Accelerated Ultraviolet Radiation-induced Carcinogenesis in Hepatocyte Growth Factor/Scatter Factor Transgenic Mice

Frances P. Noonan, Toshiyuki Otsuka, Stacey Bang, Miriam R. Anver, and Glenn Merlino

ABSTRACT

The dramatic rise in incidence of malignant melanoma experienced by populations both within the United States and throughout the world over the last several decades has been attributed to enhanced exposure to the UV spectrum of sunlight radiation. This hypothesis can now be tested using genetically engineered mouse models predisposed to malignant melanoma. Here we use melanoma-prone transgenic mice inappropriately expressing hepatocyte growth factor/scatter factor (HGF/SF) in the skin as an experimental model system to ascertain the consequences of a chronic regimen of suberythemal UV radiation on melanoma genesis. HGF/SF is a multifunctional regulator capable of stimulating growth, motility, invasiveness, and morphogenetic transformation in cells, including melanocytes, expressing its receptor tyrosine kinase Met. HGF/SF transgenic mice demonstrate ectopic interfollicular localization and accumulation of melanocytes within the truncal dermis, epidermis, and junction and if untreated develop primary cutaneous melanomas with a mean onset age of ~21 months. Transgenic mice and their wild-type littermates subjected to UV radiation three times weekly using FS40 sunlamps (60% UVB and 40% UVA), with daily UV doses graded from 2.25 to 6.0 kJ/m², developed skin tumors with a mean onset age of 26 and 37 weeks, respectively (P < 0.001, Kaplan-Meier log rank test). However, the repeated doses of suberythemal UV radiation used in this study failed to accelerate melanoma genesis, instead inducing the development of nonmelanoma tumors that included squamous cell carcinomas, squamous papillomas, and sarcomas. The conspicuous absence of melanocytic tumors occurred despite the immunohistochemical detection of a significant stimulation (P < 0.001) in melanocyte-specific bromodeoxyuridine incorporation in response to only 2 weeks of UV irradiation (total UV dose of 13.5 kJ/m²), resulting in 2.6- and 4.6-fold increases in the number of melanocytes in the dermis and epidermis, respectively (< 0.001, Kaplan-Meier log rank test). However, the repeated doses of suberythemal UV radiation used in this study failed to accelerate melanoma genesis, instead inducing the development of nonmelanoma tumors that included squamous cell carcinomas, squamous papillomas, and sarcomas. The conspicuous absence of melanocytic tumors occurred despite the immunohistochemical detection of a significant stimulation (P < 0.001) in melanocyte-specific bromodeoxyuridine incorporation in response to only 2 weeks of UV irradiation (total UV dose of 13.5 kJ/m²), resulting in 2.6- and 4.6-fold increases in the number of melanocytes in the dermis and epidermis, respectively. These data indicate that chronic suberythemal UV radiation preferentially favors the development of nonmelanocytic over melanocytic neoplasms in this transgenic animal, consistent with the pathogenesis proposed for sun exposure-associated skin cancer based on retrospective studies in the human population. Our findings suggest that the HGF/SF transgenic mouse will be useful as an experimental model for determining the consequences of exposure to various regimens of UV radiation and for elucidating the mechanisms by which such consequences are realized.

INTRODUCTION

The incidence of all types of skin cancer is rising worldwide, and malignant melanoma, notorious for its aggressive nature and poor response to currently available therapeutics, is one of the fastest increasing cancers in the United States (1–4). These trends have made the identification of risk factors for, and the elucidation of mechanisms underlying, melanoma a high priority (reviewed in Ref. 5). A relationship between sunlight exposure and human skin cancer is now well established, although the quantitative parameters that govern this relationship are not well understood (3, 6, 7). UVB radiation (290–320 nm) exposure plays a major role in the induction of nonmelanoma skin cancer, and the preponderance of evidence indicates that UV radiation also plays a major role in the etiology of melanoma (6). However, in contrast to most types of skin cancer, which appear to occur in relation to chronic or elevated total sun exposure, melanoma is thought to arise as a consequence of an altered or intermittent pattern of more intense exposures (8, 9). Sun exposure during childhood has been suggested to be a highly significant risk factor (see Ref. 10 and references therein). These conclusions are based almost entirely on retrospective, case-control studies among the human population (reviewed in Ref. 11). There has been little support from suitable experimental animal models, which are rare (reviewed in Ref. 12). Availability of such models would greatly facilitate investigations of outstanding questions on the biology and photobiology of malignant melanoma.

Melanoma can be initiated in platyfish-swordtail hybrid fish (13) and in adult opossums (14) with UV radiation alone. However, inbred mice exposed only to UVB radiation develop SCCs and some sarcomas; to induce melanoma, inbred mice must be subjected to UV radiation in combination with chemical carcinogens and/or croton oil (15–17). A human melanoma was reported to arise from human skin xenografts in mice subjected to both 7,12-dimethylbenz(a)anthracene and chronic UVB irradiation (18). Bradl et al. (19) have described a melanoma-susceptible line of C57BL/6 mice harboring a transgene in which the tyrosinase promoter was used to target expression of SV-40 viral oncogenic sequences to melanocytes. Mice from this transgenic line successfully demonstrated enhanced melanoma genesis in response to protocols in which 2- to 3-day-old neonates were exposed to multiple rounds of UV radiation, totaling up to 3.7 J/cm² (20, 21). Powell et al. (22) reported that a small number of transgenic mice expressing a mutationally activated c-Ha-ras gene developed nevi and melanoma when chronically exposed to UV alone, but that treatment with 7,12-dimethylbenz(a)anthracene resulted in a much more robust response.

We have developed transgenic mice with a cancer susceptibility syndrome in which mouse HGF/SF cDNA sequences were overexpressed by virtue of a mouse MT gene promoter and associated locus control regions (23, 24). This model has several advantages; the Met signaling pathway chronically activated in the HGF/SF mice has been implicated in human melanoma (25, 26). Moreover, the truncal skin in these transgenic mice is “humanized,” in that melanocytes arise within the murine epidermis, dermis, and junction and outside their normal confinement within the hair follicles (27). We have reported that ~20% of 15-month-old HGF/SF transgenic animals develop primary cutaneous melanoma, ~20% of which acquire a metastatic phenotype (27). Here we treat adult HGF/SF transgenic mice with a carcinogenic
regimen of UV radiation consisting of chronic suberythemal doses to determine whether cumulative UV exposure can accelerate melanoma genesis in this experimental model system.

MATERIALS AND METHODS

Mice. MT-HGF/SF line MH19 transgenic mice were created on the FVB/N inbred background and are therefore albino (23). Transgenic and control mice used in this study were typically produced from the mating of HGF/SF heterozygote transgenic males with FVB/N females. In one experiment, MH19 males were mated to C57BL/6 females to place the MT-HGF/SF transgene on an FVB-BL/6 F1 background. Genotyping was carried out by PCR, as described elsewhere (27). Littermates of the same sex were housed together and UV irradiated, or shaved only, starting at 4–6 weeks of age. Animals were maintained on mouse chow (Laboratory Rodent Diet 5001; Purina Mills Inc., Richmond, IN) and were kept under a strict 12-h light/dark cycle. All animal studies were carried out in accordance with guidelines established by the NIH.

UV Radiation-induced Carcinogenesis. Animals had their backs shaved with electric clippers and were UV irradiated in separate compartments of a modified mouse cage. The UV source was a bank of six FS40 sunlamps (60% UVB, 290–320 nm; 40% UVA, 320–400 nm; and <1% UVC, 250–290 nm). UV doses were determined as described previously (28). An incrementally graded UV protocol was used: three times weekly a UV dose was delivered of 2.25 kJ/m² (7.5 min) for a total UV dose of 13.5 kJ/m².

Skin Tumors. Mice were monitored for tumor formation; detailed notes on skin and eyes and general condition were taken each week. Time to tumor development was taken as time to appearance of a palpable swelling >1 mm subsequently diagnosed as a tumor on histopathological examination. Tumors were excised before reaching 1 cm in any dimension, and portions were preserved in 10% formalin, sectioned, stained with H&E, and examined microscopically. As described previously (24, 27), melanomas were immunohistochemically identified with the immunoperoxidase technique using antibodies produced in rabbits specific for tyrosinase and TRP1 (antibodies PEP7 and PEP1, respectively), generously provided by Dr. Vince Hearing (National Cancer Institute). In some instances, a portion of tumors and adjacent skin was frozen in dry ice and used to make total RNA for Northern blot analysis (see below).

RNA Analysis. Total RNA was prepared using guanidine thiocyanate as described previously (29). For Northern blot analysis 15 μg of total RNA were electrophoretically resolved on a denaturing 1% agarose-formaldehyde gel and then transferred to nitrocellulose membrane (Schleicher & Schuell, Keene, NH). Hybridization and washing conditions are detailed elsewhere (27, 30). Both a 2.2-kbp mouse HGF/SF cDNA probe and a 1.5-kbp mouse c-met cDNA probe were synthesized by PCR as described (24, 27). To control for variation in RNA loading and transfer, filters were routinely rehybridized with a glyceraldehyde-3-phosphate dehydrogenase cDNA probe.

Melanocyte Proliferation Analysis. Mice were subjected to the short-term six-UV exposure treatment described above and injected with BrdUrd 1 h before killing, as recommended by the manufacturer (Amersham Pharmacia Biotech, Arlington Heights, IL), and their dorsal and ventral skin was processed for immunohistochemical detection of BrdUrd (mouse monoclonal antibody clone Bu20a; Dako, Glostrup, Denmark) and TRP1. A brown

**Table 1** UV-induced tumors in HGF/SF transgenic and FVB/N wild-type mice

<table>
<thead>
<tr>
<th>Strain</th>
<th>No. of mice</th>
<th>No. of tumor bearers</th>
<th>SCC*</th>
<th>Sarcoma</th>
<th>Papilloma</th>
<th>Days to first tumor</th>
<th>Mean ± SE</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVB/N</td>
<td>32</td>
<td>27</td>
<td>19</td>
<td>7</td>
<td>10</td>
<td>248 ± 35</td>
<td>252</td>
<td></td>
</tr>
<tr>
<td>HGF/SF transgenic</td>
<td>27</td>
<td>16*</td>
<td>25</td>
<td>2</td>
<td>9</td>
<td>180 ± 35</td>
<td>183</td>
<td></td>
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*(Five tumors arising in FVB/N mice were not histologically identified.)

Squamous cell carcinoma.

*Eight transgenic mice died tumor-free before day 64 from apparent gastrointestinal obstruction or kidney failure, irrespective of UV irradiation, consistent with known phenotypes associated with these mice (23). Twenty unirradiated FVB/N mice and 15 unirradiated HGF/SF transgenic mice developed no tumors during the course of this study.

![Fig. 1](cancerres.aacrjournals.org) Development of skin cancer in HGF/SF transgenic mice. Survival analysis is shown for melanoma (open circle) and nonmelanoma (filled circle) skin cancers arising in all lines of HGF/SF transgenic mice; curves for line MH19 alone looked essentially the same. This study on the combined transgenic lines consisted of 142 mice, of which 26 developed melanoma and 14 developed nonmelanoma skin tumors. There was no statistical difference between the appearance of melanoma and nonmelanoma tumors in untreated transgenic mice. Note that the mean onset of melanoma in untreated transgenic mice was -21 months of age.

![Fig. 2](cancerres.aacrjournals.org) Accelerated carcinogenesis in UV-irradiated HGF/SF transgenic mice. Survival analysis is shown for UV-treated MH19 HGF/SF transgenic mice (open circles) and wild-type FVB/N mice (filled circles). Time to first tumor appearance, after initiation of UV irradiation, was evaluated by Kaplan-Meier survival analysis using censoring to account for animals that died tumor-free. The difference between strains was significant, *P* < 0.001. Only nonmelanocytic tumors were observed, as detailed in Table 1.
Fig. 3. Severe melanosis in UV-irradiated HGF/SF transgenic skin. Albino MH19 transgenic mice were crossed with C57BL/6 wild-type mice, and the F1 population was subjected to 5.5 months of chronic suberythemal UV radiation, as described in “Materials and Methods.” The total dose of UV radiation in this study was 307 kJ/m². Shown are F1 transgenic (Tg) and wild-type (Wt) littermates with and without UV treatment. Melanin deposits were found in both the reticular and papillary dermis of treated transgenic skin. Magnification, ×100.

Fig. 4. Melanocyte proliferation in response to UV irradiation in albino MH19 transgenic (Tg) and FVB/N wild type (Wt) mouse skin. Two-week UV treatment (total UV dose of 13.5 kJ/m²) was as described in Table 2 and “Materials and Methods.” The red cytoplasmic staining represents immunohistochemical detection of melanocytes using the PEP1 anti-TRP1 antibody, and the brown nuclear staining represents the immunohistochemical detection of BrdUrd incorporation. The black arrow shows a TRP1-positive melanocyte without BrdUrd staining, and the white arrow indicates a double-stained melanocyte. Magnification, ×250.
chromagen (diaminobenzidine) was used to visualize nuclear BrdUrd incorporation in S phase cells, and a red chromagen (Vector alkaline phosphatase substrate Kit 1; Vector Laboratories, Burlingame, CA) in the cytoplasm marking TRP1 was used to identify melanocytes. Double-labeled cells lying outside the hair follicle were microscopically scored in all sections.

RESULTS

Several independently derived lines of mice had been generated harboring the MT-HGF/SF transgene (23). Malignant melanoma arose in the various lines with similar latency and frequency. Fig. 1 shows the combined skin tumor-free survival of all HGF/SF transgenic mice. Melanoma developed in untreated transgenic animals with a mean onset of 21 months of age, whereas other skin tumors, including squamous papilloma, SCC, and fibrosarcoma, appeared somewhat later (Fig. 1). MH19, with a mean melanoma onset of 20 months (data not shown), was chosen as a representative transgenic line to test for sensitivity to a UV carcinogenesis protocol. In response to chronic suberythemal UV radiation, the majority of both HGF/SF transgenic and FVB/N wild-type mice developed skin tumors in an accelerated manner; however, time to tumor development was significantly shorter ($P < 0.001$) in HGF/SF transgenic mice (Table 1 and Fig. 2) using either mean time to tumor development (~6 months after initiating UV irradiation) or Kaplan-Meier survival analysis as criteria. Tumor locations and types were similar in both wild-type and transgenic animals. Tumors were found in FVB/N mice on the back and ear (in 25 and 16 mice, respectively) and in HGF/SF transgenic mice on the back, ear, and eye (in 30, 3, and 3 mice, respectively). SCCs were the predominant tumor type in both wild-type and transgenic mice. Remarkably, melanomas were not observed in the irradiated transgenic cohort, members of which died by 230 days after initiating UV irradiation, the time at which melanomas begin to appear in untreated MH19 mice. Therefore, chronic suberythemal UV radiation did not significantly accelerate melanoma genesis in this mouse model.

To determine whether the dose of UV radiation used in this study was sufficient to overtly affect melanocytes in the HGF/SF transgenic mouse skin, albino MH19 mice were crossed with C57BL/6 mice, HGF/SF was sufficient to overtly affect melanocytes in the radiation did not significantly accelerate melanoma genesis in this appear in untreated MH19 mice. Therefore, chronic suberythemal UV

Fig. 2 shows that chronic UV radiation for 5.5 months induced severe melanosis throughout the epidermis and dermis of the dorsal skin from transgenic F1 mice but not in the wild-type F1 animals. Note that melanin was restricted to the hair shaft in wild-type dorsal skin, irrespective of UV irradiation. The melanosis observed in the transgenic skin could be the result of melanin overproduction rather than melanocyte accumulation. To determine the effects of UV irradiation on melanocyte proliferation, we gave a short course (2 weeks) of identical UV radiation to albino FVB/N and HGF/SF transgenic littermates, injected both groups with BrdUrd 1 h before irradiation on melanocyte proliferation, we gave a short course (2 weeks) of identical UV radiation to albino FVB/N and HGF/SF transgenic littermates, injected both groups with BrdUrd 1 h before

<table>
<thead>
<tr>
<th>Epidermis*</th>
<th>Dermis*</th>
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<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>No UV</td>
<td>UV</td>
</tr>
<tr>
<td>HGF/SF</td>
<td>Met</td>
</tr>
<tr>
<td>0.31 ± 0.04</td>
<td>1.44 ± 0.13</td>
</tr>
<tr>
<td>0/90</td>
<td>11/324</td>
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<tr>
<td>Labeling index (%)</td>
<td>0</td>
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* $P < 0.001$ for differences in BrdUrd+ melanocytes in UV-treated versus untreated for both dermis alone and total skin.

* Mice were UV irradiated for a total of six treatments, each of 2.25 kJ/m2, over a 2-week period and were sacrificed 24 h after the last treatment.

* Melanocytes identified by PEPI-positive staining and proliferating cells identified by BrdUrd uptake (Fig. 2) were counted at $≥50$ magnification. Melanocytes in hair follicles and adnexa were not counted, and BrdUrd+ nuclei in epidermal cells and hair follicles were not counted.

These results suggested that melanocytes experiencing chronic Met activation in this transgenic mouse model were responding to UV irradiation in a manner consistent with that seen in human skin. However, it was also possible that UV radiation caused melanocyte proliferation simply by stimulating the stress-inducible MT promoter, thereby enhancing expression of the MT-HGF/SF transgene and the resulting mitogenic factor. In fact, analysis of total RNA isolated from some samples of skin and SCC from long-term UV irradiated mice

![Image](341x429 to 527x741)

Fig. 5. Northern blot analysis of expression of the HGF/SF transgene and the endogenous $c$-met gene in UV-irradiated and untreated skin. A, effect of exposure to chronic UV radiation on expression in skin and tumors (Tu). Although HGF/SF transgene expression could be up-regulated (as in skin and SCC tumor in animal 701), this was not a universal observation (as shown for animal 620). Consistent changes in expression of $c$-met were not seen. B, acute response to UV exposure of expression in skin. No consistent relationship between UV exposure (2-week course at total dosage of 13.5 kJ/m2) and expression of either the HGF/SF transgene or the endogenous $c$-met gene was noted. The ventral skin served as an untreated control in mice whose dorsal surface was exposed to UV. Reblotting with a labeled probe for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) served as a control for RNA loading and transfer.
demonstrated relatively high levels of HGF/SF transgene expression (Fig. 5A). To distinguish between these two possibilities, HGF/SF expression was analyzed from transgenic mice given the shorter 2-week course of UV radiation of equivalent total dosage (13.5 kJ/m²). Fig. 5B shows that no consistent increase in mRNA encoding transgenic HGF/SF could be detected in the skin of mice exposed to the dose of UV radiation capable of inducing significant melanocyte proliferation. Therefore, the mechanism by which exposure to UV radiation synergized with Met signaling to enhance tumorigenesis in this animal model was not through epigenetic augmentation of HGF/SF transgene expression. Furthermore, c-met transcript levels were also not consistently altered in response to UV irradiation (Fig. 5).

**DISCUSSION**

In this study we demonstrate that the skin of mice overexpressing HGF/SF was characterized by significantly enhanced sensitivity to the development of carcinomas and sarcomas induced by chronic suberythermal UV radiation. It has long been appreciated that the HGF/SF-MET signaling pathway plays an important role in human sarcoma genesis (for example, see Refs. 31, 32), including development of HIV-related Kaposi’s sarcoma (33, 34). c-MET has also been implicated in the genesis and progression of human SCCs and their derived cell lines, particularly in regulating motility and invasiveness (35–38). Most recently, activating somatic mutations in c-MET have been detected in metastatic SCCs of the head and neck (39). The observed sensitivity of HGF/SF transgenic skin to UV irradiation suggests the existence of a synergistic relationship between oncogenic pathways associated with constitutive Met signaling and UV-induced DNA damage. The enhanced proliferative response to irradiation in transgenic relative to wild-type keratinocytes may contribute to the enhanced carcinoma development observed in UV-treated HGF/SF mice, but other viable mechanisms must be considered.

The tumor suppressor p53 is known to play a pivotal role in the response of mammalian cells to DNA damage (reviewed in Refs. 40–43). Typically, exposure to mutagenic levels of UV radiation induces the p53 DNA damage response pathways in skin cells, as well as melanogenesis (44–46). UV-irradiated keratinocytes suffer DNA damage, forming thymine dimers and other photoproducts, which in turn induce p53. Such damaged keratinocytes then experience either apoptosis, if the damage is severe, or enhanced DNA repair through an SOS-like response, resulting in the survival of cells with minimal, but perhaps phenotypically significant, mutations (reviewed in Ref. 47). Notably, it has been reported that HGF/SF can inhibit UV-induced apoptosis in human keratinocytes through stimulation of the phosphatidylinositol-3-OH kinase pathway downstream of Met (48). This would provide a rational explanation for the elevated sensitivity of HGF/SF transgenic mice to SCC. Chronic stimulation of phosphatidylinositol-3-OH kinase and other Met signaling pathways in transgenic keratinocytes may permit the survival of damaged keratinocytes that would normally be targeted for apoptotic destruction, creating an expanded population of keratinocytes harboring potentially oncogenic mutations. Moreover, mutations in p53, thought to occur relatively early in the development of many nonmelanoma cancers, could exacerbate this situation (reviewed in ref. 49).

An alternative explanation concerns the effect of UV irradiation on the c-met gene, which has recently been shown in cultured cells to be a target of the p53 transactivator and up-regulated in response to UV exposure through a p53-dependent mechanism (50). In this scenario, exposure to UV radiation would further enhance HGF/SF-Met autocrine signaling in p53-competent transgenic keratinocytes. However, our RNA analysis revealed no consistent changes in c-met expression in the mouse skin in response to short- or long-term UV irradiation.

Remarkably, despite the fact that transgenic melanocytes throughout the skin vigorously responded to our chosen regimen of UV irradiation, and that this dose was clearly carcinogenic in the skin, melanocytic tumors failed to develop in this melanoma-prone HGF/SF transgenic mouse model. Instead, chronic suberythermal UV radiation preferentially favored the development of nonmelanocytic over melanocytic skin neoplasms. This conclusion from our transgenic animal model supports the notion that melanocytes are more resistant than keratinocytes to UV-induced neoplastic transformation (see review in Ref. 47). Moreover, our data are consistent with important retrospective studies in the human population that suggest that the incidence of melanoma is not associated so much with total UV dosage, as in nonmelanoma skin cancer, but rather with intermittent, burning UV exposure to naïve skin (11, 47, 51). This prediction is currently being tested in our HGF/SF transgenic mouse.

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**REFERENCES**


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