Development of a New Mouse Model (Xeroderma Pigmentosum A-Deficient, Stem Cell Factor-Transgenic) of Ultraviolet B-Induced Melanoma

Fumikazu Yamazaki,* Hiroyuki Okamoto,† Yasuhiro Matsumura,* Kiyoji Tanaka,† Takahiro Kunisada,† and Takeshi Horio*†

*Department of Dermatology, Kansai Medical University, Moriguchi, Osaka, Japan; †Institute for Molecular and Cellular Biology, Graduate School of Frontier Biosciences, Osaka University, CREST, Japan Science and Technology Agency, Suita, Osaka, Japan; ‡Structure and Organ Formation Control, Regeneration Medicine and Bioethics, Gifu University, Gifu, Japan

It is well established that exposure to sunlight or ultraviolet radiation (UVR) is the major environmental risk factor for the development of skin neoplasms. To date, however, there have been few appropriate mouse models available for studying the role of UVR in melanoma carcinogenesis, mainly because of the murine lack of the epidermal melanocyte, which is a major source of origin of human melanoma. In this study, we established xeroderma pigmentosum group A gene-deficient, stem cell factor-transgenic mice, which are defective in the repair of damaged DNA and do have epidermal melanocytes. The mice were exposed to UVR three times a week for 10 wk. More than 30% of the irradiated mice developed tumors of melanocyte origin that metastasized to the lymph nodes. Histologically, proliferated cells exhibited lentigo maligna melanoma or nodular melanoma. Immunohistochemistry confirmed that the tumor cells were characteristic of melanoma. Non-irradiated mice did not develop skin tumors spontaneously. The newly generated model mouse might be useful for studying the photobiological aspects of human melanoma, because the mice developed melanoma from epidermal melanocytes only after UVR exposures.

Key words: lentigo maligna/melanocyte/melanoma/stem cell factor/UV-carcinogenesis/xeroderma pigmentosum A


Melanoma, which originates from skin melanocytes, is one of the most morbid and lethal diseases among the skin disorders. The incidence rates of melanoma have steadily increased for the past several decades all over the world (Serraino et al., 1998). The rising incidence may be due in part to increased recreational exposure to sunlight (Langley et al., 1997). Elwood et al. (1997) reviewed that a number of retrospective epidemiological studies strongly suggest that sunlight plays a critical role in the induction of cutaneous melanoma. Investigations to clarify the functional role of ultraviolet radiation (UVR) in melanoma carcinogenesis, however, have been limited because of the lack of experimental animals in which to induce experimental melanomas with UVR. In contrast, the UVR mechanisms of action in the development of non-melanoma skin cancers have been extensively studied in mammals (Blum, 1959; Fisher et al., 1987). It is now well established that DNA is a major chromophore for UVR-induced cancers. Xeroderma pigmentosum (XP) is characterized by a high frequency of skin cancers, including melanoma, on sun-exposed areas of the body as a result of a defect in the early steps of nucleotide-excision repair (Kraemer et al., 1994). We have previously reported that group-A XP gene-deficient, XPA (−/−), mice are highly susceptible to UVR-induced skin carcinogenesis (Nakane et al., 1995). These mice easily and rapidly develop squamous cell carcinoma (SCC) of keratinocyte origin, but do not develop melanoma, presumably because of a lack in epidermal melanocytes. The growth and differentiation of melanocytes require stem cell factor (SCF), the ligand for the c-Kit receptor tyrosine kinase. The lack of SCF expression in keratinocytes is thought to result in the amelanocytic skin of the mouse except for the hair follicle. In contrast, the continuous expression of SCF in human keratinocytes maintains melanocytes in the skin (Yoshida et al., 1996; Kunisada et al., 1998). Targeting the expression of SCF-transgenic (SCF-Tg) to epidermal keratinocytes in mice with transgenes under the control of the human keratin 14 promoter resulted in epidermal melanocytosis, which produces and transfers melanins to keratinocytes (Kunisada et al., 1998). We crossed XPA (−/−) mice and SCF-Tg mice, and generated XPA (−/−), SCF-Tg mice (Yamazaki et al., 2004). These mice possess a hairless and deeply black skin. The epidermal melanin in the mice revealed a high inhibitory activity against the enhanced photosensitivity of XPA (−/−) mice. Although the epidermal melanocytes of XPA (−/−), SCF-Tg mice can sufficiently protect keratinocytes against UVR; melanocytes in these mice may be more sensitive
than those in XPA (+/+ ) mice because of a defect in the nucleotide excision repair of UVR-induced DNA damage. In this study, an effort was made to induce cutaneous melanoma by repeated exposures of a higher dose of UVR onto XPA (−/−), SCF-Tg mice.1

Results and Discussion

Non-treated XPA (−/−), SCF-Tg mice, and XPA-normal, SCF-Tg mice had black skin and hairs (Fig 1A, B). The epidermal cells consisted of keratinocytes and clear cells with abundant melanin in hematoxylin and eosin (HE)-stained sections (Yamazaki et al, 2004). The latter were positive to anti-S100 antibody immunohistochemically, thus consistent with melanocytes or Langerhans cells (Fig 1C). No cells in the epidermis or dermis are positive to anti-melanoma antibodies (anti-HMB45 antibody and anti-MART-1 or Melan A antibody) on immunohistochemical study (Fig 1D).

The mice at 8–10 wk of age were irradiated with UVB at a dose of 5 J per cm² on the back three times a week for 10 wk (total dose: 150 J per cm²). At the termination of the exposure protocol, scaling, crust ing, and/or ulceration was observed on the irradiated back areas. At 4 mo after the termination of UVR exposure, an unevenly pigmented macule with an irregular border appeared on the depigmented skin that had re-epithelized after ulceration (Fig 2A). Histologically, a proliferation of large, pleomorphic melanocytes was found at the epidermal–dermal junction, revealing the findings of lentigo maligna melanoma (LMM) in the HE-stained sections (Fig 2B). When these skin lesions were left untreated, the black macules became elevated tumors with ulceration and an irregular border (Fig 2C, white arrow). On histology, tumor cells with abundant melanin were found to have invaded vertically into the deep dermis (Fig 2D). At 6 mo, some of the other mice developed black nodules on the back (Fig 2E, white arrow) and ulcerated tumors appeared (Fig 2E, dotted white arrow). Histology of the black nodules revealed the findings of nodular melanoma (NM), with a proliferation of atypical melanocytes in the dermis in connection with the epidermis (Fig 2F). Tumor cells were so heavily pigmented that bleaching was required for immunohistochemical observations (Fig 3D–F). Monoclonal antibodies revealed that they were positive for the S-100 protein, melanoma antibodies (a mixture of anti-HMB-45 antibody and MART-1 or Melan A antibody), and the Ki-67 antibody (Fig 3D–F). The Ki-67 positive ratio was 18.1% in NM and 9.8% in LMM. Basal melanocytes of the non-exposed XPA (−/−), SCF-Tg mouse were positive only for the S-100 protein. The number of mice bearing LMM or NM gradually increased over time. At the end of the observation period of 58 wk after the start of UVR exposure, 12 and eight of 61 irradiated mice had developed LMM and NM, respectively. Twenty-three mice died before developing tumors because of persistent severe sunburn. Consequently, 33% (20 of 61) of irradiated mice, and 53% (20 of 38) of the survivors developed melanoma. Interestingly, individual mice developed either LMM or NM, but not both. All of the

LMM were induced on the re-epithelized skin after severe burning, whereas NM appeared on the skin without preceding ulceration. Six of eight mice with NM developed multiple tumors, and the remaining two bore a solitary tumor. Four mice developed skin tumors that were histologically confirmed to be SCC revealing proliferation of atypical keratinocytes with frequent mitotic figures, during a period of 4–8 mo after the discontinuation of UVR exposure (Fig 2E, dotted white arrow, Fig 2G). Three of these also developed NM associated with SCC, but the remaining mouse had NM alone. No tumor was induced in the non-irradiated XPA (−/−), SCF-Tg mouse, or the irradiated SCF-Tg (XPA normal (+/+ )) mice. Animals with skin melanomas were euthanized at various time points. The axillary and inguinal lymph nodes were collected for histological examination. Melanoma metastasis was found in four of 12 mice with LMM and in seven of eight with NM (Fig 4A and B). There were possible melanocytes bearing melanin granules in lymph nodes in the HE-stained section from non-irradiated mice. But they were negative immunohistochemically for anti-melanoma antibodies. Two pathologists experienced with melanoma evaluated the HE and immunohistochemically stained sections.

Walker reviewed that the development of animal models of melanoma has been undertaken by a number of investigators (Walker et al, 2002). There have been few previous reports of melanoma induction in experimental animals by UVR alone. Melanoma was induced in platyfish–swordtail hybrid fish by UVA and a visible range of light, which are not absorbed directly in DNA (Setlow et al, 1993). The question remains, however, whether these experimental data are directly applicable to human melanoma since there are great differences in the structure of the skin and in the environment between fish and humans. In terms of a mammalian model, the South American opossum, Monodelphis domestica, was exposed to UVR three times a week for 70 wk, and

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developed melanotic tumors in the dermis at 100 wk after the first exposure (Ley et al., 1989). Since this animal possesses a photoreactivation repair pathway for DNA damage, the induction of melanocyte hyperplasia was suppressed by the exposure of photoreactivating light (>320 nm) after UVR, indicating that DNA damage by UVR is linked to the melanoma development. The mouse has been used as a useful and convenient experimental animal to study the effect of UVR on non-melanoma skin cancers. To investigate the role of UVR on melanomagenicity, it is helpful to generate an animal model that develops melanoma after UVR exposure. The wild-type mouse, however, has not been a suitable model for melanoma investigation, since the mouse does not have epidermal melanocytes, although the colored mouse has melanocytes in the dermis and hair follicle. In inbred mice, melanoma has been induced by UVR in combination with chemical carcinogens (Romerdhall et al., 1989; Hussain et al., 1991). Recently, genetically engineered mouse models have been established for the study of melanomagenesis. In some of them, melanomas developed spontaneously, but were enhanced by UVR (hepatocyte growth factor/scatter factor (HGF/SF mouse)) (Noonan et al., 2000), and in other mice melanomas occurred only when chronically exposed to UVR, but from dermal melanocytes (Keless et al., 1998; Powell et al., 1999). Until recently, the responsible spectrum of UVR for melanomagenesis has not been experimentally identified. De Fabo revealed that UVB but not UVA radiation-initiated melanomas in HGF/SF mouse (De Fabo et al., 2004). In this experiment, the mice were exposed mainly to UVB, since XPA (C0/C0/C0) mice have a defect in repair of DNA damage of cyclobutane pyrimidine dimer-type that is induced by UVB. The melanin pigment...
melanoma. The dose of UV was very high compared with models that have been established by other investigators. This discrepancy seems to be mainly because of extremely dark skin with heavily pigmented epidermal keratinocytes and melanocytes in the present mice. The severe inflammation, which was induced by repeated exposures to high doses of UVR, may be partly involved in melanoma progression, as it is widely accepted that inflammation is a critical factor in tumor progression (Coussens et al, 2002). We rarely observed SCC in DNA repair-deficient mice even after exposures to a huge amount of UVB, indicating that melanocytes effectively protect keratinocytes against malignant transformation. The mouse generated in this study is a new animal model of melanoma in that melanoma occurred from epidermal melanocytes only after and as a result of experimental UVR exposure. Although melanoma was experimentally induced in the mice by exposure to an unusually high dose of UVB, we are currently generating XPA (−/−), SCF-Tg mice with a lighter skin color for clinically relevant investigations. This is the initial step toward establishing a model of melanoma that will allow close investigation of the development of the pathology in connection to light exposure. There have been several reports that mutation patterns were different (deletion, insertion, and point mutation) among clinical stages of melanomas in the tumor suppressor genes (Kannan et al, 2003). We are going to analyze the mutation of tumor suppressor genes in melanomas experimentally developed in XPA (−/−), SCF-Tg mice.

In conclusion, we have generated a mammalian model that can develop melanocyte tumor after UVR exposure with proliferating and metastasizable ability.

Materials and Methods

Mice  Hairless XPA (−/−) mice with a CBA, C57BL/6, and CD-1 chimeric genetic background (Nakane et al, 1995) were backcrossed with hairless albino mice of the inbred strains Hos/HR-1, and the resultant hairless XPA (−/−). A hairy SCF-Tg mouse has a C57BL/6 and SJL background (Kunisada et al, 1998). XPA (−/−) mice and SCF-Tg were crossed and the resultant hairless XPA (−/+), SCF-Tg mice were generated. Thereafter, offspring of XPA (+/−), SCF-Tg mice, and hairless XPA (−/−) mice were backcrossed, resulting in hairless XPA (−/−), SCF-Tg mice (Yamazaki et al, 2004). We used 61 XPA (−/−), SCF-Tg mice in this experiment. As control mouse, we used 20 XPA normal, SCF-Tg mice (Kunisada et al, 1998) and unexposed 20 XPA (−/−), SCF-Tg mice. All mice were 8–10 wk of age at the beginning of each experiment. The maximum observation interval was 2 y. Mouse experiments were approved by the Kansai Medical University Subcommittee on Research Animal Care.

Induction of skin tumors by UV irradiation  The UVB source was a bank of fluorescent sunlamps (FL.20SE.30; Toshiba Medical Supply, Tokyo, Japan) that emit approximately 55% of their radiation within the UVB range peaking at 305 nm, 25% and less than 1%, within UVA and UVC, respectively. The irradiance of UVB was measured by a radiometer (UVR-305/365D (II); Toshiba Medical Supply). XPA (−/−) SCF-Tg mice, XPA normal, and SCF-Tg mice were irradiated with 5 J per cm² of UVB on the back three times a week.

Histological examination of tumors  Skin samples and tumor samples were prepared from mice. A 5 μm section was stained with HE. For the immunohistochemical analysis, melanin in the...

References


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Address correspondence to: Fumikazu Yamazaki, MD, Department of Dermatology, Kansai Medical University, Osaka 570-8507, Japan. Email: yamazakf@takii.kmu.ac.jp