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Induction of melanoma in TPras transgenic mice

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In order to study the oncogenesis of melanocytes, transgenic mouse lines were established that express a mutated human Ha-*ras* (TPras) gene in pigment producing cells. The *ras* transgenic mice exhibit an altered phenotype, including melanocytic hyperplasia and a muted agouti coat, indicative of hyperproliferative melanocytes. These mice and their wild-type littermates have been subjected to a variety of carcinogenesis protocols, including 7,12-dimethylbenz-[*a*]anthracene (DMBA), 12-*O*-tetradecanoylphorbol-13-acetate (TPA) and UV radiation exposure. Topical DMBA treatment of TPras mice resulted in a high incidence of melanomas. Metastatic lesions were observed in skin, lungs and lymph nodes. TPA treatment of TPras mice induced a small number of papillomas but no nevi or melanomas. UV light exposures induced papillomas in negative littermate and melanomas in some albino TPras mice. These results show that melanocytes expressing an activated Ha-*ras* in the TPras transgenic mice are susceptible to induction of melanoma by DMBA.

Introduction

Melanoma is the fastest-growing cancer in the USA with a steady increase in incidence rate since the 1970s (1). The increase in incidence and an increase in mortality rate makes this disease an issue of both clinical and economic importance (2). Despite increased efforts for public awareness and improved detection and diagnosis, no treatments have increased the 5 year survival rate. There is a need for more basic research and increased efforts to identify new therapeutic or chemopreventive strategies.

The use of animal models in the study of many forms of cancer has been invaluable for dissecting the molecular events in cells as they progress towards malignancy. Animals also provide the opportunity to study the systemic effects of treatment and prevention strategies. There has been a lack of suitable melanoma models for these purposes. Reports of animal models for melanoma include the opossum (3), hamster (4), guinea pig (5) and mouse (6,7). Melanomas develop in 5–40% of these hosts after treatment with the phorbol ester, 12-*O*-decanoylphorbol-13-acetate (TPA), croton oil or 7,12-dimethylbenz[*a*]anthracene (DMBA). UV light exposure alone or with additional chemical tumor promoters and initiators has also been used to induce melanoma. The model with the highest incidence was guinea pigs exposed to DMBA (5). Melanoma developed in 40% of the animals after a long 18 month latency period. Hussain *et al.* (6) used a hairless SKHR mouse initiated with DMBA followed by repeated exposure to UV light (280–320 nm) to achieve an incidence of 35%. Romerdahl *et al.* (7) observed that C3He mice initiated with DMBA followed by topical treatment with croton oil (or TPA) plus UV irradiation (280–320 nm) resulted in a similar incidence of melanoma. The latency period of ~35 weeks was far shorter than was seen in other species.

The advent of transgenic technology has aided the development of mice that develop melanocytic lesions or tumors. Several groups targeted expression of oncogenes to pigment producing cells (8–10). The transgenic mice described by Mintz *et al.* (8) and Klein-Szanto *et al.* (11) express a transgene that contains the oncogenic sequences of the SV40 large T/small t complex driven by a mouse tyrosinase promoter. The mice are categorized according to susceptibility of tumor development, with transgene copy number delineating the groups. The more susceptible lines develop cutaneous tumors when exposed to UV radiation, but due to aggressive malignant ocular tumors they do not live long enough for the investigators to observe full progression of the cutaneous tumors. The tumors are instead transplanted to less susceptible mice where they progress, sometimes to metastasis.

The transgenic mouse line that we have developed expresses a transgene using a mouse tyrosinase promoter sequence to drive the expression of the activated human T-24 Ha-*ras* gene in pigment producing cells. The resulting mouse has an altered phenotype including mild melanocytic hyperplasia, a muted agouti coat and pigmented skin, as described previously (9). We hypothesized that the expression of an activated T24 Ha-*ras* gene would cause the melanocytes to be susceptible to the development of melanoma. The mice do not spontaneously develop cutaneous melanoma or other skin tumors; however, ~12% develop ocular melanoma (12).

More recently, Chin *et al.* (10) developed several transgenic mouse lines to study gene function in the development of melanoma. Mice that were deficient for INK4a (p16) did not develop melanoma. However, when they generated mice on the INK4a deficient background that express an activated Ha-*ras* in the melanocytes, the mice spontaneously developed cutaneous melanoma at a high penetrance. A fourth transgenic mouse model that has been reported expresses a transgene containing a metallothionein gene promoter regulating expression of hepatocyte growth factor/scatter factor (13). Over expression of the transgene was observed in a number of tissues and a broad array of tumors developed including melanoma.

In our model, the TPras transgenic mice, we examined the induction of melanoma. We report in this manuscript the results of a variety of carcinogenesis protocols and their efficiencies of inducing melanoma.

Abbreviations: DMBA, 7,12-dimethylbenz[*a*]anthracene; SCID, severe combined immunodeficient; TPA, 12-*O*-tetradecanoylphorbol-13-acetate.

Materials and methods

Ras transgenic mice

Production of the TPras transgenic mice was described previously (9). The pigment producing cells in the mice express a mutated human T24 Ha-*ras* gene (14) driven by a 2.5 kb promoter region from the mouse tyrosinase gene (15). The mice showed stable passage of the transgene and were backcrossed with C3He/N mice. The phenotype of the mice included pigmented, bluish gray skin and a muted agouti coat color.

Treatment groups

All mice were housed in an AALAC approved University Animal Facility with 12 h light cycles. Food and water were provided *ad libitum*. The mice were shaved dorsally for treatments once a week with an electric razor, or as needed to remove hair. Studies were begun when the mice were 3–4 weeks old.

UV irradiation. Mice were placed in open polystyrene cages with wire lids and irradiated with a light panel containing four FS40T12/UVB lamps (National Biological Corporation, Twinsburg, OH). Greater than 90% of the emitted light from the bulbs was in the 280–340 nm range, as determined by a scanning spectrophotometer (model 440; Spectral Instruments, Tucson, AZ). The initial dose was 5.6 kJ/m^2 per treatment, which was increased twice by 20% for a total final dose of 8.06 kJ/m^2 per treatment. Doses were determined with the wire lid in place using a UVX Radiometer with a 310 nm probe (Ultraviolet Products, Upland, CA). The UV treatments were performed twice a week for 38 weeks or until tumors developed.

TPA. Five micrograms of TPA (LC Services, Woburn, MA) were applied topically to the entire dorsal section of the mouse four times per week. The TPA was dissolved in acetone to a concentration of 100 μg/ml. An aliquot of 50 μl (5 μg TPA) was administered per treatment for 38 weeks.

UV/TPA. A combination treatment of UV and TPA was administered as described above. The mice were treated topically with TPA (5 μg) three times (Monday, Wednesday and Friday) per week. UV treatments were performed twice (Tuesday and Thursday) per week.

DMBA. Fifty or 100 μg DMBA (Sigma, St Louis, MO) were applied once per week. The duration of the treatments was either 5 or 16 weeks. The DMBA was dissolved in acetone to a concentration of 0.5 mg/ml or 1.0 mg/ml. Aliquots of 100 μl of the appropriate DMBA solution were applied to the shaved backs of the mice. Due to the photosensitivity of DMBA, the solutions were prepared and applied in minimal, low-level lighting. Additionally, the cages were covered with dark cloth sheets for 24 h after treatment.

DMBA–TPA. An aliquot of 100 μg DMBA was applied topically to the dorsal section of the mouse once a week, as described above, for 2 weeks. Additionally, 5 μg TPA was applied twice per week, on days when DMBA was not administered. The TPA treatments were performed for 38 weeks.

Cell lines

Cell lines from DMBA induced melanoma from the TPras mice were established and cultured as described previously (16). Briefly, cell lines from tumors were derived by placing the tumors in trypsin/EDTA and then mincing the tumor into a cell suspension. During early passage the cells were maintained either in melanocyte media with 5 ng/ml TPA or in M15 with the addition of 3.5% fetal bovine serum and 3.5% newborn calf serum (16). Later passage cells were maintained in M15 media supplemented with fetal bovine and newborn calf serum.

Severe combined immunodeficient (SCID) mouse injections

Cell lines (passage 10 to 20) were grown in tissue culture flasks to ~80% confluency, removed by trypsinization, and counted using a hemocytometer with Trypan blue staining (Sigma) to determine the number of viable cells. Cells were centrifuged and resuspended in sterile saline. Five million cells were then injected subcutaneously into SCID mice (obtained from the Arizona Cancer Center SCID mouse facility). Mice were observed for tumor development and killed when tumors progressed to 1.5 cm in diameter or were present for 45 days. Mice were killed and examined for the presence of metastatic lesions.

Histology

Histological sections were prepared from skin samples and tumors from each group of mice and for each treatment. Tissues were fixed in 10% buffered formalin, parafin embedded and cut into 4–5 μm sections. The histologically prepared samples were stained with hematoxylin and eosin (H&E) or Mason Fontana stain. Selected tumor samples were fixed in glutaraldehyde for examination by electron microscopy.

TPras transgene and mouse Ha-ras expression

Tumors from the DMBA-treated TPras mice and tumors that developed on SCID mice injected with cell lines established from TPras mouse melanomas were screened for expression of the TPras transgene and expression of the endogenous mouse Ha-*ras* gene using RT–PCR. RNA was extracted by using a RNeasy kit (Qiagen, Chatsworth, CA) with a modified protocol (17). Briefly, fresh or frozen pieces of tumors were homogenized in RNeasy lysis buffer and subjected to a phenol–chloroform extraction. RNA was precipitated in isopropanol, resuspended in RNeasy lysis buffer, and purified using RNeasy spin columns. DNA contaminates were removed by DNase I digestion prior to RT reaction. Two micrograms of RNA were reverse transcribed with the addition of Superscript Reverse Transcriptase (Gibco BRL, Grand Island, NY), 10 mM dNTPs, 25 mM $MgCl_2$, 10× PCR Buffer II, 0.5 mg/ml oligo$(dT)_{16}$, and Rnasin (40 U/μl). The cDNA PCR was carried out in a 25 μl reaction mixture containing 2 μl of a 1:20 dilution of the RT reaction product, 10× NH_4 reaction buffer, 2.0 mM $MgCl_2$ (TPras) or 3.0 mM $MgCl_2$ (endogenous Ha-*ras*), 10 mM dNTP (2.5 mM of each) and 1.25 U Biolase DNA polymerase (Intermountain Science, Kaysville, UT). Reaction mixtures were heated to 95°C for 5 min and amplified for 30 cycles at 95°C for 35 s, 60°C for 35 s, and 72°C for 45 s. Samples were then held at 72°C for 10 min. The primer sequences for the TPras transgene and mouse Ha-*ras* were as follows: TPras 30, 5′-GTTGCTGGAAAAGAAGTCTGTG-3′; Ras 433, 5′-CCGTTTGATCTGCTCCCTGTAC-3′; and MRas 226, 5′-CCACTTTGTGGACGAGTATGAT-3′; and Ras 433 as described above.

Results

Induction of skin tumors

To induce skin tumors in the TPras mice, several different treatment protocols were carried out including: topical treatment with TPA (5 μg) four times a week for 38 weeks; UV light irradiation (280–340 nm, 5.6 kJ/m^2) twice weekly for 38 weeks; a combination of the UV and TPA treatments; topically applied DMBA (50 and 100 μg) administered once a week for 5 or 16 weeks; and a combination of DMBA and TPA treatments. Observations were made on the study groups through week 45. The results of these studies are summarized in Tables I and II. TPras-negative littermates treated with the same protocols as the TPras transgenic mice served as controls.

With the TPA treatments, we observed the development of papilloma in a small number (10–13%) of the TPras mice and negative littermates. No nevi or melanoma were induced.

The UV light (280–340 nm) exposures induced papillomas in the negative littermates (22%). Melanocytic nevi and melanoma developed on 20% of the TPras mice. When the UV light exposure was combined with TPA treatment, papillomas were induced on the TPras mice (33%) and negative littermates (11%). Carcinoma developed on 11% of the negative littermates. Nevi (67%) and melanoma (11%) were induced on the TPras mice by this combined protocol with 15% of the negative littermates also developing melanocytic nevi. Of particular interest was the development of melanomas in the UV-treated TPras group and the UV–TPA treated TPras group. The TPras mice that developed melanomas all had an albino phenotype.

Our observations with DMBA treatments demonstrated a high incidence of melanoma in the TPras mice. A total of 86–88% of the mice developed both nevi and melanoma with 50 or 100 μg DMBA administered once per week for 5 weeks (Figure 1B and C). With 16 weekly doses of 100 μg DMBA, 100% of the TPras mice developed melanoma; however, we also observed some ulcerated lesions due to toxicity of DMBA. Papillomas and carcinomas were observed with the higher doses of DMBA (100 μg). With 16 weeks of the high dose DMBA treatment, papilloma (100%) and carcinoma (75%) developed on the negative littermates. Nine percent of the TPras mice developed carcinomas and 55% developed papillomas on this protocol. When the number of DMBA treatments was reduced to 5 weeks with 50 or 100 μg, the incidence of papillomas on the TPras mice dropped to 13% and no carcinoma were observed. No carcinomas were observed with the 5 week

Table I. Percent of mice with nevi and melanoma

	Mice[a]	Treatment[b]			DMBA			DMBA–TPA
		5 μg TPA	5.6 kJ/m^2 UVB	TPA–UVB	50 μg (5)[c]	100 μg (5)[c]	100 μg (16)[c]	
% Nevi (n)[d]	ras+	0 (15)	20 (10)	67 (9)	86 (7)	88 (8)	100 (11)	44 (9)
	–littermates	0 (20)	0 (18)	15 (27)	57 (7)	64 (11)	13 (16)	13 (16)
% Melanoma (n)[d]	ras+	0 (15)	20[e] (10)	11[e] (9)	86 (7)	88 (8)	100 (11)	44 (9)
	–littermates	0 (20)	0 (18)	0 (27)	0 (7)	0 (11)	0 (16)	0 (16)

[a]ras+, TPras mice; –littermates, negative littermates from the TPras breeding colony which have the TPras transgene.
[b]Treatments are described in Materials and methods. TPA and DMBA were dissolved in acetone and applied topically. Source of UVB irradiation was a bank of four FS40T12 lamps.
[c]Number in parentheses indicates the number of weeks DMBA was administered.
[d]Number of mice in each treatment group.
[e]The TPras mice that developed melanoma in these groups had an albino coat color.

Table II. Percent of mice with papilloma and carcinoma

	Mice[a]	Treatment[b]			DMBA			DMBA–TPA
		5 μg TPA	5.2 kJ/m^2 UVB	TPA–UVB	50 μg (5)[c]	100 μg (5)[c]	100 μg (16)[c]	
% Papilloma (n)[d]	ras+	13 (15)	0 (10)	33 (9)	0 (7)	13 (8)	55 (11)	22 (9)
	–littermates	10 (20)	22 (18)	11 (27)	0 (7)	82 (11)	100 (16)	88 (16)
% Carcinoma (n)[d]	ras+	0 (15)	0 (10)	0 (9)	0 (7)	0 (8)	9 (11)	0 (9)
	–littermates	0 (20)	0 (18)	11 (27)	0 (7)	0 (11)	75 (16)	50 (16)

[a]ras+, TPras mice; –littermates, negative littermates.
[b]Treatments are described in Materials and methods. TPA and DMBA were dissolved in acetone and applied topically. Source of UVB irradiation was a bank of four FS40T12 lamps.
[c]Number in parentheses indicates the number of weeks DMBA was administered.
[d]Number of mice in each treatment group.

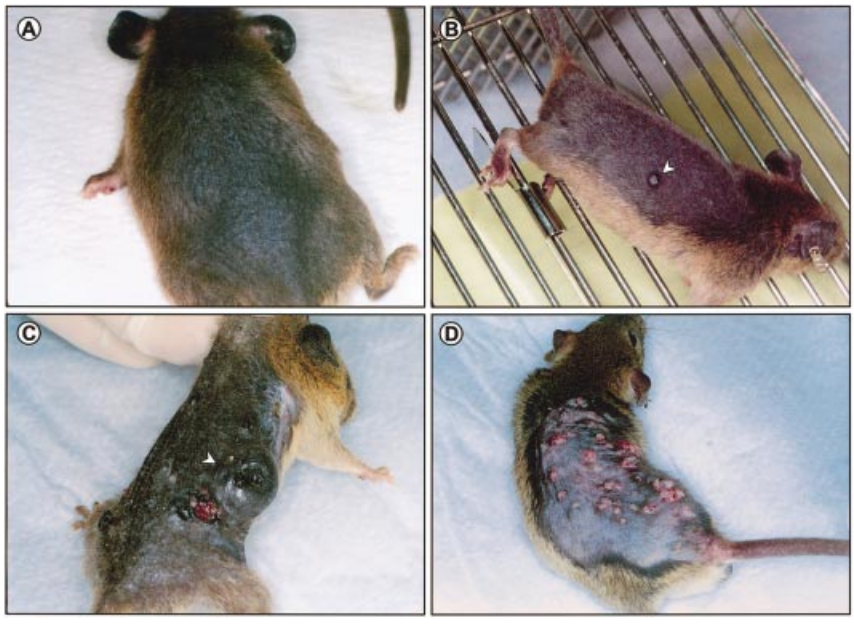

Fig. 1. Induction of skin tumors in TPras transgenic mouse and negative littermate treated with five weekly doses of 100 μg of DMBA. The dorsal area of the mice was shaved 24 h prior to treatment or as needed. (**A**) Acetone control. (**B**) Early melanocytic lesion at 12 weeks. (**C**) Melanoma with ulceration at 24 weeks. (**D**) Negative littermate treated with DMBA showing multiple papillomas at 24 weeks.

DMBA treatment in the negative littermates; however, papillomas were observed on 82% of the negative littermates treated with 100 μg DMBA (Figure 1D). When the mice were given an even lower dose of DMBA (20 μg for 16 weeks) none of the mice developed any tumors (data not shown).

The initiation/promotion protocol of two weekly doses of DMBA (100 μg) followed by TPA (5 μg) applied topically twice a week for 38 weeks also resulted in tumor development. The negative littermates developed papillomas (88%) and carcinomas (50%). Twenty-two percent of the TPras mice

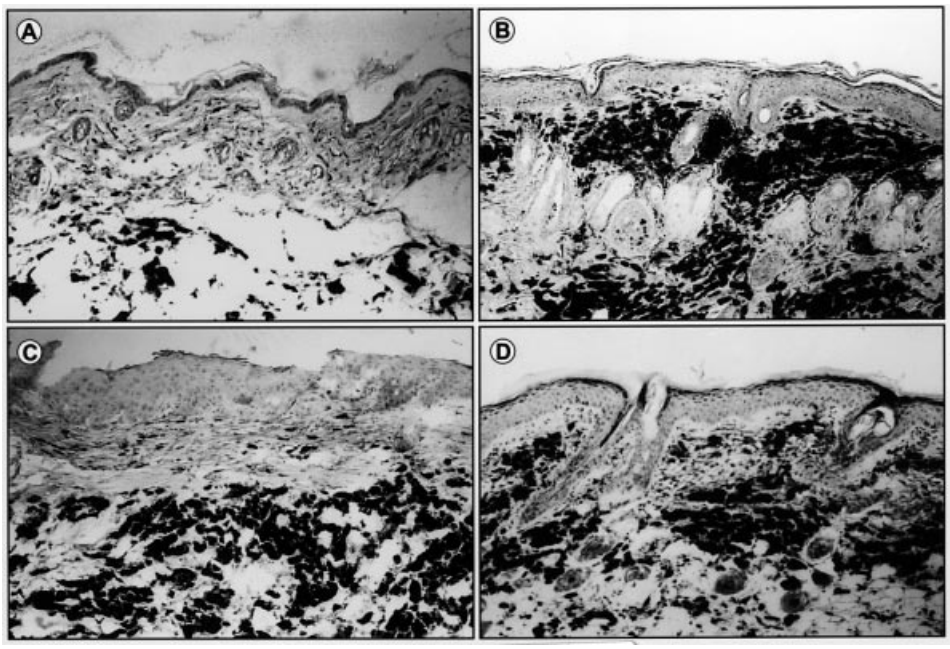

Fig. 2. TPras transgenic mice were exposed to either (A) acetone, (B) TPA (5 μg, four times per week), (C) UV-B (5.6 kJ/m^2 which was gradually increased to 8.06 kJ/m^2, twice per week) or (D) TPA and UV-B [at doses described for (B) and (C)]. Skin biopsies were sampled at 27 weeks and fixed in 10% buffered formalin. Sections were stained with H&E. (**A**) Skin treated with acetone alone. Melanin stimulation was observed. (**B**) In mice treated with TPA, there was acanthosis of the epidermis. Increased melanin depositions were observed in both the reticular and papillary dermis. (**C**) UV-B exposure in the absence of TPA resulted in a thickening of the epidermis layer with some cellular disorganization. Melanin was observed primarily in the reticular dermis. (**D**) With the combination treatment of UV-B and TPA, melanin deposits were observed in the reticular dermis with sparse melanin deposits appearing in the papillary dermis.

developed papillomas but no carcinomas were observed. Of interest was the development of nevi and melanomas in only 44% of the TPras mice.

Histopathology of the skin lesions and tumors

Histological sections were prepared from skin samples or cutaneous tumors after 27 weeks of treatment. The control, TPras mice treated with acetone alone, showed no stimulation of melanin (Figure 2A). In the TPras mice treated with TPA, we observed a marked acanthosis of the epidermis along with increased melanin depositions in the reticular and papillary dermal layers (Figure 2B). UV irradiation caused similar epidermal thickening as was observed in TPA treated mice, but UV also induced some cellular disorganization (Figure 2C). The combination of UV and TPA treatments resulted in significant melanin deposits in the reticular dermis and to a lesser extent, in the papillary dermis (Figure 2D).

The control mice, negative littermates, showed similar histological responses to the treatment protocols, though the tumors that developed were mainly papillomas, of which some progressed to carcinomas. The control, acetone-treated, mice showed no observable alterations (Figure 3A). TPA treatment caused a slight thickening of the epidermis (Figure 3B). UV exposure caused slight cellular disorganization to occur (Figure 3C). Combined TPA and UV treatment resulted in significant acanthotic thickening of the epidermis (Figure 3D).

In the DMBA-treated TPras mice, nevi developed between 7 and 13 weeks after the first treatment and melanoma occurred between weeks 12 and 29. The cutaneous melanoma contained large pleomorphic cells with hyperchromatic nuclei and dense pigment granules (Figure 4A and B). These cutaneous melanomas had a propensity to metastasize to areas including lymph node and lung. Metastatic tumors were observed in >40% of the mice that developed cutaneous primary melanomas with the DMBA treatments. In lungs (Figure 4C) and lymph nodes (Figure 4D), the metastasized tumors were observed to have multiple foci of melanin-positive cells. UV irradiation was successful in inducing melanoma only in albino TPras mice, which lack the dark, photoprotective pigmentation. Metastatic lesions were also observed in the lungs of 50% of these mice. Electron microscopy was used to confirm the presence of premelanosomes in the amelanotic tumors.

DMBA treatment protocols resulted in significant papilloma development in the negative littermates with histologic evidence of conversion to carcinoma in some of the mice. Histology of the papillomas demonstrated squamous maturation and retention of polarization. The carcinomas appeared to develop from the papillomas and were invasive, spreading into the surrounding dermis.

Evaluation of tumorigenicity in SCID mice

Four cell lines established from the DMBA-induced melanoma in TPras mice, including cutaneous tumors and lung metastases, were evaluated for tumorigenicity in SCID mice. Cells harvested from tissue culture were injected into SCID mice and the animals were observed for tumor formation. Tumors formed in mice injected with melanoma cells within 1 to 2 weeks. The tumors were invasive to adjacent muscle and metastasized in some of the mice. The lesions had histologically identifiable melanoma characteristics. The non-malignant melanocyte line derived from the untreated transgenic mouse did not form tumors when injected into SCID mice.

Cell lines designated 1998 and 1984-1, both derived from DMBA-induced primary cutaneous melanoma, formed tumors within 1 week of injection. The tumors invaded muscle and the body cavity, though no attachment to internal organs was observed. Additionally, 1984-1 was very vascularized and formed metastatic lesions in both lungs. Cell lines designated

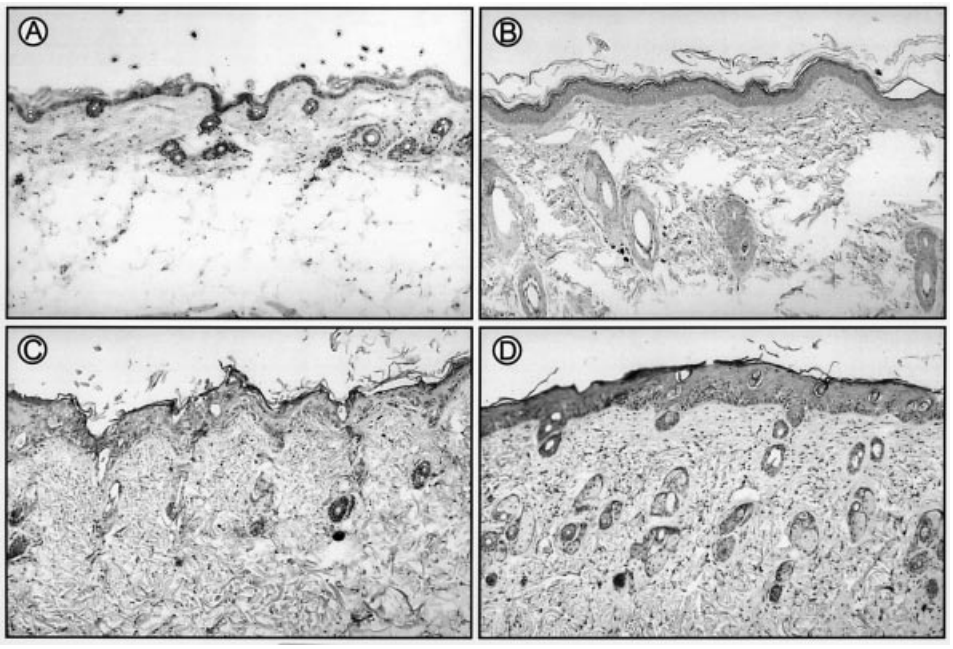

Fig. 3. Negative littermates of the TPras mice were treated with either (A) acetone, (B) TPA (5 μg, four times per week), (C) UV-B (5.6 kJ/m^2 which was gradually increased to 8.06 kJ/m^2, twice per week) or (D) TPA and UV-B [at doses described for (B) and (C)]. Skin biopsies were taken at 27 weeks and fixed in 10% buffered formalin. Sections were stained with H&E stains. (**A**) Acetone treatment of dorsal skin with no significant alterations observed. (**B**) TPA treatment caused acanthosis of the epidermis when compared with the acetone treated skin. (**C**) UV-B exposure resulted in a slight cellular disorganization of the epidermis. (**D**) When the mice were treated with both TPA and UV-B there was a prominent acanthotic thickening of the epidermis.

1996 and 1984-2, from a DMBA-induced cutaneous lesion and a lung metastasis (from mouse with 1984-1 cutaneous lesion), respectively, formed tumors by 2 weeks after injection. The tumors demonstrated slower progression than the 1998 and 1984-1 cell lines.

Expression of the TPras transgene in the melanoma

Expression of the transgene and endogenous Ha-*ras* in tumors was analyzed by RT–PCR (Fig. 5). cDNAs were made from pieces of tumors from the DMBA-treated TPras mice and tumors that developed when SCID mice were injected with cells lines derived from the TPras mice tumors. Skin samples taken from regions that had not been treated were also analyzed. As shown in Figure 5, tumors that developed on the DMBA-treated TPras mice (1984-1, 2391a, 2391b, 2395a, 2421a, 2452a) and the tumors on the SCID mice that developed from TPras melanoma cells (SC1984-1a, SC1984-1b, SC1998a) all expressed the TPras transgene. In most samples of untreated TPras skin (Sk2391, Sk2395, Sk2421 and Sk2425) we did not detect the transgene using the conditions described. However, if the number of cycles that the samples were amplified was increased then low levels of the TPras transgene were detected in some samples (data not presented). Endogenous mouse Ha-*ras* was detected in all of the tissues and tumors analyzed.

Discussion

We have observed that melanoma are induced in the TPras transgenic mice and not in negative littermates when exposed to DMBA (50 or 100 μg, 5 or 16 weeks). Melanoma developed when TPA was added to the DMBA protocol, however at a significantly lower incidence. This observation would agree with earlier studies by us and others (18,19) that TPA can be growth inhibitory for some melanomas. In several mice we observed tumor regression with the application of TPA. The development of carcinomas on the TPras mice was very infrequent. Only high doses of DMBA resulted in a few carcinomas, and the number of TPras mice with papillomas was also about half that observed in the negative littermates. We do not know if the TPras mice are protected against development of the other skin tumor types or if the changes in melanocyte numbers affect the behavior of the keratinocytes.

These studies demonstrate that the TPras mice are highly susceptible to developing melanoma and represent a model in which melanoma is induced in multiple stages of progression, from nevi to metastatic tumors. This model provides an ideal system for the evaluation of chemopreventive and therapeutic treatments due to the presence of multiple stages, a high incidence (>80%) and a moderate latency period (12–25 weeks).

We chose a mutated *ras* gene for the transgenic construct because of its integral role in the mitogenic response pathway and a high rate of mutation in many forms of cancer. In melanomas, *ras* mutation rates of 5–25% have been reported by several groups (20–22), and Platz *et al.* (21) observed increased ras expression in 70% of the melanomas they studied. The Ha-*ras* gene has been shown to be involved in non-melanoma skin cancers (23–25) initiated by DMBA and promoted by TPA. DMBA, unlike many other carcinogens, preferentially causes A→T point mutations through its epoxide metabolites. As shown by Cheng *et al.* (26), a point mutation in codon 61 of ras causes oncogenic conversion of the protein, which is integral in the transformation of the cells. We have screened the tumors for additional mutations in endogenous ras and the Ha-*ras* transgene (M.B.Powell, J.Morreale and L.Martin, manuscript in preparation). No mutations were observed in codons 12, 13 or 61. The evaluation of the DMBA-induced tumors in SCID mice demonstrated that the melanoma cell lines are malignant, invasive and have a propensity for metastasis.

We also have observed the induction of melanoma with UV

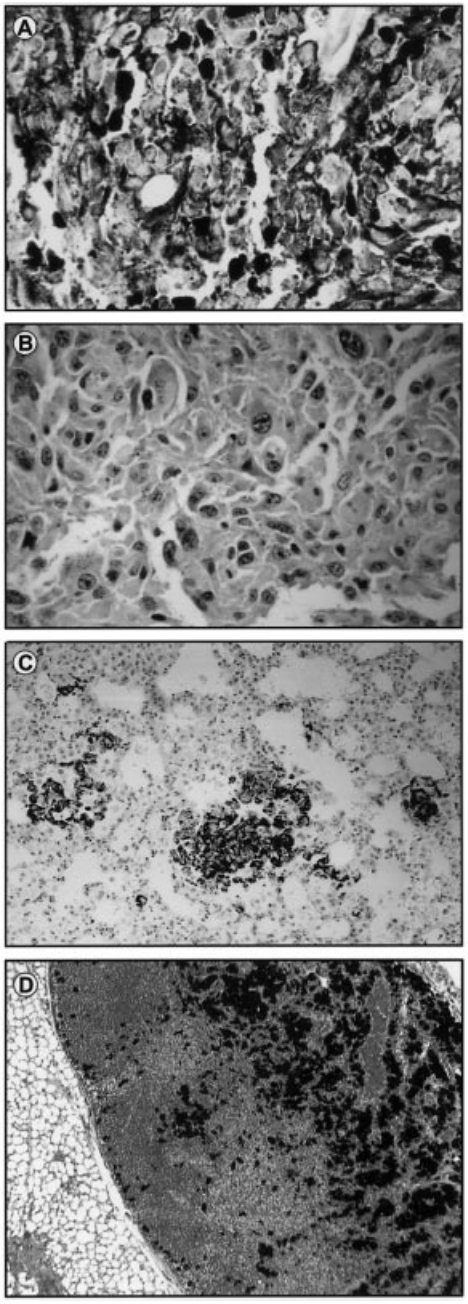

Fig. 4. TPras mice treated topically with DMBA (50 or 100 μg, once per week) for 5 or 16 weeks developed cutaneous melanomas with some metastasis to the lungs and lymph nodes. (**A**) Cutaneous melanoma. Lesion contains large pleomorphic pigmented cells with hyperchromatic nuclei. (**B**) Bleached section of the melanoma shown in (A) illustrates nuclear pleomorphism. (**C**) Section of lung with multiple foci of melanin positive tumors. (**D**) Section of lymph node showing significant invasion by melanin-positive neoplastic cells.

alone and UV–TPA treatments. The melanoma developed in a small number of TPras mice that had an albino phenotype. These tumors developed after 25–35 weeks of treatments. Several other models of UV-induced melanoma have been reported. Mintz *et al.* (8) observed in their SV40 transgenic mice that UV exposure increased the incidence of melanoma in transplanted skin grafts from 25 to 39%. This model represented a major step forward in mouse melanoma models, as the incidence reached nearly 40% and the latency period was between 24 and 37 weeks in UV-irradiated subjects.

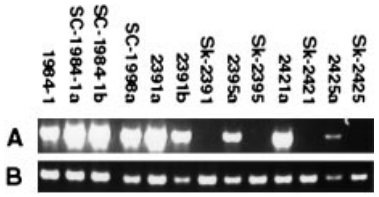

Fig. 5. Melanocytic tumors that developed in the TPras mice (1984-1, 2391a, 2391b, 2395a, 2421a, 2425a) and tumors taken from SCID mice injected with cell lines from the TPras mouse melanomas (SD1984-1a, SD1984-1b, SD1998a) expressed the TPras transgene (A) and endogenous mouse Ha-*ras* (B). Skin samples (Sk2391, Sk2395, Sk2421, Sk2425) from abdominal region of TPras mice that had no melanocytic lesions expressed only the mouse Ha-*ras*. Expression of the transgene and Ha-*ras* gene was analyzed by RT–PCR.

Ley *et al.* (3) observed in an opossum model, *Monodelphis domestica*, that UVB induced melanoma. Ley reported recently that UVA is nearly as efficient as UVB in inducing melanocytic lesions in these animals (27). A third group, Setlow *et al.* (28), have observed the induction of melanoma at UV wavelengths of 365, 405 and 436 nm in platyfish. We are presently developing several lines of TPras mice with different pigmentation phenotypes to allow a more careful evaluation of the role of UV and pigments in melanoma development.

We have characterized the melanoma cell lines for cytogenetic abnormalities (16). A key feature discovered during the characterization was a conserved alteration in chromosome 4. Accompanying this alteration was a loss of gene material for two tumor suppressor genes, *p15* and *p16*, which have been identified by several studies as being involved in melanoma development (29,30). A transgenic system developed by Chin *et al.* (10) examines the functional role of *ras* and *INK4a* (*p16*) in melanoma development. They generated *p16* deficient mice that alone did not develop melanoma. However, when they crossed the *p16*-deficient mice with a transgenic mouse with an activated Ha-*ras* controlled by a tyrosinase promoter (similar to our TPras mice), they observed multiple spontaneous, locally invasive melanomas. Chin's observations support our report (16) that melanoma development in the DMBA treated TPras mice was accompanied by either complete or partial loss of the p16 gene.

These studies provide evidence that malignant melanoma is inducible in the TPras transgenic mice that we have developed. The most effective carcinogenesis protocol, five weekly doses of 100 μg of DMBA, that we have identified has yielded a >80% incidence of malignant melanoma, including some invasive metastatic disease. These studies suggest that expression of an activated ras in the melanocytes contributes to their susceptibility to develop melanoma.

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