[Frontiers in Bioscience 7, d1531-1534, June 1, 2002]

ANIMAL MODELS OF ULTRAVIOLET RADIATION (UVR)-INDUCED CUTANEOUS MELANOMA

Ronald D. Ley

Department of Cell Biology and Physiology and the Cancer Research and Treatment Center, University of New Mexico Health Sciences Center, Albuquerque, NM

TABLE OF CONTENTS

1. ABSTRACT

The incidence of cutaneous melanoma continues to increase in many parts of the world including the United States. The American Cancer Society predicts that there will be approximately 53,600 new cases of melanoma in the United States during 2002 and that there will be 7,400 deaths from melanoma during the same time period. Increased understanding of the underlying mechanisms and risk factors involved in the induction of this deadly disease will require the use of suitable animal models of melanoma. To date, the induction of cutaneous melanoma with ultraviolet radiation (UVR) alone has been observed only in a few diverse animal models: a South American opossum, *Monodelphis domestica*; a hybrid fish, *Xiphophorus*; several stocks of transgenic mice; and in Angora goats. Most of these models are not completely suitable due to: 1) the target cell for melanoma formation; 2) the location of the melanocytes in the skin (i.e.- dermal as opposed to epidermal in humans); or 3) problems associated with husbandry and experimental manipulation. Recent studies have identified a mouse, the hepatocyte growth factor/scatter factor (HGF/SF) transgenic mouse, as an attractive model with which to study the induction of melanoma following a single, neonatal exposure to a moderate dose of UVR.

2. INTRODUCTION

The incidence of cutaneous melanoma has increased at an alarming rate in fair-skinned individuals over the last 3 decades. For the United States, Rigel and Carucci (1) predict that 1 of 74 people born in 2000 will develop melanoma during his or her lifetime. In Queensland, Australia, the cumulative risk of cutaneous melanoma currently stands at 1 in 14 for men and 1 in 17 for women (2). The rapid rate of increase in cutaneous melanoma makes it imperative that we understand fully the etiology of this most lethal form of skin cancer.

The exact role of UVR in the etiology of malignant melanoma of the skin is not clear (3-6). A

number of observations argue against UVR as an etiologic factor in the induction of melanoma (3). First, melanomas are not commonly found on the face and hands, which are anatomical sites of chronic UVR exposure. Second, in spite of higher chronic exposure to UVR and a higher incidence of non-melanoma skin cancer, outdoor workers have lower rates of melanoma than indoor workers. Third, a latitude gradient of melanoma induction is not apparent in some parts of the world. The observation, however, that xeroderma pigmentosum patients are at high risk for malignant melanoma induction supports a role for UVR in the etiology of malignant melanoma. The role of UVR in the induction of malignant melanoma appears to be more complex than in the induction of non-melanoma skin cancer. Some epidemiological data suggest that intermittent, high-intensity exposure to UVR, especially early in life, may be a risk factor in melanoma development (4). Defining a precise role for UVR in the induction of malignant melanoma has been hampered by the lack of completely suitable animal models susceptible to induction of malignant melanoma by UVR alone.

3. ANIMAL MODELS OF UVR-INDUCED CUTANEOUS MELANOMA

3.1. Hybrid Fish

Some hybrid and backcross strains of *Xiphophorus* are susceptible to the induction of melanoma following single and multiple exposures to UVR wavelengths (290-400 nm) (7). The susceptibility of the hybrids seems to be associated with two genes. One gene is the sex-linked oncogene, Xmrk (8), and the second gene resides within a putative suppressor locus, designated DIFF. The tumor suppressor gene within this locus appears to be a homologue of the p16 (CDKN2) gene, the gene frequently altered in human melanoma (9). The hybrid fish model has been used by Setlow and his co-workers (7) to generate an action spectrum (wavelength dependency) for the induction of melanoma. In the UVB (290-320 nm) region of the UVR spectrum, the action spectra for the induction of melanoma in fish, erythema in humans (10), and non-melanoma (NMSC) skin cancer in mice (11) are similar. Wavelengths in the UVA (320-400 nm) are far more efficient in the induction of fish melanoma than erythema in humans or NMSC in mice. It is not clear whether the results obtained with the fish model are relevant to the induction of melanoma in humans as the melanomas induced in the fish may arise from "non-typical" melanocytes and do not resemble human melanoma.

In addition to the CDKN2 gene, another tumor suppressor gene, the *p53* gene, has been cloned and sequenced from *Xiphophorus* fish hybrids (12). The induction and repair of cyclobutane pyrimidine dimers and pyrimidine (6-4) pyrimidine dimers by excision repair and photoenzymatic repair has been characterized in *Xiphophorus* (13, 14).

3.2. Monodelphis domestica

Monodelphis domestica is a South American opossum that has been used in our laboratory since 1984 for photodermatological studies. The animal was originally selected for these studies because it possesses a light-activated DNA repair system, photoreactivation, that repairs specifically UVR-induced pyrimidine dimers (15). The photoreactivation repair pathway was used to demonstrate a role for pyrimidine dimers in the induction of a number of pathologic conditions of the skin.

Monodelphis is a small (~100 g) pouchless opossum that has been maintained in outbred colonies since 1978. *Monodelphis* has been shown by our group (16) and by others (17, 18) to be susceptible to the induction of melanoma upon exposure to UVR alone. In these studies, chronic exposure to sub-erythemal doses of UVR (primarily UVB) three times per week for up to 70 weeks resulted in the appearance of areas of dermal melanocytic hyperplasia in the exposed skin. Some of these pigmented lesions progressed to melanotic tumors with pigmented cells metastasizing to regional and distal lymph nodes (16). One melanotic tumor was reported to have metastasized to the spleen (17). UVA can induce areas of focal, melanocytic hyperplasia, the putative precursor of melanoma in this animal (19). Contrary to what was observed with the fish model, the ability of UVA to induce melanoma (or melanoma precursors) in the opossum (15) is not substantially greater than would be predicted from its capacity to induce nonmelanoma skin cancer in mice (11) or erythema in humans (10). Thus, the action spectrum for melanoma induction in the fish model would *not* be expected to predict susceptibility to melanoma in mammals.

Strengths of the model include that it is: 1) one of only a few non-transgenic, mammalian models where melanoma can be induced with UVR alone; 2) relatively easy laboratory animal to maintain and to bred; 3) possesses the pyrimidine dimer-specific repair pathway, photoreactivation; and, 4) a number of relevant genes have been cloned. Disadvantages of the model include: 1) the immune system has been only partially characterized; 2) it is an out-bred animal which precludes transplantation experimentation; 3) unlike with human melanoma, opossum melanomas develop in the dermis and metastasize infrequently; and 4) animals must be maintained under red lighting to prevent photoreactivation.

3.3. Mouse

Early attempts to induce melanoma in non-transgenic mice with UVB (20-22) or UVA (21, 23) alone were unsuccessful. In one study, daily exposure to a dose of UVA sufficient to induce squamous cell carcinomas in the exposed mice failed to induce melanoma (23). UVR has been used in combination with chemical carcinogens or chemical promoters to induce melanoma in experimental animals. Epstein and co-workers (24) reported in 1967 that DMBA-induced melanocytic hyperplasia in mice could be promoted to melanomas with chronic exposure to a radiation source rich in UVB wavelengths. Kripke (25) produced one melanoma in a group of mice chronically exposed to UVR followed by repeated application of the tumor promoter, croton oil. Later studies by Romerdahl and co-workers (20) used the two-stage initiation-promotion

approach to show in a murine model that UVR (primarily UVB) can act as a weak initiator or promoter, but not as a complete carcinogen. Similarly, Husain et al. (21) reported that UVB and UVA promoted the appearance of cutaneous melanomas in mice initiated with DMBA. The results obtained with the UVA exposures should be interpreted with caution. The source of UVA in the Husain study was used without additional filtration to remove UVB. Biologically significant levels of UVB may be emitted from various sources of UVA (26). Thus, what was interpreted as a UVA effect may in fact have resulted from UVB exposure.

One of the earlier transgenic mice used in UVR-induced skin cancer studies was a strain of mouse that carried the Tyr-SV40E gene construct (27). In this mouse, the SV40 T antigen is under control of the tyrosine promoter and expressed only in melanocytes. The transgenic animals develop spontaneous cutaneous and ocular melanoma and short-term exposures to relatively low doses of UVR accelerate the appearance of melanotic lesions. Other transgenic mouse lines have been established with a mutated human Ha-ras gene also under control of the tyrosinase promoter (28). These mice are susceptible to the induction of melanomas upon exposure to UVR alone. As with the opossum, the melanomas appear to arise from dermal melanocytes.

A recent report from Noonan and co-workers has identified a transgenic mouse with considerable potential for use in studies on mechanisms and risk factors for the induction of melanoma (29). The mouse, the hepatocyte growth factor/scatter factor (HGF/SF) transgenic mouse, has melanocytes in the dermis, epidermis and dermal-epidermal junction and is thus similar to human skin. Chronic exposure of mice with sub-erythemal doses of UVR starting at 4-6 weeks of age accelerated the appearance of nonmelanoma skin tumors but had no effect on melanoma formation. In a later study by the same group, it was observed that a single, neonatal exposure to a moderate, but sunburning dose of UVR, accelerated the appearance of melanoma in the HGF/SF mouse (30). The UVR-induced melanomas were similar to human melanoma histopathologically and in molecular pathogenesis. This model may prove to be of considerable value in determining risk factors for human melanoma and characterizing the underlying events involved in melanoma formation.

3.4. Angora goat

An Australian group has observed that a vast majority of melanomas in the Angora goat occur on the dorsal, sun-exposed surface of the ear (31). Histopathologically the lesions resemble human melanoma. This animal model may provide insight into the mechanisms involved in the induction of melanoma upon exposure to natural sunlight. Husbandry considerations may limit the use of this model for studies with strictly controlled exposures to artificial sources of UVR.

4. CONCLUSION

Our understanding of the pathogenesis of melanoma has been hampered by a lack of suitable animal models (32). However, recent studies have demonstrated the utility of transgenic and knockout mice as animal models for defining risk factors and underlying events in the induction of melanoma by UVR (30,33).

5. ACKNOWLEDGEMENTS

I wish to thank Ms. Leslie Lund and Ms. Rose Marie Grace for critical review of the manuscript.

6. REFERENCES

1. Rigel DS, J.A. Carucci: Malignant melanoma: prevention, early detection, and treatment in the 21st century. *CA Cancer J Clin* 50 (4), 215-36 (2000).

2. MacLennan R, A.C. Green, G. R. McLeod, N. G. Martin: Increasing incidence of cutaneous melanoma in Queensland, Australia. *J Natl Cancer Inst* 84(18), 1427-32 (1992)

3. Cascinelli, N, R. Marchesini: Increasing incidence of cutaneous melanoma, ultraviolet radiation and the clinician, *Photochem. Photobiol.*, 50, 497-505 (1989)

4. Armstrong BK: Epidemiology of malignant melanoma: intermittent or total accumulated exposure to the clinician. *Dermatol. Surg. Onc*. 14, 835-49 (1988)

5. Lee JA: The relationship between malignant melanoma of skin and exposure to sunlight, *Photochem Photobiol.* 50, 493-96 (1989)

6. Sober A J: Solar exposure in the etiology of cutaneous melanoma, *Photodermatol*, 4, 23-31 (1987)

7. Setlow RB, E. Grist, K. Thompson, A.D. Woodhead: Wavelengths effective in induction of malignant melanoma. *Proc Nat Acad Sci USA* 90, 6666-6670 (1993)

8. Schartl M: Homology of melanoma-inducing loci in the genus *Xiphophorus. Genetics* 126, 1083-1091 (1990)

9. Kazianis S, D.C. Morizot, L.D. Coletta, D.A. Johnston, B. Woolcock, J.R. Vielkind, R.S. Nairn: Comparative structure and characterization of a CDKN2 gene in a Xiphophorus fish melanoma model. *Oncogene* 18(36), 5088-99 (1999)

10. Parrish JA, K.F. Jaenicke, R. R. Anderson: Erythema and melanogenesis action spectra of normal human skin. *Photochem Photobiol* 36, 187-91 (1982)

11. de Gruijl, FR, H.J.C.M. Sterenborg, P.D. Forbes, R. E. Davies, C. Cole, G. Kelfkens, H. van Weelden, H. Slaper, J.C. van der Leun : Wavelength dependence of skin cancer induction by ultraviolet irradiation of albino hairless mice. *Cancer Res* 53, 53-60 (1993)

12. Kazianis S, L. Gan, B. Della Coletta, D. C. Santi, R.S. Morizot, R.S. Nairn: Cloning and comparative sequence analysis of TP53 in Xiphophorus fish hybrid melanoma models. *Gene* 212 (1), 31-8 (1998)

13. Ahmed FE, R.B. Setlow: Ultraviolet radiation-induced DNA damage and its photorepair in the skin of the platyfish Xiphophorus. *Cancer Res* 53 (10 Suppl), 2249-55 (1993)

14. Meador JA, R.B. Walter, D.L. Mitchell: Induction, distribution and repair of UV photodamage in the platyfish, Xiphophorus signum. *Photochem Photobiol* 72(2), 260-6 (2000)

15. Ley RD: Photorepair of pyrimidine dimers in the epidermis of the marsupial *Monodelphis domestica*. *Photochem Photobiol* 40, 141-3 (1984)

16. Ley RD, L. A. Applegate, R. S. Padilla, T. D. Stuart: Ultraviolet radiation-induced malignant melanoma in Monodelphis domestica. *Photochem Photobiol* 50(1), 1-5 (1989)

17. VandeBerg, JL, S. Williams-Blanger, G. B. Hubbard, R.D. Ley, E.S. Robinson: Genetic analysis of ultraviolet radiation-induced skin hyperplasia and neoplasia in a laboratory marsupial model (*Monodelphis domestica*) *Arch Dermatol Res*, 286, 12-7 (1994)

18. Robinson, ES, J.L. VandeBerg, G.B. Hubbard, T.P. Dooley: Malignant melanoma in ultraviolet irradiated laboratory opossums: Initiation in suckling young, metastasis in adults, and xenograft behavior in nude mice. *Cancer Res* 54, 5986-91 (1994)

19. Ley RD: Dose response for ultraviolet radiation A-induced focal melanocytic hyperplasia and nonmelanoma skin tumors in *Monodelphis domestica*. *Photochem Photobiol* 73(1), 20-3 (2001)

20. Romerdahl CA, L.C. Stephens, C. Bucana, M. L. Kripke: The role of ultraviolet radiation in the induction of melanocytic skin tumors in inbred mice. *Cancer Commun* 1(4), 209-16 (1989)

21. Husain Z, M.A. Pathak, T. Flotte, M.M. Wick: Role of ultraviolet radiation in the induction of melanocytic tumors in hairless mice following 7,12-dimethylbenz(a)anthracene application and ultraviolet irradiation. *Cancer Res* 51(18), 4964-70 (1991)

22. van Weelden H, van der S.C. Putte, J. Toonstra, J.C. van der Leun: Ultraviolet B-induced tumors in pigmented hairless mice, with an unsuccessful attempt to induce cutaneous melanoma. *Photodermatol Photoimmunol Photomed* 7(2), 68-72 (1990)

23. van Weelden H, S.C. van der Putte, J. Toonstra, J.C. van der Leun: UVA-induced tumours in pigmented hairless mice and the carcinogenic risks of tanning with UVA. *Arch Dermatol Res* 282(5), 289-94 (1990)

24. Epstein JH, W.L. Epstein, T. Nakai: Production of melanomas from DMBA-induced "blue nevi" in hairless mice with ultraviolet light. *J Natl Cancer Inst* 38, 19-30 (1967)

25 Kripke ML: Speculations on the role of ultraviolet radiation in the development of malignant melanoma. *J Natl Cancer Inst* 63, 541-8 (1979)

26. McKinlay AF: Artificial sources of UVA radiation: Uses and emission characteristics. In: Biological Responses to Ultravioleet A Radiation. Eds: Urbach F, Valdenmar Publishing Company, Overlandd Park, Kansas (1992)

27. Klein-Szanto AJ, W.K. Silvers, B. Mintz: Ultraviolet radiation-induced malignant skin melanoma in melanoma-susceptible mice. *Cancer Re*s 17, 4569-72 (1994)

28. Broome Powell M, P.R. Gause, P. Hyman, J. Gregus, M. Lluria-Prevatt, R. Nagle, G.T. Bowden: Induction of melanoma in TPras transgenic mice. *Carcinogenesis* 20(9), 1747-53 (1999)

29. Noonan FP, T. Otsuka, S. Bang, M.R. Anver, G. Merlino: Accelerated ultraviolet radiation-induced carcinogenesis in hepatocyte growth factor/scatter factor transgenic mice. *Cancer Res* 15;60(14), 3738-43 (2000)

30. Noonan FP, J.A. Recio, H. Takayama, P. Duray, M. R. Anver, W.L. Rush, E.C. De Fabo: Neonatal sunburn and melanoma in mice. *Nature*, 413 (6853), 271-2 (2001)

31. Green A, R. Neale, R. Kelly, I. Smith, E. Ablett, B. Meyers, P. Parsons: An animal model for human melanoma. *Photochem Photobiol* 64(3), 577-80 (1996)

32. Kusewitt DF, R.D. Ley: Animal models of melanoma. In: Cancer Surveys – Skin Cancer. Eds: J. Tooze. Cold Spring Harbor Laboratory Press, Plainview, NY. Volume 26, 35-70 (1996)

33. Yang FC, G. Merlino, L. Chin: Genetic dissection of melanoma pathways in the mouse. *Semin Cancer Biol* 11(3), 261-8 (2001)

Key Words: Melanoma, Animal Models, Ultraviolet Radiation, Transgenic Mice, *Monodelphis domestica*, opossum, Angora goat, Hybrid Fish, Review

Send correspondence to: Dr. Ronald D. Ley, Department of Cell Biology and Physiology, Room 149 BMSB, 915 Camino de Salud, NE, Health Sciences Center, University of New Mexico, Albuquerque, NM 87131, USA, Tel: 505-272-5142, Fax: 505-272-9105, E-mail: rley@unm.edu