiation-induced apoptosis by 45% in HDFs (Fig. 6A). In contrast, XPA fibroblasts were not protected by α-MSH from UVB radiation-induced apoptosis (Fig. 6B), indicating that the reduction of CPDs by α-MSH may depend on a functional NER. To exclude the possibility that the failure of α-MSH to reduce apoptosis in XPA fibroblasts is due to a non-functional α-MSH receptor, genotyping of the MC-1R gene was performed. The utilized XPA fibroblasts contained a single variant allele (V60L). Based on the presence of one wild type allele, it is highly likely that these cells are able to signal via the wild type receptor upon the binding of α-MSH (38).

**DISCUSSION**

UVB radiation-induced apoptosis has been recognized as a protective mechanism because it contributes to the elimination of cells carrying DNA damage, thereby preventing malignant transformation (39). In this context, UVB radiation-induced apoptosis has been most intensively studied in NHKs, the major cellular target for solar/UVB radiation. In contrast to NHKs, which are quite prone to apoptosis and appear in situ as sunburn cells, NHMs have been reported to be quite resistant to UV radiation-induced apoptosis (33). This is based on the fact that apoptotic NHMs are hardly observed in situ and also on the fact that higher doses are required to induce apoptosis in vitro in NHMs than in NHKs. The resistance to UVB radiation-induced apoptosis of NHMs may be due to the high expression of the anti-apoptotic protein Bcl-2 (34, 40–43). The higher vulnerability of NHKs to UV radiation as compared with that of NHMs may also be due to the fact that the former cell type is more likely to undergo active cycling during UV radiation exposure than the normally non-mitotic NHMs, be-
cause cells are prone to undergo apoptosis when synthesizing DNA (44). All of these observations gave rise to the speculation that nature may accept a certain degree of risk of mutation induced by UV radiation in NHMs in order to maintain their melanin-generating photoprotective role in the skin (33). However, this also implies that severely UV radiation-damaged NHKs, which are at risk for incomplete repair of DNA and subsequent mutation, may be eliminated by apoptosis, whereas similarly damaged NHMs are retained and, thus, are at some risk for subsequent mutations. Because the apoptotic protection pathway obviously may not be as efficient in NHMs, the identification of any pathway or strategy to reduce the load of DNA damage in NHMs may be of relevance for the understanding of the pathogenesis of malignant melanoma and for the development of preventive/therapeutic measurements. Here, we demonstrate that this may apply for the neuropeptide α-MSH.

The present data demonstrate that α-MSH reduces UV radiation-induced apoptosis of NHMs in vitro. Elucidating the underlying mechanism, we observed that α-MSH neither exerts its effect on UVB radiation-induced apoptosis via altering the cell cycle distribution nor via changing the expression of apoptosis-related proteins including Bcl2, Bcl-x, p53, CD95, and CD95L. In contrast, as demonstrated by Southwestern dot blot analysis, α-MSH significantly reduced the amounts of CPDs, the major DNA lesion induced by UV. The reduction of CPDs by α-MSH was detected when DNA was extracted 8 h after UV radiation exposure. However, when DNA was extracted immediately after UV irradiation, no differences were observed between α-MSH-exposed and untreated UV-light-irradiated NHMs. This finding indicates that the reducing effect of α-MSH is not due to a filtering effect of α-MSH. This is also in accordance with spectrophotometric analyses that did not demonstrate any absorbing capacity of α-MSH within the UVB range.

The fact that the amounts of CPDs were the same in the α-MSH-treated and untreated NHMs immediately after UV radiation exposure but were remarkably reduced at 8 h after UV radiation exposure can only be explained by the fact that α-MSH might accelerate the removal of UVB radiation-induced DNA lesions. NER is the major DNA repair system in mammalian cells for the removal of UV radiation-induced DNA damage. To determine whether α-MSH may exert its reducing effect on DNA damage by affecting NER, NER-deficient cells were used. Cells derived from patients suffering from xeroderma pigmentosum either lack or have reduced DNA repair capacity due to genetic mutations in several components of the NER. The XPA complementation type represents the most severe phenotype, because the Xpa gene is the most crucial component in the repair process and, thus, cells lacking the Xpa gene are completely deficient in NER (45, 46). Because XPA melanocytes are not available, we used XPA fibroblasts for our purposes. Previous studies have demonstrated that HDFs also express receptors for α-MSH and are susceptible to the effects of this neuropeptide (25). In fact, the reducing effect of α-MSH on UV radiation-induced DNA damage was observed in wild type but not in XPA fibroblasts. This implies that the effect of MSH on DNA damage may be mediated via NER or at least linked to a properly functioning NER system.

As a crucial protection mechanism, NER was regarded for a long time as being constitutively expressed and was thought not to be regulated by external stimuli. However, it was recently described that the immunomodulatory cytokine IL-12 may modulate the intracellular amount of reactive oxygen species targeted by α-MSH (47). Regarding the protective effect of IL-12 on UVB radiation-induced apoptosis, it was shown that IL-12 affects several components of the NER (11). We have not yet checked whether α-MSH exerts a similar effect. The fact that the reduction of DNA damage by α-MSH may be mediated via NER is also supported by the observation that the induction of apoptosis of NHMs by bleomycin, a radiomimetic DNA-damaging anticancer drug inducing both single- and double-strand DNA breaks that are not repaired by NER (52), is not prevented by α-MSH. In contrast, α-MSH significantly suppressed apoptosis of NHMs exposed by cisplatin (53). Cisplatin-induced DNA lesions are at least partially repaired by NER, thus supporting our assumption that α-MSH may modulate NER.

Nevertheless, at this stage we cannot exclude with absolute certainty that α-MSH may mediate its anti-apoptotic activity via additional mechanisms other than NER. It will be interesting to check whether α-MSH affects other apoptosis regulators such as Bax, Mcl-1, c-IAP1/2, XIAP, Livin, and Apaf-1, all of which are expressed in NHMs (43). UVB light has been recently recognized to be able to induce reactive oxygen species, and this may contribute to UV radiation-induced apoptosis (7). Because α-MSH can modulate the intracellular amount of reactive oxygen species in HaCaT keratinocytes exposed to hydrogen peroxide (53), it also will be worth to investigate if α-MSH reduces UVB radiation-mediated apoptosis by acting on the formation of reactive oxygen species.

Taken together, these data demonstrate that UVB-induced apoptosis of NHMs can be modulated in vitro by the neuropeptide α-MSH and that this effect is primarily due to a reduction of UV radiation-induced DNA damage, presumably via activation of the NER. The fact that POMC peptides are expressed in the skin and are induced by UVB radiation in vitro and in vivo (18, 19, 28) suggests that α-MSH might function as a paracrine or autocrine “protection factor” against the harmful effects of UVB radiation on epidermal homeostasis and genomic stability. This protective effect may be due to its melanin-generating capacity primarily affecting NHKs but, as demonstrated here, also to its capacity to reduce DNA damage. The latter capacity may be of relevance for the homeostatic genomic stability of NHMs and may represent a physiological mechanism reducing thymidine dinucleotides that enhance the repair of UV radiation-induced DNA damage in a p53-dependent manner (47). In contrast, the effect of IL-12 appeared to be independent of p53 because it was also observed in HaCaT cells, which contain two p53 mutations (48). Likewise, we did not observe an alteration of the p53 protein levels by α-MSH, suggesting that the effect may be independent of p53. Our findings are in accordance with data from others, who also could not detect a modulating effect of α-MSH on p53 expression in UVB light-irradiated NHMs (49). However, we cannot completely rule out an involvement of p53 in the cytoprotective mechanism of α-MSH. Interestingly, α-MSH is not protective against UVB radiation-induced apoptosis of HaCaT keratinocytes, which carry p53 mutations (50). It is thus possible that the anti-apoptotic pathways targeted by α-MSH exhibit cell type-specific differences. On the other hand, it has been reported that HaCaT keratinocytes exhibit deviations in the signaling events modulated by α-MSH, i.e. the NF-κB pathway (51) being also involved in the regulation of apoptosis. Therefore, the lack of an anti-apoptotic effect of α-MSH in HaCaT cells may be related to deviations in the NF-κB signaling pathway. Further studies are needed to fully clarify the mechanistic role of p53 in regulation of UVB radiation-induced apoptosis by α-MSH.

α-MSH Protects from UV Radiation-induced Apoptosis

5801

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the risk of the malignant transformation of NHMs. Whether MSH[Nle4-D-Phe7] (54) in UV protection, because any strategy that reduces the cumulative load of UV radiation-damaged DNA in melanocytes in particular may contribute to lowering the risk of developing UV radiation-induced skin cancer.

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