Development of Heritable Melanoma in Transgenic Mice

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Transfer of genetic material into recipient cells by transfection has been used successfully to isolate genes responsible for particular phenotypic traits. By using this strategy, DNA fragments were isolated that when transfected into appropriate uncommitted cells will commit the recipient cells to undergo adipocyte differentiation. Transgenic mice were generated with one of the active DNA clones, Clone B. The transgenic mice were expected to display an adipocyte-related phenotype; however, the animals developed melanin containing tumors at a young age. Insertion of Clone B into the mouse DNA probably interrupted a gene(s) that is involved in the regulation of cell growth, specifically regulation of cell growth in melanin-producing cells. Histopathologic analysis of these mice showed dark spots on the ear lobes of the animals as early as 10–12 d of age. By 3 mo, in addition to the ear lobes, pigmented tumors could be observed in other organs. A significant number of these transgenic mice died within 1 y of age. The melanomas developed spontaneously in these animals in the absence of any known chemical carcinogen or ultraviolet radiation. This line of mice provides a way of identifying genes involved in regulation of cell growth control and differentiation. These mice also serve as a model system to investigate the molecular, genetic, and phenotypic characterization and development of melanomas. ***Key words: insertion mutagenesis/melanocytes/spontaneous skin lesions. J Invest Dermatol 110:247–252, 1998***

Melanoma is the result of the neoplastic transformation of melanocytes of the skin or other tissues. The incidence and mortality rate of melanoma have increased dramatically among Caucasians during the last decade. By the end of this century, approximately one in 100 Caucasians in the United States will develop melanoma in their lifetime. Multiple etiologic factors lead to the development of melanoma. One set of the factors is genetic, whereas other factors include several carcinogenic stimuli applied to that genetic predisposition.

Several oncogenes and tumor suppressor genes have been implicated in the progression of melanoma. Cytogenetic analysis of primary melanomas often shows loss of heterozygosity of specific regions of several chromosomes, strongly suggesting the presence of tumor suppressor genes (Becher *et al*, 1983; Balaban *et al*, 1984; Trent, 1991; Thompson *et al*, 1995). One such putative tumor suppressor gene, Multiple Tumor Suppressor 1 (MTS1), located on chromosome 9p21, is frequently rearranged or deleted in familial melanoma (Hussussian *et al*, 1994; Kamb *et al*, 1994; Wolfel *et al*, 1995). MTS1 has been shown to encode a previously identified protein, p16, which is known to bind to and inhibit the activity of cyclin-dependent-kinase 4 (cdk4) during the cell cycle (Hussussian *et al*, 1994; Kamb *et al*, 1994; Wolfel *et al*, 1995). Analysis of p16 protein in over 100 melanomas showed that a large number of the tumors examined retained expression of p16 (Wick *et al*, 1995); however, these melanoma-associated p16 proteins are mutated and are thus defective in cdk4 binding. These results suggest that p16 mutation, via its deregulation of the cdk4 pathway, is of biologic significance in the development of melanoma (Wick *et al*, 1995). In addition to chromosome 9, chromosomes 1, 3, 6, 7, and 11 have been shown to be altered in malignant melanomas (Healy *et al*, 1995). The long arm of chromosome 6 shows preferential loss of heterozygosity through deletion or rearrangement in a large number of cases of human malignant melanoma. The re-introduction of a normal human chromosome 6 into human melanoma cells results in the loss of the malignant phenotype (Trent *et al*, 1990; Church *et al*, 1993; Zhang *et al*, 1995; Ray *et al*, 1996), suggesting the presence of a "melanoma suppressor gene" within this region of chromosome 6. At least two putative "tumor suppressor genes" on the long arm of chromosome 6 have been identified (Church *et al*, 1993; Ray *et al*, 1997). Taken together, it is clear that melanoma is a complex disease whose etiology may involve several possible pathways controlling normal melanocytic functions. Data describing the proto-oncogenes and tumor suppressor genes suggest that mutations in any of the genes controlling growth and/or differentiation of cells may lead to neoplastic transformation (Trent, 1991). Other as yet unidentified genes, such as those involved in the control of proliferation and differentiation of pigment cells, if altered, would be candidates for transforming genes.

To date, the animal model system best characterized for the development of melanoma at the molecular level is the hybrid between the Mexican platyfish (*Platypoecilus maculatus*) and the swordtail (*Xiphophorus hellerii*). The hybrid fish develop benign and malignant melanocytic lesions. A "genetic imbalance" in the black spotted members of the hybrid fish is associated with an atypical growth of melanocytes (Schwab, 1987; Wittbrodt *et al*, 1989; Anders, 1991). Selective breeding of the fish abolished a tumor suppressor gene, thus deregulating an oncogene and inducing melanoma (Schwab, 1987; Wittbrodt *et al*, 1989). The putative oncogene (Xmrk) was cloned and shown to be homologous to human epidermal growth factor receptor (Schwab, 1987; Wittbrodt *et al*, 1989).

Currently, very few experimental animal models exist for melanoma

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Abbreviations: cdk4, cyclin-dependent-kinase 4; MTS1, Multiple Tumor Suppressor 1.

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formation and most murine models require a combination of carcinogen and ultraviolet radiation treatments (Bickers and Low, 1989; Romerdahl *et al*, 1989; Yuspa and Dlugosz, 1991). The majority of neoplasias resulting from these treatments are papillomas, squamous cell carcinomas, and fibrosarcomas; the development of melanoma is very infrequent (Bickers and Low, 1989; Romerdahl *et al*, 1989; Yuspa and Dlugosz, 1991). There have been several transgenic mouse models for development of melanocytic tumors (Bradl *et al*, 1991; Iwamoto *et al*, 1991; Larue *et al*, 1992; Taniguchi *et al*, 1992; Mintz *et al*, 1993). One transgenic mouse model for melanoma carried the early region of SV40 regulated by the tyrosinase promoter (Bradl *et al*, 1991; Larue *et al*, 1992; Mintz *et al*, 1993). Another transgenic mouse model carried the *ret* oncogene under the regulation of metallothionein promoter–enhancer (Iwamoto *et al*, 1991; Taniguchi *et al*, 1992). Ocular tumors developed in both types of transgenics (Bradl *et al*, 1991; Iwamoto *et al*, 1991; Larue *et al*, 1992; Taniguchi *et al*, 1992; Mintz *et al*, 1993) and melanocytic tumors arose frequently in the dermis of *ret*-transgenics (Iwamoto *et al*, 1991; Taniguchi *et al*, 1992). In both studies the transgenic mice were constructed with a known oncogene.

We have established five independent transgenic mouse lines with the insertion of a piece of genomic DNA, Clone B (Teicher *et al*, 1993). Transfection of Clone B DNA into several cells results in the differentiation of the cells to adipocytes (Teicher *et al*, 1993). Of these five independent transgenic lines, one spontaneously develops heritable melanoma. We hypothesize that the site of the host gene where the transgene is inserted must play an important role in the development of the tumor. The interruption of host sequences by the inserted transgene may have activated an "off" oncogene to the "on" state, thus modulating its expression and resulting in the transformation of cells. Alternatively, our transgene may have interrupted a tumor suppressor gene, leading to unregulated expansion of targeted cells. A third possibility is that specific interaction(s) occur between our transgene and the interrupted host sequences; such interaction(s) resulted in tumor development.

Earlier we reported a preliminary description of the development of melanotic lesions in one of the lines of the transgenic mice described above (Chen *et al*, 1996). This paper describes in detail the distribution, histology, and clinical progression of the melanotic lesions in these transgenic mice. These lesions resemble human cuboid (P. Durray, National Cancer Institute, Bethesda, MD, personal communication) cell melanoma and are detected in skin, eyes, lymph nodes, lung, inner ear, brain, and muscle.

MATERIALS AND METHODS

Generation of transgenic mouse lines The founder transgenic mice were generated by insertion of Clone B DNA in C57BL/6 J × SJL F1 hybrid mice (DNX, Princeton, NJ). Initially, the transgenic lines were maintained by back crossing to C57BL/6 J. After six generations, the transgenic mice were maintained by brother–sister mating. All animals were maintained in a germ-free environment and provided food and water *ad libitum*. Transgenic mice were identified by Southern hybridization to Clone B DNA with genomic DNA prepared from tail biopsies (Chen *et al*, 1996).

Histologic analysis Animals were inspected for tumor development twice weekly. Mice were sacrificed with CO_2 according to the Rutgers University policy on animal welfare. Complete autopsies were performed on all animals, and tissue samples were fixed in 10% neutral buffered formalin. Skull, including eye and bone, were decalcified for 3 d in phosphate-buffered saline with 10% formaldehyde and 5% formic acid. Standard procedures were followed for paraffin embedding, sectioning and hematoxylin and eosin staining as described (Luna, 1992).

For bleaching of the melanin in the tissues, 6 μm sections were deparaffinized and hydrated with distilled water. Samples were then bleached overnight in a solution containing 20 ml benzyl alcohol, 10 ml acetone, 5 ml 10% hydrogen peroxide, and four drops of 25% ammonia solution at 37°C. The samples were subsequently immersed in acetone:distilled water (1:1) for 5 min, rinsed in distilled water for 5 min, treated in 20% $N_2S_2O_3$ for 30 min, rinsed again in distilled water for 5 min, and dried at 37°C for 30 min. The bleached slides were then stained with hematoxylin and eosin.

Electron microscopy Tumor samples for electron microscopy were trimmed into 1 mm^3 cubes and fixed in 2.5% phosphate-buffered glutaraldehyde for 12 h. Following postfixation in osmium tetroxide for 90 min, samples were dehydrated and embedded in Epon-Araldite by standard procedures (Hayat, 1989). Thin sections (60 nm) were prepared, stained, and examined on a Zeiss 10CA electron microscope.

Table I. Affected organs in transgenic mice

Organs examined for lesions	Number of animals affected/total (%)			
	Stage 1	Stage 2	Stage 3	Stage 4
Skin:pinnae of the ear	15/15 (100)	25/25 (100)	63/63 (100)	106/106 (100)
Skin:perianal region	13/15 (87)	25/25 (100)	63/63 (100)	106/106 (100)
Skin:other areas	4/15 (27)	22/25 (88)	63/63 (100)	106/106 (100)
Eye	5/15 (33)	25/25 (100)	63/63 (100)	106/106 (100)
Skull	0/15 (0)	2/25 (8)	26/63 (41)	78/106 (74)
Lymph node	9/15 (60)	24/25 (96)	63/63 (100)	106/106 (100)
Lung	0/15 (0)	1/25 (4)	12/63 (19)	65/106 (61)
Brain	0/15 (0)	0/25 (0)	7/63 (11)	14/106 (13)
Muscle	0/15 (0)	0/25 (0)	9/63 (14)	58/106 (55)
Spleen	0/15 (0)	1/25 (4)	19/63 (30)	79/106 (75)
Liver	0/15 (0)	0/25 (0)	17/63 (27)	92/106 (87)

RESULTS

Generation of transgenic lines In all five independent lines of transgenic founder mice (TG1–5) bearing insertion of the Clone B DNA, the transgene was found to be in head-to-head, head-to-tail, or tail-to-tail arrangements. There were 5–7 copies of the transgene inserted in each line. In one of the five transgenic lines, TG-3, the founder mother developed pigmented lesions at about 8 mo and was killed at 14 mo of age. Mice from the TG-3 transgenic line were backcrossed to C57BL/6 J for the first six generations, then maintained by brother–sister mating. This paper describes studies done with mice derived from the TG-3 line.

In this study a total of 519 mice (282 males and 237 females) were examined for their phenotypic appearance. Tissue samples from some animals were further analyzed by histopathologic means. There were 84 nontransgenic mice (52 males and 32 females) and the remainder of the animals were transgenic and were divided into four cohorts based on the age of the animals when the appearance of the external lesions was detected. The first cohort had 70 animals (33 males and 37 females). These mice developed externally visible pigmented lesions in perianal region, pinnae of the ear, eyes, snout, legs, tail, and other parts of the skin within 2–4 wk of birth. The lesions rapidly increased in size (to 0.5–1.5 cm) and most of the mice died between 2 and 5 mo of age. The second cohort had 257 animals (144 males and 113 females). These animals showed an anatomical distribution of lesions similar to that described in the first cohort, but exhibited a delay of the development of the lesions until 5–7 mo of age. Animals in this cohort died by 6–8 mo of age. The third cohort had 43 animals (15 males and 28 females). Animals in this cohort showed the pigmented lesions at about 8 mo of age, a time-course similar to the founder mother, and all died by 14 mo of age. The fourth cohort had 65 animals (38 males and 27 females). These animals did not manifest pigmented lesions at any point during their lifetime. The life span of these mice was similar to that of the nontransgenic mice.

Because the animals in the first cohort became sick and either died or were killed while relatively young, it was difficult to obtain progeny from them. Subsequently all animals are derived by crosses of the animals within the second and third cohorts. To assess the progression of the lesions, histologic examinations were carried out on mice randomly chosen from cohorts one, two, or three. A rating system of four stages was used for the histologic analysis, based on morphology, size of the tumor, and site of the lesions, with stage 1 being the least and stage 4 being the most extensively involved, as defined by the number of organs affected and the severity in the appearance of the lesions (**Table I**). A transgenic mouse manifesting typical stage 3 and stage 4 lesions is shown in **Fig 1**(***A***, ***B***, respectively).

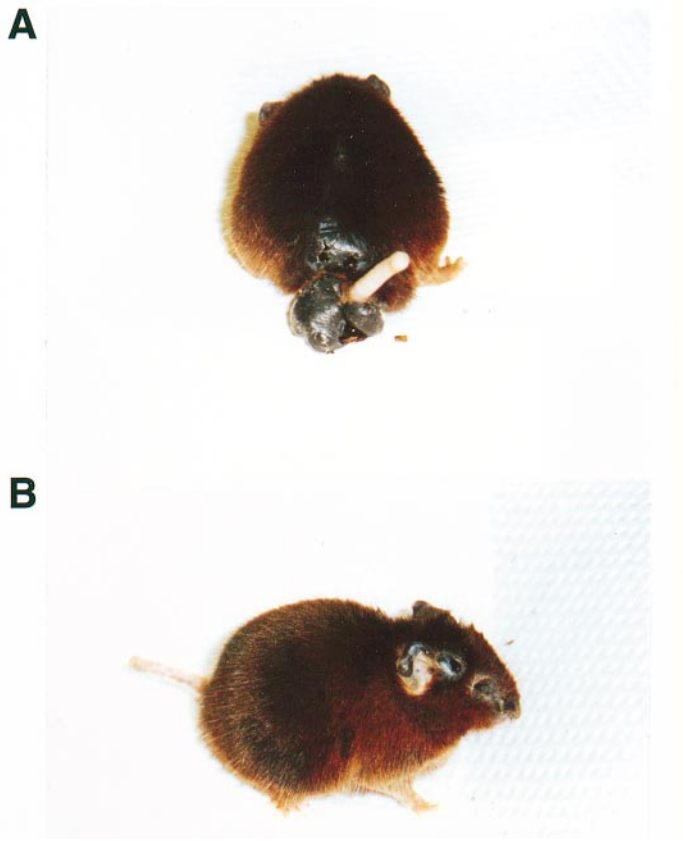

Figure 1. Transgenic mice. (*A*) Tumors on the perianal region of a 1-y-old transgenic mouse at stage 4. (*B*) Tumors on the ear, eye, and snout of an 8-mo old transgenic mouse at stage 3.

Gross morphologic evaluation of transgenic and control mice Control, nontransgenic mice (10 animals) were included in this evaluation and no lesions were detected in any organ. A total of 209 animals from the first, second, and third cohorts were analyzed histopathologically. Mice of similar ages from each cohort were analyzed and compared. Regardless of the cohort, the lesions were always first detected grossly on the skin of the ear and the perianal region, and then other parts of the body including the snout, the tail, the back, the eyes, and the legs. The results of necropsies of affected mice at different stages and the number of organs affected grossly are shown in **Table I**.

Gross pathology of transgenic mice *Stage 1* Animals began to show pigmented spots on the ear and perianal region, but the lesions did not appear as raised spots on the skin. The Harderian glands were darker and slightly larger than those of normal mice. The lymph nodes were black or enlarged in nine mice (60% of animals in stage 1). No other organs were found to be affected. A total of 15 mice were assigned to stage 1.

Stage 2 Raised pigmented lesions were seen on the ears and perianal region. The thickness of the lesions was about 1 mm on the ears and 1–3 mm at the perianal region. Additional lesions appear on the snout, eyelid, and back of the animals. The Harderian glands of the eye were about twice the normal size and were completely black. Exophthalmia was not apparent. Stage 2 mice generally presented initial lymph node involvement in the aortic, axillary, ilioinguinal, and cervical nodes (96% of affected animals in stage 2). A small number of animals showed pigmented lesions in skull, lung, and spleen. A total of 25 mice were assigned to stage 2.

Stage 3 The thickness of the pigmented lesions on the ears was 1–3 mm and 3–8 mm at the perianal region. The lesions on the snout and eyelid were raised and enlarged. Additional pigmented spots were detected on other parts of the skin. Eyes were exophthalmic due to the progressive enlargement of the Harderian glands and increased thickness of the choroid. These affected mice were not as active as control nontransgenic mice of the same age. Virtually all lymph nodes were affected. Of 63 animals assigned to stage 3, 19% had lesions within the lung, 41% had lesions of the skull, 11% had lesions of the brain, 14% had lesions of muscle, and 30% had lesions of spleen; in 27% of animals, livers were markedly discolored, appearing gray-black.

Stage 4 The size and thickness of the pigmented lesions on the ears, the eyelid, the snout, and the perianal region were even larger and thicker than those in stage 3. Ulceration was seen in some of the lesions. The lesions on other parts of the skin continued to increase in number and many of them were raised. Bilateral exophthalmia was more prominent. Tumor involvement of the Harderian glands, if allowed to progress, would likely have occupied the entire orbital fossa. When mice reached this stage, they usually died within 2 wk or were killed for humane reasons. There were 106 mice assigned to this cohort.

Histopathology *Skin* All affected transgenic mice had pigmented lesions on their skin. The number and the size of the lesions increased as the condition of the animal worsened. Histologically, multifocal pigmented tumor nests or nodules were detected in the dermis of skin in all affected mice. Small tumor nests were surrounded by compressed connective tissue. The tumors contained a mixture of melanin-containing, spindle-shaped, or oval-shaped cells grouped in irregular bundles with interspersed fibroblasts and connective tissue (**Fig 2*A***). Large tumors showed invasion of adjacent adipose tissue and skeletal muscle. The cells in large tumors were mainly round and extraordinarily heavily pigmented. The melanin pigment frequently obscured the nuclear profile and other cytoplasmic features, which became discernible only after bleaching (**Fig 2*B***). Epidermis and hair follicles were intact except in regions of ulceration. Infiltration of inflammatory cells into the tumors was uncommon.

Lymph node In some of the animals in stage 1, the lymph nodes appeared to be normal during gross dissection; however, by light microscopy pigmented tumor cells were seen scattered throughout the lymph nodes. Generally the cervical, thoracic, abdominal, and inguinal nodes were pigmented and enlarged. Lymph node involvement progressed with time (**Fig 2*C***), increasing from 2 mm in diameter in stage 1 to 4–10 mm in diameter in stage 4.

Muscle Single pigmented tumor cells were observed infiltrating between skeletal muscle fibers in mice of stage 2. Mice in stages 3 and 4 possessed pigmented tumor nests or nodules in the interstitial space between skeletal muscle fibers, but did not appear to invade the individual fibers (**Fig 2*D***).

Spleen No tumor cells were found in the spleen either grossly or microscopically when the mice were in stage 1. With the mice in stage 2, the spleens looked normal grossly but light microscopy revealed many round, heavily pigmented cells scattered within the parenchyma. With the mice in stages 3 and 4, the color of the spleen was frequently gray or black and significantly enlarged. In these spleens, the tumor cells formed nests or nodules.

Liver In the livers of stage 2 animals, melanin pigmentation was observed in Kupffer cells, but not hepatocytes. In stage 3 and stage 4 mice, some of the livers were gray or black. Microscopically, an increase of pigmented Kupffer cells was seen in the liver sinusoids. The changes in the color of the livers appeared to reflect the phagocytosis of melanosomes or melanotic debris originally present in other cells.

Lung Small groups of pigmented cells in the lung were first detected in stage 2 animals. With animals in stages 3 and 4, the lesions in the lung appeared as multifocal pigmented nodules. These nodules, measuring ≈1 mm in diameter, were frequently bilateral and located peripheral in the lung parenchyma. These nodules compressed the adjacent pulminary parenchyma (**Fig 3*A***), and were comprised of large, oval tumor cells.

Brain Heavily pigmented tumor masses were first detected in the brain of the mice in stage 3. Grossly, the pigmented lesions appeared to be located only in the midline brain structures, particularly the choroid

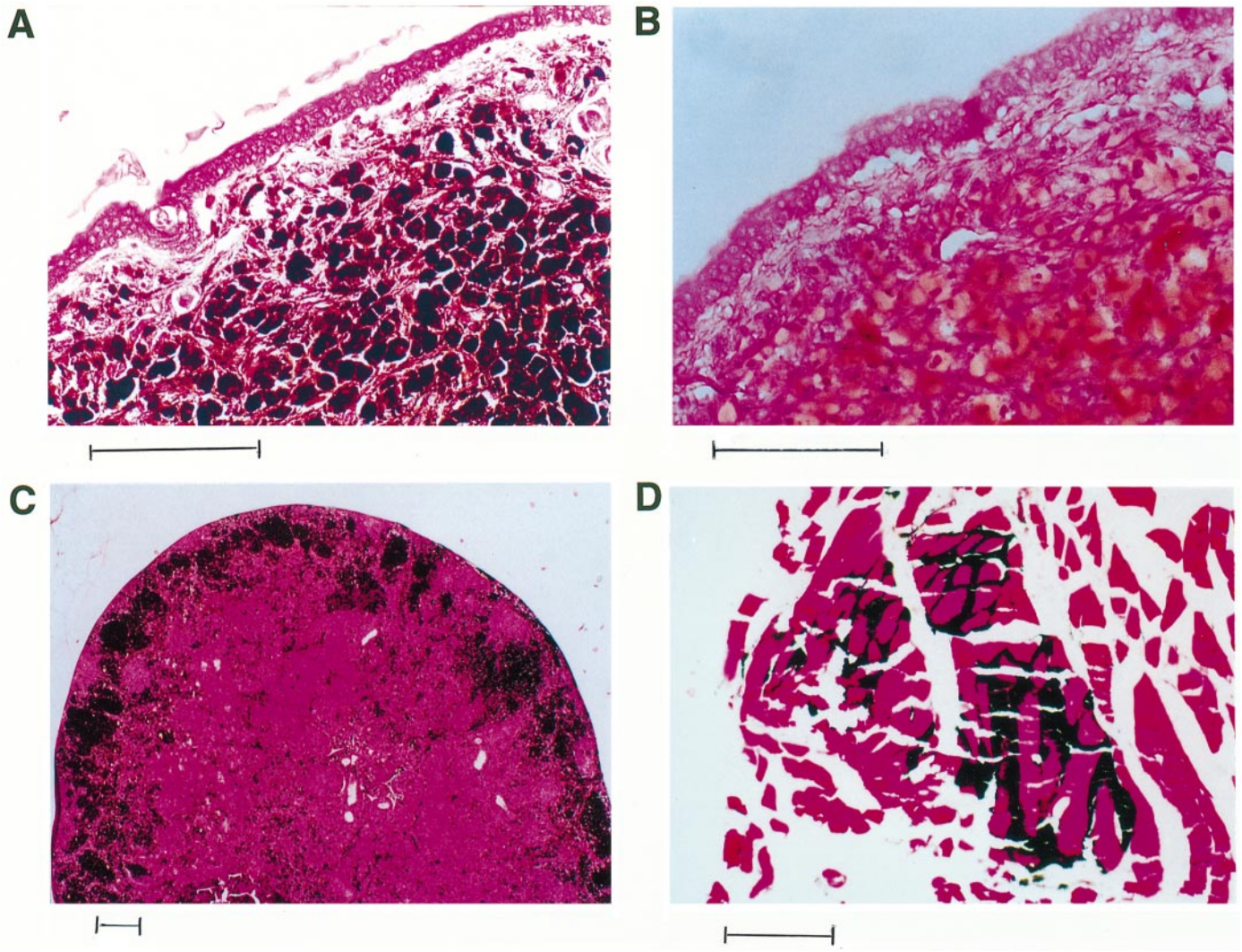

Figure 2. Histopathologic evaluation of transgenic mice. (*A*) Light micrograph of 5 μm sections of tumor from the perianal region from a 6 mo old mouse at stage 3. Accumulation of large, densely pigmented cells was observed within the dermis but sparing the epidermis. (*B*) Treatment of specimen from (*A*) following chemical bleaching of melanin. Nuclear detail is now visible, revealing large oval nuclei with prominent nucleoli. Mitotic figures were infrequent. (*C*) Cervical lymph node of a 5 mo old stage 3 mouse. Densely pigmented tumor cells formed nests, most evident at the periphery of the node. (*D*) Skeletal muscle from a stage 2 mouse at 7 mo of age. Infiltration of tumor cells between the muscle fibers is apparent. *Scale bar,* 100 μm (hematoxylin and eosin).

plexus. These lesions were composed of cells morphologically similar to those in the skin, eye, and lung (**Fig 3*B***).

Eye The majority of the mice showed pigmented ocular lesions. Upon dissection, thickening of the choroid and enlargement and darkening of the Harderian glands were observed bilaterally. As the condition of the animals progressed, the choroid and the Harderian glands became larger. Microscopically, the choroid of the eye and the interstitial space of the Harderian glands were extensively involved by tumor infiltrate (**Fig 3*C***, **D**). For the animals in stages 3 and 4, the tumors in the Harderian glands compressed the glandular tissues. Pigmented cells appeared to invade the optical tissues and optic nerve in these animals. The cells of the retinal pigmented epithelium were intact in all animals.

Inner ear Visible pigmented lesions at the base of the skull were first detected in occasional stage 2 animals. Microscopically, an increased number of round pigmented cells were present on the vestibular membrane of the inner ears of stage 1 and stage 2 animals. These pigmented cells appeared to involve the marrow cavity of the cranial bone near the inner ear in stage 3 and stage 4 mice. These pigmented lesions presumably originated from the pigmented cells of the stria vascularis.

Other tissues There was no evidence of involvement of the salivary gland, thymus, thyroid, testis/ovary, kidney, adrenal gland, gastrointestinal tract, or cardiac muscle in any of the mice examined. Blood smears of all the animals examined appeared to be unremarkable.

Electron microscopy Electron microscopy of pigmented lesions from skin, muscle, lymph node, and Harderian gland showed similar findings (**Fig 4**). In all lesions, tumor cells became engorged with large numbers of dense, membrane-bound melanotic bodies that in stage 3 and stage 4 animals obscured or displaced other organelles. The melanosomes were 0.2–0.8 μM in greatest aspect and were usually uniformly dense. Occasionally, immature melanosomes containing a folded or filamentous interior were identified. In liver, melanosomes were most common within Kupffer cells. In these macrophages, the melanosomes were usually present within secondary lysosomes, suggesting that they were phagocytosed debris from other cells.

DISCUSSION

We have developed a line of transgenic mice (TG-3) displaying a strong genetic predisposition to develop melanoma, whereas the nontransgenic littermates remain disease free. Results from these studies strongly suggest cosegregation of the susceptibility to melanoma development and the presence of the transgene, Clone B. In young TG-3 animals, the pigmented lesions were first detected on the skin, where they appeared as multifocal lesions resembling dysplastic melanocytic nevi. These lesions were progressive, increasing in size and invading nearby tissues, and histologically resembled human cuboidal cell melanoma (P. Durray, personal communication).

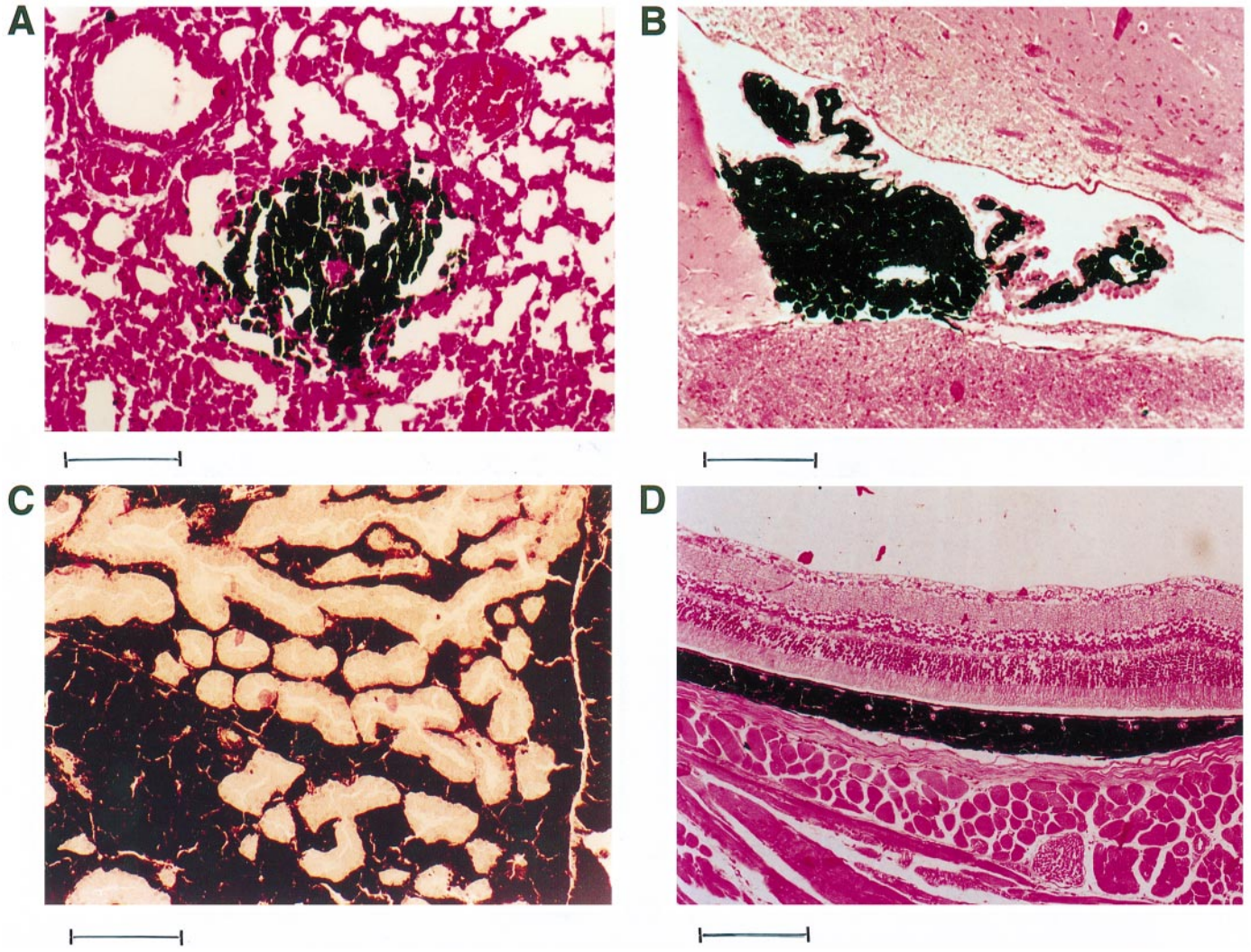

Figure 3. Histopathologic evaluation of transgenic mice. (*A*) A metastatic pigmented tumor in the lung of a 4 mo old mouse at stage 3. (*B*) Heavily pigmented cellular aggregation in the interstitial space of the choroid plexus of the brain from a 6 mo old mouse at stage 3. The overlying choroid epithelium is not involved. (*C*) and (*D*) Ocular lesions in a 7 mo old mouse at stage 1. (*C*) Pigmented cells have proliferated in the interstitial space of the Harderian gland. (*D*) Hypercellularity of heavily pigmented round cells in the choroid layer of the eye. Retinal epithelium remained intact. *Scale bars*, 100 μm (hematoxylin and eosin).

With additional time more organs became involved, either as sites of metastasis and/or as additional sites of primary tumor development.

In the mouse the skin melanocytes are located primarily in the dermis except for the melanocytes of the hair follicles. The pigmented lesions in the skin of TG-3 transgenic mice originated within the dermis. In humans, the skin melanocytes reside mainly in the basal layer of the epidermis. In human cutaneous melanoma, the tumors always originate in the basal layer of epidermis. Histologic analysis of early pigmented lesions detected in the skin, the Harderian gland, and the choroid of TG-3 mice suggested they were of neural crest-derived melanocytes. All skin lesions appear to derive from melanocytes in the dermis and none from melanocytes of the hair follicles. Perhaps the microenvironment surrounding the hair follicle melanocytes precluded them from becoming tumors. We never detected lesions in retinal pigment epithelium that is derived from local neuroectoderm.

The transgenic mice in this study were divided into four cohorts based on the age of the animals when the external pigmented lesions were first detected. A direct correlation was found between the life span of the animals and the age at the appearance of the first lesions. Mice in the first cohort showing the external lesions very early had a very short life span, whereas those in the fourth cohort never developed lesions and had life spans similar to those of control nontransgenics. By Southern blots using our transgene as a probe, no differences were detected among the four cohorts. Presumably the genetic element that is critical in the development of the pigmented lesions is inactivated or lost in cohort four, and we do not yet have the appropriate probe to identify this element.

The histologic rating of the affected mice was based on the size of the tumors and the number of organs involved. Tissue samples from mice in the first cohort were always assigned to stage 3 or 4, because of the severity of their lesions at a young age. Lesions from younger animals in the second or third cohort were assigned to stage 1 or 2. With time, mice in these two cohorts developed more lesions, and more organs were affected. Histologically the tissue samples were then assigned to stage 3 or 4. At the level of Southern blot analysis no differences among animals with mild or severe lesions were detected with respect to the integrated transgene. Identification and characterization of the disrupted host sequences are needed to understand the complex mechanisms underlying the development of these lesions.

This paper describes the development of heritable melanomas in transgenic mice made with a DNA fragment that is not a known oncogene. Our results suggest that the site of integration of the transgene plays a vital role in the development of the tumors. The availability of these transgenic animals affords an excellent model system with which to investigate the host gene(s) that are involved in the development of melanoma.

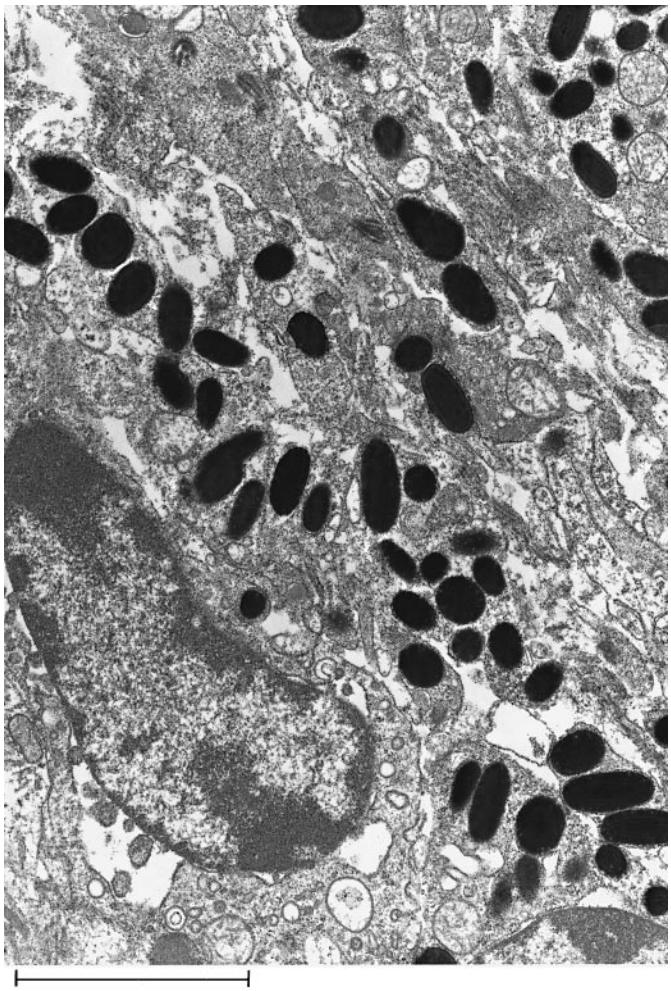

Figure 4. Electron micrograph from stage 2 lesion. Multiple membrane-bound melanosomes and premelanosomes are present within the cytoplasm. With progression of the lesion, neoplastic cells became filled with melanosomes, which displace and obscure other organelles. *Scale bar,* 5 μm.

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