Role of Ultraviolet Radiation in the Induction of Melanocytic Tumors in Hairless Mice following 7,12-Dimethylbenz(a)anthracene Application and Ultraviolet Irradiation

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ABSTRACT

We examined the role of UVR (UV radiation) (UVA, 320-400 nm; UVB, 290-320 nm; and the combination of UVA and UVB) as a promoter in the induction of cutaneous melanoma. One hundred and seventy hairless mice (Skm-hr2), 6-8 weeks old, were treated in 8 groups: group I, DMBA [7,12-dimethylbenz(a)anthracene] plus UVA; group II, DMBA plus UVA plus UVB; group III, DMBA plus UVB; group IV, DMBA; group V, UVA; group VI, UVA plus UVB; group VII, UVB; group VIII, control. DMBA (0.5% solution) was applied once to promote the formation of dermal melanocytic nevus-like lesions while UVR treatments were conducted 3 times/week for 30 weeks. The mice were examined periodically for the development of multiple pigmented lesions, papillomas, squamous cell carcinomas, melanomas, and lymphomas. Treatment with DMBA plus UVA, DMBA plus UVB, and DMBA plus UVA plus UVB stimulated the development of multiple pigmented nevus-like lesions (85-100%) in mice of groups I, II, III, and IV. Upon necropsy, 27-33% of animals in groups I, II, and III, and receiving UVR treatments developed clinically and histologically characterized melanomas. Treatment with DMBA alone did not produce melanomas. DMBA-treated animals in groups I, II, and III which received UVR treatments also developed lymphomas (21-50%). Animals treated with DMBA alone or those that received UVB or the combination of UVA plus UVB (without DMBA) developed only papillomas and squamous cell carcinomas (25-47%). Skin tumors were analyzed for the presence of point mutations in the ras gene. Polymerase chain reaction amplification of DNA and selective oligonucleotide hybridization revealed mutations in the 61st codon of the N-ras gene in the precursor nevus-like lesions and melanoma samples studied. This study suggests that UVR (both UVA and UVB) plays a role as a promoter in the stimulation of melanoma and lymphoma development in hairless mice.

INTRODUCTION

In the United States the incidence of cutaneous melanoma has increased by nearly 100% in the past decade, which is a faster rate of increase than that for any other cancer except lung cancer in women (1, 2). The increased incidence appears to be significant and unrelated to better registration of patients and changing trends in histopathological diagnosis. In an attempt to understand the basis of this increase, a number of factors have been implicated in the etiology of cutaneous melanoma including genetic factors related to skin color, exposure to oncogenic viruses, solar radiation, trauma, chemical carcinogens, and hormones (3-7).

There is considerable circumstantial evidence that suggests exposure to sunlight, particularly UVR, contributes to the induction of cutaneous melanomas in humans, although the relative importance of UVR in the induction of melanoma remains controversial (4, 5).

There is a consensus on the role of solar UVR in the etiology of nonmelanoma skin cancer (basal and squamous cell carcinomas), and the principal action spectrum for the pathogenesis of nonmelanoma skin cancer has been shown to be UVB (290-320 nm). In nonmelanoma skin cancers, cumulative lifetime exposure to the sun correlates well with the development of basal and squamous cell carcinomas (8, 9). Furthermore, the action spectrum studies in relation to the UVR dose-response data in experimental animal model carcinogenesis are convincing and confirm UVB radiation as the major carcinogenic factor.

The relationship between solar radiation exposure and causation of melanoma is not as well understood. Until recently cutaneous melanoma had not been experimentally induced in mammalian species in a predictable manner by exposure to UVR alone or in combination with an initiator or photosensitizer. Therefore, it has not been possible to demonstrate experimentally whether UVR is a promoter or inducer of malignant melanoma and what spectrum of UVR could plausibly be related to the induction of melanoma.

Studies by Epstein et al. (10) suggest that small benign pigmented growths in the skin of hairless mice could be stimulated to form melanocytic tumors by DMBA application and UVR exposure. Five of 32 treated mice exhibited melanomas with no distant metastasis. These studies demonstrating the role of UVR were not reproduced by other investigators. Kripke (11) exposed mice to UVB radiation followed by croton oil application. After 92 weeks, one mouse developed melanoma. Using the concept developed by Berkelhammer et al. (12), Romerdahl et al. (13) investigated whether exposure to UVR at a site treated with DMBA and croton oil or at a distant site influenced or modified the induction of melanocytic tumors. Chronic doses of UVR to the croton oil-treated site modified the course of development of melanomas. The investigators concluded that UVR accelerated the development of melanocytic tumors by serving as a weak initiator or promoter. Ley et al. (14) recently reported the induction of melanoma in a marsupial, the South American opossum (Monodelphis domestica). They observed the development of melanotic tumors in 10 of 46 surviving animals, 100 weeks after the first UVR exposure.

Additional reports of the production of melanotic tumors in several rodent species after topical application of carcinogens, but without exposure to UVR, have also appeared (12, 15-19).

There appears to be a significant epidemiological relationship between solar radiation and the development of malignant melanoma in humans (5, 20). Within fair-skinned populations, several factors have been identified that relate UVR to increased risk of developing melanoma (e.g., the prevalences of nonmelanoma skin cancer and malignant melanoma are similar in...
latitude dependence and in their relatively higher occurrence in light-skinned individuals and those who have experienced intermittent sun exposure or acute blistering reaction. A strong association with melanoma risk is related to the presence of a certain type of pigmented nevi (dysplastic) in patients with a family history of melanoma (21-23).

The identification of the role of oncogenes has provided a way of understanding the molecular basis for tumor development. A family of genes frequently found to harbor mutations in human tumors is the ras gene family (24). Mutations of the 61st codon of the H-ras gene have been reported in mouse and human skin tumors, and ras mutations have been reported in about 20% of human melanomas (25, 26). While the role of UV light in inducing ras mutations has been suggested, ras gene mutations in melanomas induced in an in vivo model system have not yet been reported.

The aim of the present investigation was to obtain experimental evidence determining the role of UVR in the induction of melanoma by promoting the formation of nevus-like lesions in the skin of hairless mice by a single topical treatment with a chemical agent and repeated UVR exposures as reported in earlier studies by Epstein et al. (10) and Clark et al. (16). We have investigated whether UVA or UVB, alone or in combination, promoted melanoma induction. We also wanted to determine whether ras gene mutations could be induced by two different carcinogens (i.e., UVR and DMBA) and to evaluate their type of mutations and possible role in cutaneous melanomas in mice.

Using topical DMBA as an initiator and repeated exposures to UVA, UVB, or the combination of UVA plus UVB as promoters, we have generated evidence to indicate that both UVA and UVB can stimulate the induction of melanomas and lymphomas in mice pretreated with DMBA.

### MATERIALS AND METHODS

Mice. Six- to 8-week-old, female hairless mice (Skh-hr2) capable of exhibiting UVR-induced epidermal skin pigmentation response (as determined by increased proliferation of melanocytes accompanied by increased formation of new melanosomes, increased melanization of melanosomes, and increased transfer of melanosomes to the keratinocytes) were obtained from Temple University Skin and Cancer Institute (Philadelphia, PA). One hundred and seventy mice were placed in eight experimental groups of 20–24 animals (Table 1).

The animals were housed in a pathogen-free barrier facility in a temperature- and humidity-controlled room. The ambient light was controlled to produce 12-h cycles of light and dark.

UV Sources. Groups of mice were exposed to different spectral bands of UVR. Two types of UV-emitting fluorescent lamps were used: (a) lamps that emitted primarily UVB (290–320 nm); and (b) lamps that emitted primarily UVA (320–400 nm).

UVR radiation was obtained from a bank of four Westinghouse sunlamps (FS40-T12) with relative emissions of 0.27, 0.69, 1.0, and 0.09 at 290, 300, 313, and 360 nm, respectively. Animals were irradiated 3 times/week for 30 weeks and received initially 30 mJ/cm² of UVB (~1 MED). Halfway through the experiment, the UVB dose was increased to 50 mJ/cm² (~2 MED). The proportion of UVA was only 5% or less of the total irradiance. The major fraction of the UVB source was 290–320 nm. The contribution of UVA to the effects of UVB were negligible since the exposure times to UVB were in the range of 10–15 s.

UVA radiation (320–400 nm) was obtained from a bank of four GTE-Sylvania high-intensity fluorescent black light tubes (FS40-T12 BL), which are similar to those used clinically. The UVA source emitted primarily in the 320–400 nm range (≥80%), with about 15–18% in the visible range (400–440 nm). The outer envelope used in these low-pressure mercury arc lamps is glass, which does not transmit any UVB radiation that might be produced. The lamps were also solarized to eliminate any possible UVB radiation. Exposure doses of UVA irradiation, 3 times/week for 30 weeks, ranged from 2 to 4 J/cm².

The UVB and UVA output of the lamps was compared with the aid of precalibrated radiometers (IL-700; International Light Company, Newburyport, MA) equipped with UBV- and UVA-detecting probes and cosine-corrected quartz windows. The total dose of UVB or UVA and the combination of UVB plus UVA received by each animal are shown in Table 1.

### Table 1 Experimental groups for the induction of skin cancers by UVR

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Exposed dose</th>
<th>Total dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>DMBA + UVA</td>
<td>22 2–4 0</td>
<td>264 0</td>
</tr>
<tr>
<td>II</td>
<td>DMBA + UVB</td>
<td>22 2–4 30–50 264 3.5</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>DMBA + UVB only</td>
<td>24 0 30–50 0 3.5</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>DMBA only</td>
<td>22 0 0 0 0</td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>UVB only</td>
<td>20 2–4 0</td>
<td>264–300 0</td>
</tr>
<tr>
<td>VI</td>
<td>UVA + UVB</td>
<td>20 2–4 30–50 264 3.5</td>
<td></td>
</tr>
<tr>
<td>VII</td>
<td>UVB only</td>
<td>20 0 30–50 0 3.5</td>
<td></td>
</tr>
<tr>
<td>VIII</td>
<td>Control</td>
<td>20 0 0 0 0</td>
<td></td>
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</tbody>
</table>

* Animals were exposed 3 times/week for 30 weeks.
* One application of 0.5% DMBA solution on day 1, applied to two-thirds of the back (below frontal scapular region).
MELANOCYTIC TUMORS IN HAIRLESS MICE

Fig. 1. A, two mice treated with DMBA and UVR showing multiple blue nevus-like lesions 1–3 mm in size. The mouse on the left was exposed to UVB, and the mouse on the right was exposed to UVA. B, mouse treated with DMBA and UVB, with an enlarged view of a few papillomas and multiple pigmented nevi. The nevus at the bottom was a 4-mm-wide, raised lesion. C, mouse upon necropsy with a melanoma growth protruding down from epidermis to dermis, indicating an intralesional transformation of blue nevus-like lesion.

Table 2 Development of multiple blue nevus-like lesions

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>20</th>
<th>25</th>
<th>30</th>
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<tr>
<td>I</td>
<td>DMBA + UVA</td>
<td>52.0</td>
<td>65.2</td>
<td>86.4</td>
<td>94.0</td>
<td>100.0</td>
<td>100.0</td>
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<tr>
<td>II</td>
<td>DMBA + UVA + UVB</td>
<td>54.5</td>
<td>82.0</td>
<td>86.0</td>
<td>90.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>III</td>
<td>DMBA + UVB</td>
<td>54.2</td>
<td>91.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>IV</td>
<td>DMBA</td>
<td>82.0</td>
<td>90.0</td>
<td>90.0</td>
<td>90.0</td>
<td>100.0</td>
<td>100.0</td>
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<tr>
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<td>UVA + UVB</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>VII</td>
<td>UVB</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
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<td>Control</td>
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<td>0</td>
<td>0</td>
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</table>

mM deoxynucleotide triphosphate, 1.5 μl of 20 mM solution of primers, and 2.5 units Taq polymerase and made up to volume with distilled water. The reaction mixture was overlaid with mineral oil. Amplification was performed with DNA Thermal Cycler (Perkin-Elmer Cetus) for 30 cycles with the following parameters: 2 min at 95°C for denaturation; 2 min at 50°C for annealing; and 2 min at 60°C for primer extension. Primers used included (a) H-ras primers for codon 12/13, GGCAGAGACCTGTAGGAG and GTATTGCTCACAAGATGGTTCT; (b) H-ras primers for codon 61, CTACCAGAAGCGGGTGGTC and TTCTACAGGAAAGGAGTAG; (c) N-ros primers for codon 12/13, GACTGAGTCAAACCGGTGG and CTCTATGGTGAGGCTTATT; and (d) N-ras primers for codon 61, TCTTACAGAAACAGTG and ATACACAGGAAAGCCTTCG (5' to 3' orientation for each primer).

PCR-amplified DNA was applied as dots on Nytran membrane, and the membranes were baked, prehybridized, and hybridized with 32P-labeled wild-type and mutated oligomer probes as described (29).

RESULTS

Induction of Nevi with DMBA and UVR

These experiments were initiated to determine whether melanocytic tumors could be induced in the skin of hairless mice in a two-step process of initiation and promotion. Scattered, small, pigmented blue-black spots less than 1 mm in diameter were observed within 7–10 days in animals of groups I, II, III, and IV receiving a single application of DMBA, and their appearance was not dependent on UVR irradiation. Prior to receiving any UVR treatments mice from groups V, VI, VII, and VIII showed no pigmentary changes. The first macroscopically recognizable change in the DMBA-treated and UVR-irradiated mice was the distinct appearance of several small (1–2 mm) bluish-black hyperpigmented lesions (Fig. 1A). Within 8–10 weeks these lesions enlarged in size and number (15–30 nevi/animal), covering an area roughly 4 cm x 3 cm (Fig. 1A). The development of these lesions in different groups is shown in Table 2. The number of multiple pigmented lesions generated was greater and their size was larger in the mice from groups I, II, and III (receiving DMBA plus UVA, DMBA plus UVB, and DMBA plus UVA plus UVB treatments, respectively) than in
the mice from group IV treated with DMBA alone. These pigmented lesions did not appear spontaneously in animals without prior treatment with DMBA or UVR. The pigmented papules gradually increased in size so that each animal on average exhibited 20–25 raised pigmented lesions (Fig. 1, A and B). The number of pigmented nevus-like lesions was greater and their size was larger in the mice from groups I, II, and III (treated with DMBA plus UVA, DMBA plus UVB, or DMBA plus UVA plus UVB, respectively). The mice from groups V, VI, and VII receiving UVR treatments only and the nonirradiated control mice kept in the ambient light/dark cycle showed no formation of similar lesions.

Continued exposures of mice three times weekly to UVA, UVB, or UVA plus UVB stimulated the eventual development of papillomas (68–100%), SCCs (32–80%), melanomas (25–33%), and lymphomas (21–50%) (Figs. 1C and 2). The distribution of tumors in each experimental group is shown in Table 3. The non-DMBA-treated mice of groups V, VI, and VII, which were also exposed repeatedly to UVB, UVA, or the combination of UVA plus UVB, developed only papillomas, SCCs, or cutaneous photoaging changes indicative of chronic UV damage. Hyperpigmentation of skin of the back, ears, and tail could be seen, but no melanomas or lymphomas could be induced in any of these groups. The prevalence of papillomas and SCCs in the animals of groups V, VI, and VII was less than that of DMBA-treated animals receiving similar UVR treatments (Table 3). One of the unexpected observations in DMBA-treated mice exposed to either UVA, UVB, or UVA plus UVB (groups I, II, and III) was the development of lymphomas (21–50%) (Table 3). Necroscopy revealed highly enlarged lymph nodes with effacement of lymph node architecture (Fig. 2 and Fig. 3, A and B).

**Histology**

Fig. 3, A–F, illustrates representative histological findings concerning the development of nevi, melanomas, lymphomas, papillomas, and SCCs.

Squamous Papillomas. These lesions were characterized by benign papillary epidermal hyperplasia, frequently with marked hyperkeratosis. The margins were rounded and well circumscribed, and there was an increased thickness of the epidermis. The keratinocytes showed normal maturation to cornified cells. The lesions did not extend past the papillary dermis.

Squamous Cell Carcinomas. These lesions (Fig. 3E) were characterized by invasive papillary keratinizing tumors. The lesions showed irregular margins with small cords and clusters of tumor cells extending past the limits of most of the tumor. There was disorderly keratinization with whorls of keratinized keratinocytes.

**Table 3 Effects of DMBA and UV treatments on the prevalence of tumors and other manifestations in mice**

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatments</th>
<th>No. of weeks</th>
<th>Blue nevi</th>
<th>Papillomas</th>
<th>SCCs</th>
<th>Melanomas</th>
<th>Lymphomas</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>DMBA + UVA</td>
<td>20</td>
<td>94.0</td>
<td>68.5</td>
<td>32.0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>II</td>
<td>DMBA + UVA + UVB</td>
<td>30</td>
<td>100.0</td>
<td>100.0</td>
<td>33.0</td>
<td>27.0</td>
<td>21.0</td>
</tr>
<tr>
<td>III</td>
<td>DMBA + UVB</td>
<td>20</td>
<td>90.0</td>
<td>85.0</td>
<td>63.0</td>
<td>0</td>
<td>5.0</td>
</tr>
<tr>
<td>IV</td>
<td>DMBA</td>
<td>30</td>
<td>100.0</td>
<td>100.0</td>
<td>80.0</td>
<td>25.0</td>
<td>32.0</td>
</tr>
<tr>
<td>V</td>
<td>UVA</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
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<tr>
<td>VI</td>
<td>UVA + UVB</td>
<td>30</td>
<td>0</td>
<td>0</td>
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<tr>
<td>VII</td>
<td>UVB</td>
<td>20</td>
<td>0</td>
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<tr>
<td>VIII</td>
<td>Control</td>
<td>30</td>
<td>0</td>
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Fig. 2. A, two mice from group II treated with DMBA, UVA, and UVB, with multiple nevus-like pigmented lesions, papillomas, and squamous cell carcinomas. Upon necroscopy one mouse also showed enlarged lymph nodes. B, mouse from group III treated with DMBA and UVB, with the development of multiple pigmented nevus-like lesions, papillomas, and a lymphoma protruding from the left side of the chest.
Fig. 3. Low-power (A) and high-power (B) views of a section of a biopsy from a mouse exhibiting UVR-induced lymphoma. This mouse received DMBA, UVA, and UVB combination treatments. C, low-power and D, high-power light microscopic views of a section of a pigmented nevus (precursor lesion). E, biopsy section of a squamous cell carcinoma from a mouse treated with DMBA and UVB. Similar types of tumors were observed in mice treated with DMBA plus UVA or UVB alone. F, low-power and G, high-power views of a section of a biopsy from a mouse exhibiting melanoma after treatment with DMBA, UVA, and UVB.
cells present at various depths of the tumors. Some cells were multinucleated with nuclei which were pleomorphic and hyperchromatic. An inflammatory cell infiltrate composed predominantly of lymphocytes was generally present. The tumors extended into the s.c. tissue.

Lymphoma. The lymph nodes were enlarged, and the lymph node architecture was effaced (Fig. 3, A and B). The lymph nodes were replaced with small to medium-sized lymphocytes. The nuclei exhibited irregular contours, and the larger nucleoli had a prominent nucleolus. The cells had a small quantity of eosinophilic cytoplasm.

Pigmented Precursor Lesions. These lesions (Fig. 3, C and D) were characterized as dermal tumors of spindle pigmented cells. The tumors were present in the dermis without an associated overlying nevus structure. The widest dimensions exceeded the thickness of the lesions, and the cells extended through the entire thickness of the dermis. High-power examination of the tumors revealed a predominance of spindle cells which were highly pigmented. There was an associated accumulation of melanophages which were either spindle or epithelioid. The nuclei of the spindle cells were not hyperchromatic or enlarged. An inflammatory cell infiltrate was generally not present.

Melanoma. The lesions were vertically oriented, and the thickness exceeded the widest dimensions (Fig. 3, F and G). The lesions formed expansile masses filling the dermis and extending into the s.c. tissue. The cells were spindle shaped, and the nuclei were generally small and hyperchromatic. Melanin was absent or minimally present in the lesions. An inflammatory cell infiltrate composed mostly of lymphocytes was present.

Sinus Histiocytosis. The lymph nodes were enlarged, but their architecture was intact. The subcapsular and medullary sinuses contained large numbers of macrophages which contained abundant melanin in their phagolysosomes (melanophages). The germinal centers were generally prominent in these nodes.

Molecular Analysis Detection of ras Gene Mutations in Blue Nevi and Melanomas

DNA isolated from dermal nevi and melanomas were used to enzymatically amplify regions around the 12th, 13th, and 61st codons of the H-ras and N-ras genes by the polymerase chain reaction technique. Three of the eight precursor lesions and one of the three melanomas showed mutations in the 61st codon of the N-ras gene only (Fig. 4). An A to T transversion in the third base of codon 61 was observed to be present in the DNA of one precursor lesion and one melanoma. The other mutations observed were for an A to T transversion in the second base and a C to A substitution in the first base of codon 61 in two separate dermal nevi analyzed during the present investigation. No mutations in the H-ras gene have been observed thus far.

DISCUSSION

Although we have not used monochromatic radiation to determine the primary wavelengths for promoting melanoma induction, the current study suggests that the melanoma-promoting UV action spectrum in DMBA-treated experimental animals not only extends from 290 to 320 nm but also includes the UVA spectrum (320–400 nm). Repeated exposures to UVR of the DMBA-treated site accelerated the development of melanoma (Table 3), suggesting that UVB radiation participated in the induction of the tumors by serving as a promoter in a two-stage process of melanomagenesis. The substitution of UVA also resulted in the development of a significant number of melanocytic tumors. The treatment with DMBA alone had no effect on the development of melanomas. These experiments demonstrated that the combined effect of UVB and UVA promoted the induction and development of cutaneous melanocytic tumors induced by treatment with the chemical carcinogen DMBA. Similarly, the data presented in Table 3 show that UVB, the combination treatment of UVA plus UVB, and UVA alone to a limited extent participated in the induction of lymphomas by serving as a promoter in this experimental model of UVR carcinogenesis. These data underscore the hitherto unproved importance of UVA as a promoter in DMBA-induced carcinogenesis.

Our observations indicate the possibility of a mechanism for UVR-induced melanoma in which UVR is able to act as a promoter for the development of melanomas following the initiation stage induced by DMBA application. This is supported by three observations: (a) melanotic tumors were induced only in mice that received DMBA treatment; (b) UVR alone (both UVA and UVB) was not effective in initiating and promoting the development of melanomas; and (c) repeated exposures to measured doses of UVB and UVA to the carcinogen (DMBA)-initiated site dramatically promoted the development of melanomas and lymphomas, as well as squamous cell carcinomas. A promoter action of UVR seems likely on the basis of increased melanoma incidence in the mice of groups I, II, and III. Cutaneous melanomas have been induced by DMBA in guinea pigs as well as mice. Some of them developed from carcinogen-induced blue nevi (10); others were developed from intraepidermal melanocytic hyperplasias (16, 17). However, intrallesional transformation to melanoma in the these animals...
was quite low (<5%), but the clinical and histological events leading to the development of melanomas were found to be similar to human melanomas in a number of aspects. The early morphological and numerical changes in the epidermal melanocyte system of mice were examined by Blog and Szabo (30) after 10 weeks of DMBA treatment. Damage to melanocytes exhibited by irregular shape, atypia, and clumped and shortened dendrites was found, but no tumor formation was observed.

It appears that initiation of tumors by a single application of DMBA requires additional promotion before manifestation or expression of tumor is achieved. The observations of Romerdahl et al. (13) in this regard are of interest.

DNA-mediated gene transfer studies have shown the presence of transforming genes in malignant melanomas (31). There have been several reports of observations of ras gene mutations in malignant melanoma (25, 26). Reports on the frequency of ras gene mutations in melanomas have also varied. Van’t Veer et al. (26) reported N-ras mutations to be present in human biopsies in areas exposed to sunlight, suggesting a role for solar radiation in promoting ras mutations. A recent report (25), however, while analyzing the role of ras mutations in human melanoma, suggests that ras mutations may be “limited to indirect involvement in the transformation of a subset of melanomas.”

Furthermore, due to the unavailability of adequate tissue samples for DNA/RNA analysis, most research has been limited to the analysis of cell cultures or NIH 3T3 transformants. While some of the recent approaches have utilized techniques involving specific oligonucleotide hybridization of DNA amplified by PCR, there has been no attempt, at the molecular genetic level, to use an in vivo model system in which induction, initial stages of transformation, malignancy, and metastasis can be studied to identify the possible role of specific cellular protooncogenes in melanomas. Our induced murine melanomas allowed us to examine tumor samples for the presence of ras mutations and to determine whether the experimental model reflected the human situation in this respect. While the stage of tumor development at which ras mutations have been observed in humans has varied from dysplastic nevi to primary and metastatic melanomas, it has generally been suggested that such mutations are associated with the early stages of pathogenesis of melanoma (31). Moreover, some evidence implicated the presence of activating ras mutations in tumors localized in sun-exposed sites (26). Our results corroborate both these observations by indicating that exposure of mouse skin to a chemical carcinogen (DMBA) followed by UVR treatments causes activating mutations in the N-ras gene and that these mutations are present in early pigmented lesions as well as malignant melanomas. To our knowledge, these results constitute the first report of the presence of ras mutations in melanomas and pigmented nevi induced in an in vivo model. They suggest that ras mutations may have some role in the induction of melanomas and demonstrate the potential utility and relevance of this murine model to the study of the development and biology of human melanoma.

REFERENCES


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