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Relevance of In Vivo Models in Melanoma Skin Cancer

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*Abbreviations: BCC, basal cell carcinoma; CHO, Chinese Hamster Ovary;
CMM, cutaneous malignant melanoma; MED, minimum erythema
dose; SCC, squamous cell carcinoma; UV-A, ultraviolet-A,
320-400 nm; UV-B, ultraviolet-B, 280-320 nm.

Key Words: action spectrum/fish/marsupials/transgenic mice

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Cutaneous malignant melanoma (CMM)* involves, as do most cancers, a number of steps such as initiation, promotion/progression, immunosurveillance, and metastasis. The incidence rate of CMM is dependent on many factors--skin type, hair color, presence of nevi, socioeconomic class and sun exposure, for example. It is a complicated disease^{1,2}, but sun exposure is the most common denominator as indicated by estimates³, from a comparison of incidence on sun exposed to unexposed sites, of the proportion of CMM resulting from sun exposure in Australia (96-97%) and a comparison of incidence in U.S. whites to blacks gives 92-96%. However, compared to non melanoma skin cancer, CMM is related to episodic rather than chronic exposures and, though it occurs on light-exposed areas, it is not principally confined to the high light-exposed areas of the body. The prevalence of CMM among individuals defective in DNA repair is orders of magnitude greater than in the average population⁴. Hence, one might expect that the wavelengths strongly absorbed by DNA--those in the UV-B--would be the most effective in inducing CMM. This conclusion is consistent with the latitude gradient of CMM--among comparable populations the incidence increases with decreasing latitude⁵--because the annual level of UV-B increases markedly as the latitude decreases. However, it is not a conclusive argument for the role of UV-B in CMM because the irradiance at all wavelengths increases as one approaches the Equator. Moreover, DNA damage may be mediated indirectly by light absorbed by other cellular chromophores absorbing at longer wavelengths. If one knew the dose-response relation for sunlight-induced CMM, one could use the latitude dependence to estimate the wavelengths important in CMM. Alternatively, if one knew the wavelengths affecting the rate-limiting step in melanoma induction, one could use the latitude data to compute the dose-response relation. Obviously, latitude data alone cannot yield both a dose-response relation and the wavelength dependence.

Model, animal experiments are needed to help determine the action spectrum for the rate-limiting step in the induction of CMM. Three animal models have been described (Table 1): 1) The laboratory opossum, Monodelphis domestica, develops melanoma after prolonged chronic exposures to UV-B, beginning at approximately 20 weeks of age^{6,7}, or by several exposures to suckling young followed by chronic exposures, beginning at 2 months, of those animals that had developed nevi⁸; 2) transgenic mice containing SV40 oncogene sequences specifically under transcriptional control by a mouse tyrosinase promoter, exposed at 4-7 days of age to UV-B, developed melanocyte hyperplasia that, upon subsequent grafting to longer lived unirradiated recipients, developed into melanomas⁹; 3) particular backcross hybrid fish of the genus Xiphophorus, containing approximately 1 melanoma suppressor gene, developed melanomas after single monochromatic light exposures¹⁰. The UV-B doses to these animal models, in multiples of the human minimal erythema dose, to give significant fractions of animals with melanomas are given in Table 2.

The high sensitivity of model 3 and the linear dose-response relation is the apparent result of the presence of only one tumor suppressor gene which, when inactivated, permits a melanoma to develop. Presumably, this model measures the initiation step in tumor induction. The wavelengths, if any, involved in the other steps have not been investigated. The step or steps that determine the responses of the other models are not apparent, and the spectral responses of these models have not been reported.

For model 3, the analysis of dose-response relations at a number of monochromatic wavelengths (302, 313, 365, 405, and 436 nm) yields an action spectrum for melanoma induction¹¹. The wavelength showing highest sensitivity

per incident photon was 302 nm, but there were significant sensitivities at all other wavelengths investigated. The calculated sensitivity of melanoma induction by individual wavelengths in sunlight is the product of the monochromatic sensitivity and the sunlight fluence rate at that wavelength. Because, at low wavelengths, sunlight intensity increases rapidly with increasing wavelength, the most effective wavelength for melanoma induction by sunlight is in the UV-A region (365 nm). If the CMM action spectrum were similar to the fish spectrum, approximately 90% of the melanoma inducing effect of sunlight would result from UV-A and visible radiation and only 10% from UV-B. Hence, this model predicts that ozone depletion would have little effect on melanoma incidence and moreover, that the use of sunscreens with maximum absorption in the UV-B region would result in users spending more time in the sun and an enhanced exposure to UV-A that would increase the probability of developing CMM¹².

Two human epidemiological findings confirm the extrapolation of the fish action spectrum to humans. First, the age adjusted incidence rates of BCC and SCC in Australia are approximately 10 and 20-fold greater than in Norway but the ratio for CMM is only approximately 2^{13,14} (Table 3). These ratios are consistent with the greater ratio of UV-B--the major sunlight component in non-melanoma skin cancer--in Australia to that in Norway and the much smaller ratio for the UV-A and visible regions. Second, a recent case control study¹⁵ indicates that sunscreen users had an odds ratio for melanoma incidence significantly greater than 1, even when corrected for hair color, skin type, and holidays in sunny resorts.

The wavelength dependence of the other steps in carcinogenesis need elucidation. Preliminary data, using mice, indicate that sunscreens give less protection against UV-B-induced immune suppression than against the direct formation of DNA damage or skin edema as if the UV-A wavelengths in the "UV-B light source" were important in immune suppression¹⁶.

Possible evidence against a very significant role of UV-A in melanoma induction comes from sequence analyses of the CDKN2 gene, a tumor suppressor gene involved in melanoma, in cell lines derived from human melanomas¹⁷. There were deletions of exon 1 and/or exon 2 in 19 of 30 lines investigated. Among the remaining 11 lines the predominant mutations were similar to those found for SCC and in cells irradiated in vitro with UV-B. The mutations were in both the transcribed and non-transcribed strand and no A-T→C-G transversion were observed. Such a transversion was characteristic (25%) of mutations introduced into the aprt gene of CHO cells by simulated solar light¹⁸. All the mutations in these unpigmented cells were in the non-transcribed strand and the transversions were very highly clustered. These discordant results may reflect an unexpected statistical anomaly, a difference between the DNA products indirectly induced by UV-A in non-pigmented cells compared to the products induced in melanocytes, the presence of an undetected T→G hot spot in deleted segments, that the CDKN2 gene does not operate at the initiation step or that CMM is more complicated than envisaged above.

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Table 1. Animal models for light-induced melanoma

1. Monodelphis domestica

- A. Chronic exposures to sunlamp radiation^{6,7}
beginning at 2-6 months of age,
3 times/week for up to 70 weeks.
[Photoreversal observed after UV-B exposure*]
- B. Eight exposures over 16 days to sunlamp radiation⁸
beginning at 1-5 days of age. Animals with nevi
at weaning (2 months) were further exposed 3 times/week
for up to 45 weeks.

2. Transgenic mice (C57BL/6)

containing SV40 oncogene sequences under control
of a tyrosinase-gene promoter⁹.
Exposed to sunlamp radiation at day 4 followed by
reexposures for 1 or 3 days. Skin containing lesions
at 20 weeks was grafted onto hemizygous hosts.

3. Xiphophorus backcross hybrids

with reduced copies of supressor genes.
Single exposures at 7 days of age^{10,11} to monochromatic
wavelengths (302, 313, 365, 405, 436 nm).
Tumors appear at 2 months, scored at 4 months.
[Photoreversal observed after UV-B exposure*]

*Photoreversal implicates cyclobutane pyrimidine dimers in DNA
involvement in melanoma induction.

Table 2. Approximate human minimum UV-B erythema dose
to induce melanomas in animal models

<u>Model</u>	<u>~ MED</u>
<u>M. domestica</u>	
chronic exposures, 2 or 6mo old for 16mo	50-100
8 exposures between 4 and 19d, followed by	3-17
chronic exposures between 2 and 10mo	10-55
Transgenic mice	
1-4 acute exposures at 4-7d	10-40
Hybrid fish	
1 acute exposure at 7d	0.5

Table 3. Annual age-adjusted incidence rates per 10^5 (1985)¹³

<u>Cancer Type</u>	<u>Australia</u>	<u>Norway</u>	<u>Australia/Norway</u>
BCC	443	40	11
SCC	112	5	22
CMM	19	11	1.7