Role of Gene Expression and Protein Synthesis of Tyrosinase, TRP-1, Lamp-1, and CD63 in UVB-Induced Melanogenesis in Human Melanomas

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Using melanotic cells (SK-MEL-23 and G361) and amelanotic cells (C32 and SK-MEL-24) of human melanoma, this study examined whether UV-B irradiation has a direct stimulatory effect on the expression of genes involved in melanogenesis. Our initial screening of methylthiazol tetrazolium (MTT)-formazan formation assay indicated a low dose of ultraviolet (UV)-B irradiation, 2.5 and 5.0 mJ/cm², can metabolically stimulate these cells. Repeated exposure of UV-B at 5.0 mJ/cm² for seven consecutive days resulted in increased tyrosinase activity and melanin synthesis in SK-MEL-23 and G361 cells, but not in C32 and SK-MEL-24 cells. On reverse-transcription–polymerase chain reaction and immunoprecipitation studies, the two melanotic cell lines exhibited upregulated expression of mRNA and antigenic epitopes of tyrosinase, tyrosinase-related protein (TRP-1; gp75/HMSA-5), and lysosomal membrane associated protein (Lamp-1). The amelanotic cell line, C32, expressed the tyrosinase gene and protein constitutively but revealed no active tyrosinase or melanin synthesis even after UV-B exposure. Another amelanotic cell line, SK-MEL-24, exhibited no expression of tyrosinase gene and protein before and after UV-B exposure and, therefore, no melanin synthesis. Both C32 and SK-MEL-24 showed no gene or protein expression of TRP-1 before or after UV exposure, but upregulation of the Lamp-1 gene and protein expressions after exposure.

We conclude that tyrosinase is the key enzyme responsible for UVB-induced melanogenesis. Both TRP-1 and Lamp-1 act together in melanogenesis, TRP-1 being essential and necessary. There is no change in the expression of CD63 lysosomal membrane protein at either the mRNA or protein level. *Key words: melanogenesis/tyrosinase/UV irradiation/lysosome. J Invest Dermatol 102:495–500, 1994*

Solar irradiation on human skin produces two major photobiologic processes of melanin pigmentation, i.e., immediate and delayed tanning reactions. Delayed tanning reaction can best be produced by repeated exposure to ultraviolet (UV)-B (290–320 nm) or UV-A (320–400 nm) in combination with psoralen and is associated with the differentiation and proliferation of epidermal melanocytes [1]. It is still not fully understood whether this new melanogenic process is mediated by a direct effect of solar radiation to melanocytes or an indirect effect through cytokines and growth regulators released by surrounding epidermal keratinocytes or dermal mesenchymal cells [2]. This study addresses whether there is any direct effect of UV-B irradiation on the melanocytes to stimulate new melanogenesis. If this is the case, how and to what extent can UV-B regulate the transcriptional, translational, and post-translational processes of melanogenesis? Specifically we wish to know the effect of UV-B irradiation on expression of tyrosinase, tyrosinase-related protein (TRP-1), lysosomal membrane protein (CD63), and lysosome-associated membrane protein (Lamp-1). The association of TRP-1, Lamp-1, and CD63 with/within the melanosomal compartment has been recently reported [3–9], but their functional role in melanogenesis has not fully been determined. If these melanogenic enzymes and proteins are regulated by UV-B irradiation, how are they coordinated to produce new melanogenesis after UV exposure? Is TRP-1 important in the induction of melanogenesis? If so, how? Similarly, is the presence of CD63 and Lamp-1 essential to melanogenesis?

To address these questions, we irradiated melanotic and amelanotic human melanocytes (melanoma cells) with UV-B and evaluated their photobiologic responses by determining gene and protein expressions of tyrosinase, TRP-1, Lamp-1, and CD63 in conjunction with tyrosinase activation and melanin synthesis.

MATERIALS AND METHODS

Cell Culture Four human melanoma cell lines were obtained from the American Type Culture Collection (ATCC, Rockville, MD) for this study. These included SK-MEL-23 (heavily pigmented melanotic line), G361 (lightly pigmented melanotic line), SK-MEL-24 (non-pigmented amelanotic line), and C32 (non-pigmented amelanotic line).

UV Irradiation The UV irradiation was carried out at room temperature by a Dermalight/Bluelight 2001 (Dr. Homle Company, Martinsried, Germany), which delivers approximately 91% of the UV spectra output in the range of UV-B (290–300 nm) and 9% in the UV-A range (320–400 nm), with the irradiance of 1.07×10^3 watts/second. During UV irradiation, the culture medium was replaced by warm phosphate-buffered saline (PBS) without Ca^{++} and Mg^{++} to avoid the formation of medium-derived toxic products. Sham-irradiated control dishes were processed identically but covered with aluminum foil.

Manuscript received June 4, 1993; accepted for publication December 2, 1993.

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Abbreviations: TRP, tyrosinase-related protein; HMSA, human melanosome-specific (associated) antigen; Lamp, lysosome-associated membrane protein; MTT, methylthiazol tetrazolium; TES, N-trishydroxylmethyl-methyl-2-aminothane sulfonic acid; RT-PCR, reverse-transcription–polymerase chain reaction.

0022-202X/94/$07.00

Table I. Tyrosinase Activity and Melanin Content in Human Melanoma Cells After Multiple UV-B Exposures[a]

	Tyrosinase Activity (nmol/h/10^6 Cell)[b]			Melanin Content (pg/10^6 Cell)[b]		
Cell Line	0.0 mJ/cm²	2.5 mJ/cm²	5.0 mJ/cm²	0.0 mJ/cm²	2.5 mJ/cm²	5.0 mJ/cm²
SK mel 23	8.18 ± 0.77	19.38 ± 3.49	26.50 ± 1.16	3.19 ± 0.06	2.91 ± 0.31	3.95 ± 0.52
G 361	2.37 ± 0.39	3.98 ± 0.32	8.72 ± 0.08	0.79 ± 0.04	1.11 ± 0.06	1.32 ± 0.05
C 32	0.13 ± 0.05	0.19 ± 0.01	0.23 ± 0.03	0.03 ± 0.01	0.03 ± 0.01	0.05 ± 0.01
SK mel 24	0.23 ± 0.06	0.22 ± 0.03	0.32 ± 0.04	0.02 ± 0.00	0.04 ± 0.01	0.06 ± 0.00

[a] Daily exposure of 7 d to UV-B with 0.0 mJ/cm², 2.5 mJ/cm², and 5.0 mJ/cm².
[b] Average ± SEM; both tyrosinase activity and melanin content were measured at 24 h after the seventh irradiation.

Colorimetric Methylthiazol Tetrazolium (MTT) Assay The MTT assay was carried out at various intervals, i.e., 3 h, 5 h, 12 h, and 24 h, with UV-B irradiation doses of 2.5, 5.0, 7.5, 10.0, 15.0, 17.5, 20.0, 25.0, and 30.0 mJ/cm². This colorimetric assay is based upon the cell's ability to reduce a tetrazolium salt, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and form a blue formazan product by a mitochondrial enzyme, succinate dehydrogenase [10]. At 24 h after irradiation, the cells were harvested and plated in 96 wells of flat-bottomed microtiter plates (5×10^3 cells/100 μl/well). The MTT solution was prepared by dissolving 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (Sigma Chemicals Inc., Toronto, Ontario) in PBS at 5 mg/ml and filtering to remove a small amount of the insoluble MTT residue. Ten microliters of the MTT solution was added to each well, incubated at 37°C for 4 h, and then 0.1 ml of 0.04 M HCl in isopropanol was added to dissolve the formazan crystals. The absorbency at 540 nm was measured with an EAR 400 AT automatic colorimeter (SLT-Lab Instruments, Australia) for microplates. The activity of formazan formation from MTT was expressed as the percentage increase compared to that of sham control.

Assay for Melanin Content Based on the results of the MTT assay, cells were exposed to UV-B at doses of 0.0, 2.5, and 5.0 mJ/cm² for seven consecutive days. Melanin content was measured according to Whittaker's method [11].

Assay for Tyrosinase Activity Tyrosinase activity was measured by Pomerantz's method [12] with some modifications. The cell pellets were processed for tyrosinase activity measurement by incubating them in the reaction solution consisting of substrates (1.0 mCi/mL of L-[ring-3-,5-^{3}H] tyrosine [Dupont, Boston, MA], 8.4 mM of L-tyrosine, and 15.2 mM of L-dopa) at 37°C for 3 h. The reaction was stopped by adding charcoal suspension (500 μl; 1% [w/v] in 0.1 M citric acid), which was removed by centrifugation. The supernatants were passed over Dowex-50 × 2-400 ionic exchange column (0.5 ml of bed volume) and collected in scintillation vials, and the amount of radioactivity was counted. The calculation formula of tyrosinase activity was as follows: 84.0 nmol × (sample dpm − blank dpm)/(total dpm − blank dpm) × 0.5 × 3 h = nmol/h.

Immunoprecipitation Study

TRP-1, Lamp-1, and CD63: The UV-irradiated cells grown in 75 cm²-cultured dishes were metabolically labeled with L-^{35}S-methionine (250 μCi, specific activity 1000 Ci/mmol, Amersham, Ontario, Canada) for 10 h and then harvested in lysis buffer (3 ml; 150 mM NaCl, 1% [v/v] NP-40, 0.5% [w/v] deoxycholic acid-sodium salt, 50 mM Tris, 0.1% [w/v] sodium dode-

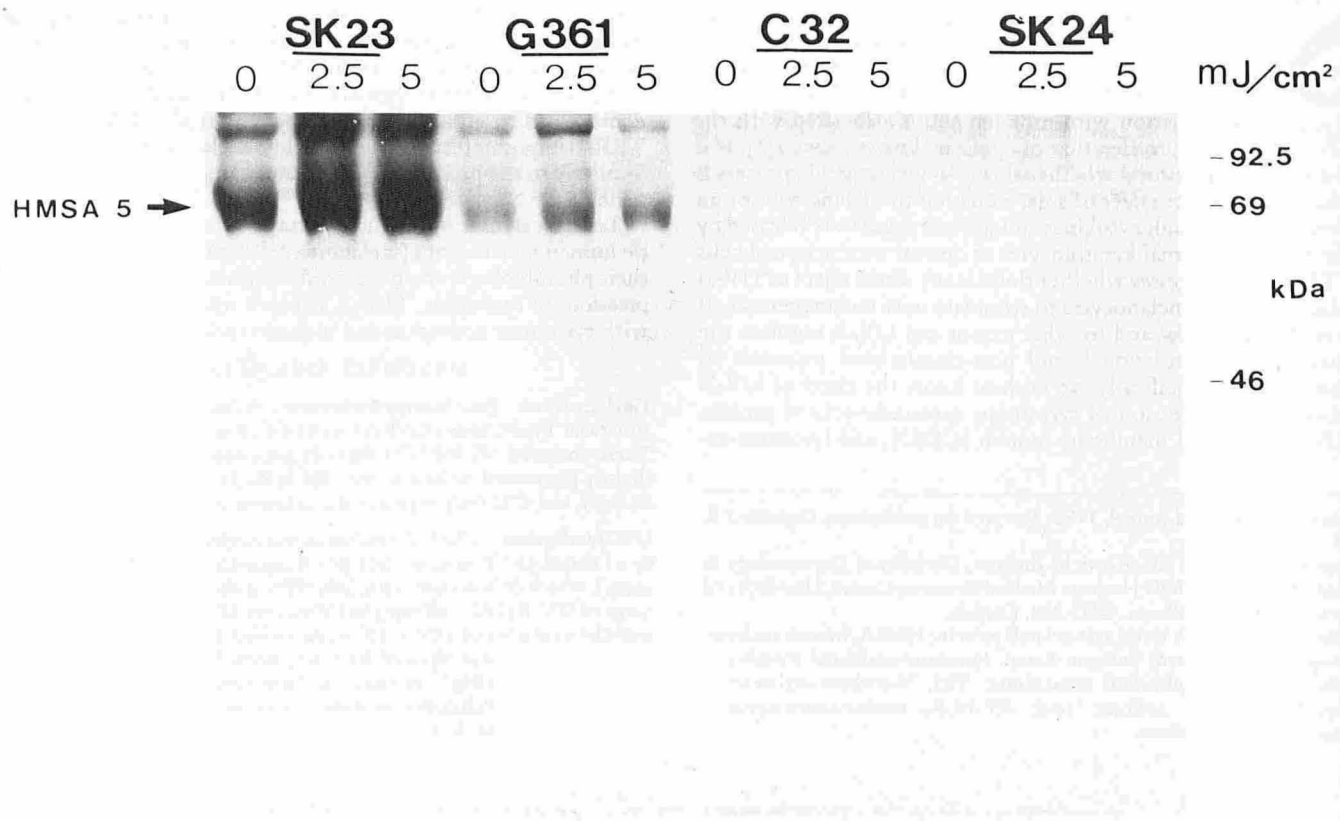

Figure 1. The expression of the TRP-1 (gp75) protein immunoprecipitated by MoAb HMSA-5. Semiconfluent, cultured melanoma cells of melanotic SK-MEL-23 and g361) and amelanotic (C32 and SK-MEL-24) lines grown in 75-cm² culture dishes were exposed daily for seven consecutive days at room temperature to UV-B at 2.5 mJ/cm² and 5.0 mJ/cm². They were then metabolically radiolabeled with ^{35}S-methionine for 10 h at 37°C, harvested in lysis buffer, subjected to immunoprecipitation, and analyzed by SDS-PAGE. An *arrow* of HMSA-5 indicates the location of TRP-1 protein (69 kDa).

cyl sulfate [SDS] and 0.25% [v/v] aprotinin at pH 8.0). Aliquots (5 μl) of lysate supernatants from the labeled cells were precipitated by 10% [w/v] trichloroacetic acid and counted by a liquid scintillation counter. All lysates were balanced for total radioactivity (counts per minute) [cpm] prior to immunoprecipitation.

The culture supernatants of hybridoma-cells producing monoclonal antibody (MoAb) human melanosome-specific (associated) antigen (HMSA)-5 or -7 or anti-Lamp-1 antibody were added to the lysate supernatants and incubated at 4°C for 18 h. The goat affinity-purified antibody to mouse immunoglobulin (Ig) (15 μl; IgG, IgA, and IgM [Organon Teknika Corp, West Chester, PA]) was added to the lysates and incubated for another 2 h. Protein-A sepharose suspension (50 μl) was then added. The immunoabsorbent beads were collected by centrifugation (1000 × *g*, 10 min) and washed with immunoprecipitation buffer (250 mM NaCl, 5 mM ethylenediamine tetra-acetic acid, 50 mM Tris, 1% [v/v] NP-40).

Immunoabsorbent beads were resuspended in Laemmli's sample buffer [13], and loaded onto 12% SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Gels containing ^{35}S-methionine were processed for fluorography. Kodak X-omat AR X-ray films (Eastman Kodak, Rochester, NY) were used to visualize the labeled proteins.

Tyrosinase: The tyrosinase immunoprecipitation study was carried out as described recently [14]. The total amount of anti-tyrosinase epitope expression was assayed by counting the radioactivity of the immunoprecipitate of protein A-sepharose complexes.

Analysis by Polymerase Chain Reaction (PCR) The total cellular RNA was prepared using the guanidium thiocyanate/phenol/chloroform method. The total RNA (2 μg) was denatured at 90°C for 5 min, and mRNA was reverse-transcribed in the reaction mixture solution containing oligo(dT)$_{21}$ (1 μl), 2.5 mM mix of four deoxynucleotide triphosphates (dNTP) (8 μl, Boeringer Mannheim, Germany), 10 × concentrated PCR buffer solution (4 μl, 500 mM KCl-100 mM Tris; pH 8.3), 25 mM $MgCl_2$ (8 μl), reverse transcriptase (1 μl, Maloney-murine leukemia virus, 20 U/μl; Stratagen, CA), RNase inhibitor (1 μl; Gibco BRL, MD). The reaction mixture was incubated at 37°C for 1 h, precipitated by ethanol solution, centrifuged, air-dried, and then resuspended in water (100 μl). The following was added for PCR amplification: the prepared cDNA solution (1 μl), 10 × PCR buffer (5 μl), 25 mM $MgCl_2$ (4 μl), deoxynucleotide triphosphate (dNTP, 4 μl), 15 μM primer A (0.5 μl), 15 μM primer B (0.5 μl), and Tag polymerase solution (0.5 μl; Perkin Elmer Cetus, Norwalk, CT). The final volume was made up to 50 μl with water. Primers for human tyrosinase, human TRP-1, CD63, and human Lamp-1 are listed in Table I. The PCR reaction was carried out under the condition of 35 cycles with denaturation (1 min at 94°C), annealing (2 min at 55°C), and extension (2 min at 72°C) using a thermal reactor (Tyler Research Instrument, Edmonton, Canada). The PCR products (10 μl per *lane*) were loaded onto 1.1% agarose gels and stained by ethidium bromide.

Electron Microscopy The cultured cells with and without UV irradiation were prefixed with 3% glutaraldehyde and then postfixed with 1% osmium tetroxide. They were then dehydrated, embedded in epoxy resins, ultrasectioned, and counterstained with lead citrate and uranyl acetate.

RESULTS

MTT Assay To ascertain the adequate UV-B dosage, human melanoma and HeLa cell cultures were exposed to various doses of UV-B irradiation and processed for MTT assays. There was a significant difference in MTT values between melanoma cells and HeLa cells after a single exposure of UV-B. Even with a low dosage of UV-B (5.0 mJ/cm²), HeLa cells did not tolerate UV-B exposure and showed a decreased MTT value immediately after UV irradiation (e.g., 90% of sham-treated control in 3 h with 5.0 mJ/cm²). In contrast, all four melanoma cells showed an increased MTT value (1.2-1.7-times increase compared to sham-treated cells) with UV-B irradiation. Increased MTT values were seen from 3-12 h post-irradiation. The C32 amelanotic melanoma cells showed the highest UV tolerance and activation (1.25-1.7-times increase), followed by an amelanotic cell line of SK-MEL-24, a heavily pigmented cell line of SK-MEL-23, and finally a lightly pigmented melanoma cell line of G361. However, after 6 h G361 showed a much higher UV tolerance than SK-MEL-23. The C32 and G361 melanoma cells showed two peaks of formazan formation at 6-12 h after UV irradiation. All four melanoma cells showed a decreased MTT value in a dose-dependent manner at 24 h post-irradiation (50-70% of control sham treatment).

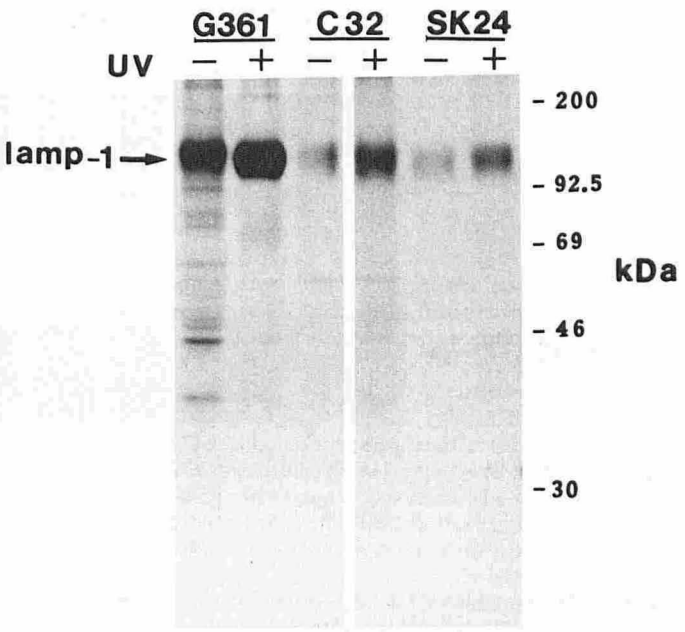

Figure 2. Effect of UV-B-induced Lamp-1 expression. Human melanoma cells of G361, C32, and SK-MEL-24 were irradiated to UV-B (5.0 mJ/cm²) for seven consecutive days at room temperature, labeled with ^{35}S-methionine, subjected to immunoprecipitation, analyzed on SDS-PAGE, and followed by fluorography. Control cells (UV minus) were sham-irradiated, being covered with aluminum foil at room temperature. An *arrow* of Lamp-1 indicates the location of Lamp-1 protein (120 kDa).

These studies indicated that melanoma cells are UV resistant compared to HeLa cells, and metabolically activated by exposure to the low dosage of UV-B (2.5-7.5 mJ/cm²). Based upon these results, the following experiments with seven consecutive days of multiple UV-B exposures were carried out with dosages of 2.5 and 5.0 mJ/cm².

Tyrosinase Activity and Melanin Contents Two melanotic melanoma cells, SK-MEL-23 and G361, showed increased tyrosinase activity and melanin synthesis after multiple UV-B exposure for seven consecutive days. Tyrosinase activity was increased approximately 3.7 times, whereas melanin synthesis was 1.7 times in G361 melanoma cells at a dosage of 5.0 mJ/cm². In contrast, there was no obvious increase of melanin synthesis in SK-MEL-23 melanoma cells, although tyrosinase activity increased approximately 3.2 times. This may be partly related to the presence of heavily loaded melanin pigments in these cells before UV irradiation. In two amelanotic melanoma cells, SK-MEL-24 and C32, there was no significant alteration in tyrosinase activity or melanin synthesis at UV-B dosages of 2.5 mJ and 5.0 mJ/cm² (Table I).

Immunoprecipitation Study for Protein Synthesis Expression The MoAb HMSA-5-immunoprecipitated bands were seen only in melanotic cell lines of SK-MEL-23 and G361, which showed increased protein expression after UB-B irradiation. No immunoprecipitated band of MoAb HMSA-5 was detected on C32 and SK-MEL-24 amelanotic melanoma cells (Fig 1). The immunoprecipitates of anti-Lamp-1 antibody revealed a UV dose-dependent increase of immunoprecipitated bands in all three UV-irradiated melanotic and amelanotic cells (Fig 2). All four melanoma cells revealed the immunoprecipitation bands with MoAb HMSA-7 (identical to CD63), but with no alteration in the total amount of immunoprecipitates before and after UV-B irradiation (Fig 3). There was a marked increase in the tyrosinase immunoprecipitate with anti-PEP 7 (tyrosinase) antibody after UV irradiation in G361

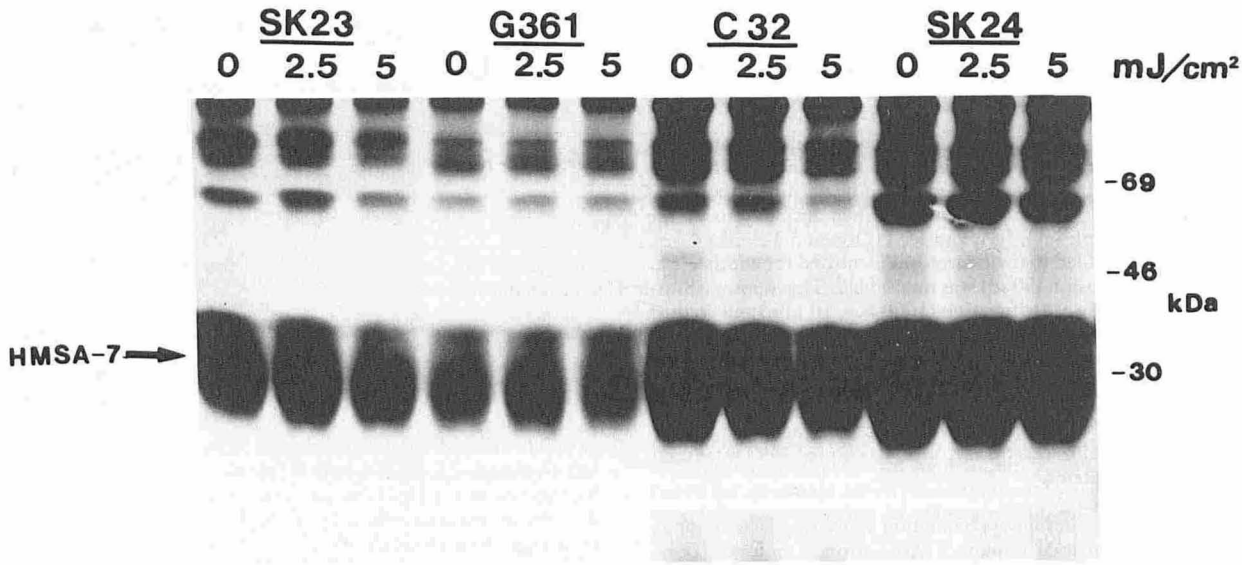

Figure 3. The expression of CD63 (lysosome membrane protein) immunoprecipitated by MoAb HMSA-7. The experimental condition (2.5 mJ/cm² and 5.0 mJ/cm²) and cell lines (SK-MEL-23, G361, C32, and SK-MEL-24 melanoma cells) were identical to those described in *Fig 1*. An *arrow* of HMSA-7 indicates the location of CD63 protein (53–21 kDa).

melanotic melanoma cells. Surprisingly, there was a minimum increase of anti-tyrosinase antibody epitope expression in C32 amelanotic melanoma cells, but no increase in SK-MEL-24 amelanotic melanoma cells (Fig 4).

RT-PCR Analysis for Gene Expression Study Total RNA isolated from UV-irradiated cells of G361, C32, and SK-MEL-24 was subjected to RT-PCR amplification with the synthetic oligonucleotide primers listed in Table II. There was a marked increase in the tyrosinase mRNA expression in G361 melanotic melanoma cells after UV irradiation. As in the immunoprecipitation studies, C32 amelanotic melanoma cells revealed a faint band of the tyrosinase mRNA expression before UV irradiation and a marked increase after UV irradiation. In contrast, there was no expression of tyrosinase mRNA in SK-MEL-24 amelanotic cells before or after UV irradiation (Fig 5). The Lamp-1 mRNA expression was present and increased in all of these melanoma cells after UV irradiation (Fig 5). In contrast to tyrosinase and Lamp-1 gene expression, the mRNA expression of TRP-1 was well correlated with melanin synthesis and tyrosinase activity. G361 melanotic melanoma cells exhibited TRP-1 gene expression before and after UV irradiation, with markedly enhanced expression after UV irradiation. There was no signal for mRNA expression of TRP-1 in C32 and SK-MEL-24 amelanotic melanoma cells. The mRNA expression of the CD63 gene was seen in all three melanoma cells, with no alteration in the degree of signals before and after irradiation (Fig 6), indicating there is no connection between CD63 expression and melanin synthesis or tyrosinase activity.

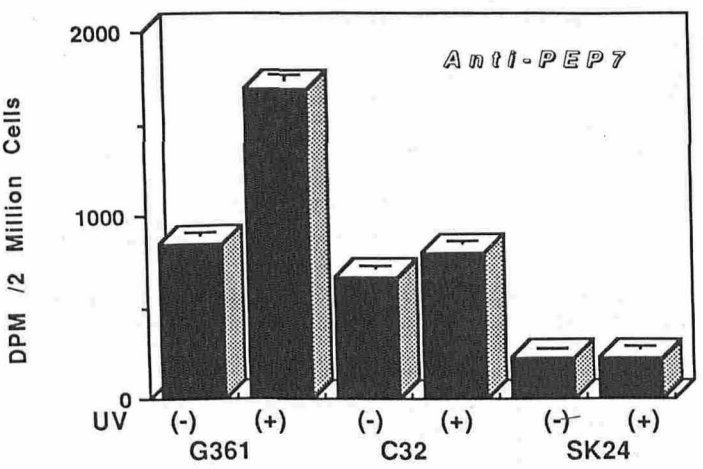

Figure 4. Expression of UV-B–induced tyrosinase protein immunoprecipitated by anti-PEP 7 tyrosinase antibody. The cells were exposed daily to UV-B, 5.0 mJ/cm² for seven consecutive days at room temperature, labeled with ^{35}S-methionine, and subjected to immunoprecipitation. The data are presented as the specific radioincorporation subtracted by both background serum (control) and protein A sepharose, and carried out in triplicate. *Bar*, SD. The experimental conditions of control cells (UV minus) are identical to those described in Fig 2.

Electron Microscopic Study for Melanosome and Melanin Synthesis

Melanotic Melanoma Cells: SK-MEL-23 melanoma cells possessed a large number of melanosomes in stages III and IV. Most of them were either spherical or ellipsoidal and contained melanized lamellar matrix. After UV-B irradiation there was some increase in the number of melanosomes and, again, most of these melanosomes were in stages III and IV. Basically the same finding was obtained in the case of G361 melanoma cells. However, the number of melanized melanosomes before UV exposure was much less, most of them being in stages I and II. After UV-B irradiation there was a marked increase in melanization of these melanosomes, revealing stage III and IV granules (data not shown).

Amelanotic Melanoma Cells: Amelanotic C32 cells were full of granules with fine structural characteristics similar to those of pre–stage I or stage I melanosomes. On rare occasions there were a number of melanosomes with internal lamellae identical to those seen in stage II melanosomes. None of these melanosomes or granules revealed any melanization. There were also a fairly large number of spherical or ellipsoidal granules with some electron density. There were not the melanosomes, rather the lysosomal granules. After UV-B irradiation none of the melanosomes revealed melanization, but showed identical spherical structures containing amorphous or proteinaceous material. There was no melanin deposition in these pre–stage I or stage I melanosomes. Other amelanotic SK-MEL-24 cells also revealed the same findings, but the number of spherical pre–stage I or stage I granules was much less. Some of these spherical granules revealed the electron-dense myelinoid figures that are often seen in lysosomal granules. There was an in-

Table II. Synthetic Oligonucleotides of 5′ and 3′ RNA-Specific Primers Used for Amplification

mRNA Species	5′ Sense Primer	3′ Anti-Sense Primer
Human TRP-1	5′-ACCGCTGTGGCTCATCATCA-3′	5′-GTCATCCGTGCAGATATCAC-3′
Human tyrosinase	5′-ATGGAGAAGGAATGCTGTCC-3′	5′-GACTGATGGCTGTTGTACTC-3′
Human Lamp-1	5′-GCCTCATGCATTGTCGTCAG-3′	5′-GCTGTGCAGCTCCAGAGTCA-3′
CD63	5′-CGGCAGCCATGGCGGTGGAA-3′	5′-ATGAGGAGGCTGAGGAGACC-3′

creased number of pre–stage I or stage I granules after UV-B exposure but, again, there was no melanin formation. There were no lamellar granules with regular striation that could be typically seen in stage II melanosomes. The number of Golgi complexes appeared to be increased after UV exposure (data not shown).

DISCUSSION

This study has clearly shown that exposure to UV-B can directly activate the cellular metabolism (as shown by MTT-formazan formation) of pigment cells under both amelanotic and melanotic conditions, and that after repeated irradiation these pigment cells can exhibit an increased mRNA and protein expression of tyrosinase, TRP-1, and Lamp-1. This increase is followed by an increased tyrosinase activity and melanin synthesis in the melanotic cells. Our finding supports the previous *in vitro* reports, indicating that UV-B can directly stimulate melanocyte mitosis and pigment production [15, 16]. Our two amelanotic cell lines did not produce any melanin pigmentation. Importantly, one of them, C32, was found to constitutively express tyrosinase mRNA and protein that was upregulated after UV irradiation; however, they showed no active tyrosinase or melanin synthesis after UV irradiation. Our finding contradicts the recent report of Cerdan *et al* [17], who indicated that diacylglycerol analogue, 1-oleoxyl-2-acetylglycerol, can induce melanin synthesis in C32 in a dose-dependent manner. Gordon and Gilchrist [18] indicated that this diacylglycerol-mediated melanogenesis in human pigment cells is regulated by protein kinase C. It appears that UV-B–induced and diacylglycerol-induced melanogenesis is regulated differently in humans inasmuch as repeated exposure of UV-B could not produce melanin pigmentation in C32.

We have recently shown that HMSA-5 possesses homology with the gp75 antigen recognized by MoAb TA99 (Mel 5) [5]. Peptide sequencing of the affinity-purified human TA99 antigen showed 90% identity with regions of the deduced amino acid sequence of one of the murine b (brown) locus gene clones, pMT4 [6, 19, 20]. When oligo-nucleotide probes, based on this gp75 amino acid information, were used to screen the human melanoma cDNA library, a clone that encoded the human counterpart of the murine b-locus gene product was isolated [6]. At the same time the full nucleotide sequence encoding human tyrosinase-related protein (TRP-1) was characterized and found to be identical to the murine b-locus gene, coordinating the function of the tyrosinase gene [21]. The mutation of this gene may cause the premature death of melanocytes in mice [22]. The TRP-1 (gp75, HMSA-5) is an N-linked glycoprotein (69–73 kDa) and glycosylated at the site of the ER-Golgi complexes, from which TRP-1 appears to be transferred to the melanosomes at an early stage of maturation by vesiculoglobular bodies [23]. The biologic role of TRP-1 in the human pigment cell system has been unknown, although at one time it was suggested to have a catalase activity [24]. Our present study in C32 amelanotic cells has, however, provided indirect evidence that the TRP-1 gene and protein expression is required in tyrosinase activation and melanin synthesis, probably by post-translational modification of tyrosinase. This assumption is further supported by our finding that G361 melanotic cells revealed coordinated upregulation of tyrosinase mRNA and protein expression as well as its enzymic activity with the upregulated TRP-1 gene and protein expression.

Another important finding from this study relates to Lamp 1. Lamp-1, a lysosome-associated membrane protein, is localized in the lysosome, a small portion of Golgi complex, and melanosomes in stages I and II [25, 26]. It is also expressed, however, by many cells other than melanocytic cells. Our study has clearly shown that the gene expression and protein synthesis of Lamp-1 in both melanotic and amelanotic cells is upregulated by exposure to UV-B irradiation. Yet there is no melanin synthesis in amelanotic cells even though the tyrosinase gene and protein is expressed (e.g., as is seen in C32). The upregulation of the Lamp-1 gene and protein expression is therefore not essential to promote tyrosinase activity and melanin synthesis (e.g., as is seen in C32 and SK-MEL-24), but likely coordinates the tyrosinase function for the melanin formation (e.g., as is seen in G361).

Lastly, our study showed that CD63 is present in all of the melanoma cell lines tested, and that there is no alteration of CD63 expression in either mRNA or protein level after UV irradiation. CD63 antigen is associated with the membrane structure and co-localized with the resident lysosomal membrane proteins Lamp-1

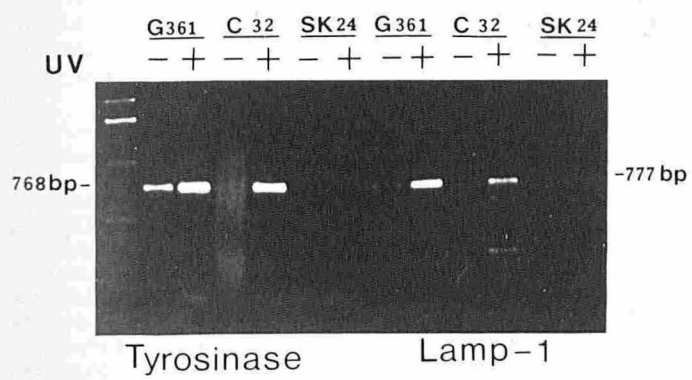

Figure 5. Semiquantitative RT-PCR analyses of tyrosinase and Lamp-1 mRNA expression in melanotic (G361) and amelanotic (C32 and SK-MEL-24) melanoma cells induced by UV-B exposure, 5.0 mJ/cm² for seven consecutive days at room temperature. Total RNAs were isolated and reverse transcribed. The resulting mRNA-cDNAs were amplified with specific tyrosinase or Lamp-1 primer set and submitted to 35 cycles. The sizes of RT-PCR product amplified from tyrosinase and Lamp-1 cDNAs are 768 bp and 777 bp, respectively.

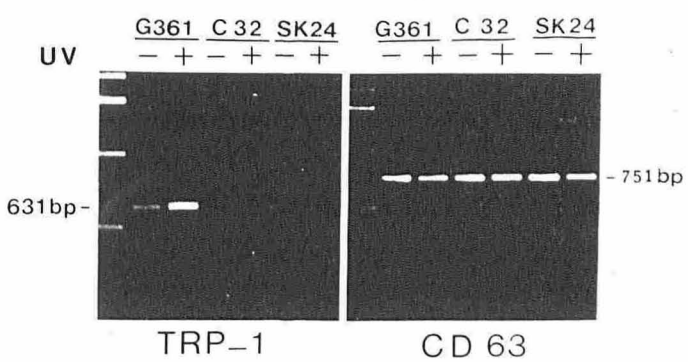

Figure 6. Effect of UV-B–induced specific mRNA expression of TRP-1 and CD63 by RT-PCR analysis. Extracts of total RNA were converted into cDNA by reverse transcriptase, and amplified with specific TRP-1 and CD63 primer set by PCR. The sources of cultured cells and methods of UV-B exposure are identical to those described in Fig 5. The sizes of RT-PCR product amplified from TRP-1 and CD63 cDNAs are 631 bp and 751 bp, respectively.

and -2 [27,28]. A recent immunochemical and molecular genetic investigation has clarified that epitopes of previously known melanoma-associated antigens, ME491, NKI/C-3, and NGA, are all identical to CD63 [8]. Our present study indicates that CD63 is a structural, but not a functional, protein of the lysosomes and is not directly involved in melanogenesis. In contrast, another lysosomal membrane protein, Lamp-1, acts together with TRP-1 to produce melanin pigments after UV exposure. This co-ordinated interaction of the TRP-1 and Lamp-1 genes is critical in UV-induced melanogenesis and the upregulation of tyrosinase mRNA expression alone cannot produce melanin pigmentation.

This study was supported by grants from the Medical Research Council of Canada, National Cancer Institute of Canada, and Steifel International Inc. HH and MHL are visiting research fellows from the Departments of Dermatology, Nihon University School of Medicine, Tokyo, Japan and the College of Medicine, Kung Hee University, Seoul, Korea, respectively. KJ is an AHFMR scientist.

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