Development of a New Melanoma Model in C57BL/6 Mice

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Abstract

In the present study, we induced melanomas in C57BL/6 mice by a single application of 7,12-dimethylbenz(a)anthracene to the scapular region of 4-day-old mice, followed by twice-weekly applications of croton oil. Of 20 mice treated, melanomas arose in two female littermates. The first melanoma (JB/MS) arose 16 weeks after initiation of treatment, and the second melanoma (JB/RH) arose 23 weeks later. The melanomas maintained their melanotic appearance after s.c. transplantation to normal C57BL/6 mice and metastasized spontaneously in the transplant recipients. To our knowledge, these are the first melanomas to have been induced in C57BL/6 mice since the B16 melanoma arose spontaneously in 1954. We feel that the JB/MS and JB/RH melanomas provide an excellent comparative system for studies done with the B16 melanoma. These melanomas of recent origin will also facilitate the investigation of biological, immunological, and biochemical parameters that influence the growth and metastasis of malignant melanomas.

Introduction

The spontaneous development of melanomas in mice is rare. Three murine melanomas, the Cloudman (12, 15), Harding-Passey (3, 14), and B16 (4, 5, 7, 13) melanomas have been used routinely for experimental studies. These melanomas have been propagated in vivo and in vitro for many years and may no longer resemble the original melanomas. Recently, Kripke (11) induced the K-1735 melanoma in a C3H mouse with UV radiation and croton oil. Fidler et al. (6) and Fidler and Kripke (8) have utilized the K-1735 melanoma to confirm many of the tumor heterogeneity studies performed previously with the B16 melanoma. The purpose of the present study was to determine if melanomas could be induced in C57BL/6 mice with DMBA and croton oil. Melanomas arose in 2 female littermates 16 and 39 weeks after initiation of treatment. The melanomas metastasized spontaneously when transplanted to normal C57BL/6 mice. We feel that these melanomas will be extremely useful as experimental models of malignant melanoma.

Materials and Methods

Mice. C57BL/6 mice were bred at the Sinclair Comparative Medicine Research Farm from a stock obtained originally from The Jackson Laboratory, Bar Harbor, Maine.

Induction of Melanomas. Melanomas were induced by application of 50 µl of 0.4% DMBA (Sigma Chemical Co., St. Louis, Mo.) (2) in acetone to the dorsal region of 4-day-old mice. This was followed 2 weeks later by twice-weekly applications of 25 µl of 2.5% croton oil (Sigma) in acetone (11) or dimethyl sulfoxide until the appearance of raised black lesions. The growth rate of the resulting tumors was determined by weekly measurements in 3 dimensions. Tumor volume was calculated as length x width x height.

Processing of Melanomas. The processing of the melanomas was done under sterile conditions. After the skin and surrounding membrane were removed, the tumor was transferred to a sterile Petri dish. A portion of the tumor was removed with a sterile scalpel blade and fixed in 10% buffered formalin for histopathological examination, while the remaining tumor was minced with curved scissors. Portions of the minced tumors were then cultured, transplanted, or cryopreserved in liquid nitrogen.

Establishment of Cell Cultures. Tumor explants were placed in a 25- or 75-cm² tissue culture flask (Falcon Plastics, Oxnard, Calif.) containing a small amount of medium. The flask was then incubated in a humidified, 5% CO₂ atmosphere to allow optimal attachment of the explants to the bottom of the flask. Additional medium was added after 24 hr, and the explants were fed twice weekly thereafter with fresh medium until subcultivation became necessary.

The medium used for all cell culture procedures was Eagle's minimum essential medium (Auto-Pow minimum essential medium; Flow Laboratories, Inc., McLean, Va.) supplemented with sodium bicarbonate, 4 mM L-glutamine, 1% vitamins, 1% sodium pyruvate, 1% nonessential amino acids, 100 IU penicillin, and 100 µg streptomycin per ml, 25 µg fungizone per ml, 30 µM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer (Flow Laboratories), 50 µg gentamycin per ml (M. A. Bioproducts, Walkersville, Md.), and 8% fetal calf serum (KC Biological, Inc., Lenexa, Kans.).

For subcultivation of cell cultures, cell monolayers were overlayed with 2 ml of 0.05% trypsin and 0.02% EDTA (Flow Laboratories). The flasks were agitated briefly to facilitate cell detachment, and the removal of attached cells was monitored under an inverted microscope (Leitz Diavert). After complete detachment of the cell monolayer, the trypsin-EDTA mixture was inactivated with complete Eagle's minimum essential medium, and the suspension was plated at appropriate dilutions in new flasks.

Results

Growth of the JB/MS and JB/RH Melanomas in the Primary Hosts. Of 20 C57BL/6 mice treated with DMBA and croton oil, melanomas arose on the scapular regions of 2 female littermates. The first melanoma (JB/MS) arose 16 weeks after initiation of treatment, and the second melanoma (JB/RH) appeared 39 weeks after initiation of treatment. The JB/MS melanoma grew progressively for 10 weeks (Chart 1A) at which time the mouse was sacrificed. Upon necropsy, 3 large melanotic masses were found in the cervical region. Histopathological analysis revealed the presence of metastatic malignant melanoma in grossly black lymph node masses. The tumor cells grew as cohesive fascicles of spindle-shaped cells supported by nests of macrophages filled with coarsely granular melanin pigment (Fig. 1).
The mouse bearing the JB/RH melanoma died 1 day after the appearance of a measurable tumor on the scapular region. Histopathological analysis of the primary tumor revealed a deeply invasive melanoma (Fig. 2). The tumor was heavily pigmented (Fig. 2a) and composed of a fairly cohesive growth of tumor cells interspersed with pigment-laden macrophages (Fig. 2b) similar to that seen in the JB/MS melanoma. Metastases were not found in the primary host.

Transplantation Characteristics. Transplantation of the primary JB/MS melanoma into the scapular s.c. tissue of normal C57BL/6 mice produced melanomas with an average latent period of 2.5 to 3 months (Chart 1B). Two subsequent in vivo passages of the MS melanoma in C57BL/6 mice reduced the tumor latent period to 3 weeks. The melanomas that arose in transplant recipients invaded into the underlying skeletal muscle (Fig. 3). The transplanted tumors grew as cohesive sheets of spindle-shaped cells and contained variable amounts of melanin pigment (Fig. 4). Tumor-bearing mice that were allowed to die of natural causes exhibited extensive melanotic metastases in the draining cervical and axillary lymph nodes.

Since the mouse bearing the JB/RH melanoma died when the tumor was still quite small (25 cu mm), the melanoma was injected into just 2 female C57BL/6 recipients. A melanotic tumor arose in one of the 2 mice 23 weeks after transplantation. The tumor grew progressively for 7 weeks (Chart 1C), at which time the mouse died. Upon necropsy, numerous melanotic metastases were found in the lung (Fig. 5).

In Vitro Morphology. Cell cultures were established from the JB/MS and JB/RH melanomas after the first in vivo passage. The cells cultured from the 2 melanomas exhibited strikingly different morphologies in vitro (Fig. 6). The JB/MS cells were relatively large, flat, and spindle shaped with dendritic processes (Fig. 6a). In contrast, cells from the JB/RH melanoma were small and round with long thin processes (Fig. 6b).

DISCUSSION

The results of the present study demonstrate that malignant melanomas could be induced in C57BL/6 mice with a single application of DMBA applied dorsally to 4-day-old mice, followed by twice-weekly application of croton oil. To our knowledge, these are the first transplantable melanomas to have been induced in C57BL/6 mice since the B16 melanoma arose spontaneously in 1954 (6).

Epstein et al. (2) obtained melanomas in random-bred pigmented hairless mice by inducing "blue newi" with a single application of DMBA, followed 13 months later by exposure to UV radiation. These melanomas, although invasive and metastatic to the regional lymph nodes in the primary hosts, were not transplantable to other hairless mice or to the hamster cheek pouch.

Goerttler et al. (9) administered DMBA and 12-O-tetradecanoylphorbol-13-acetate to adult C57BL/6 mice and obtained a variety of tumors, including papillomas, lymphomas, and carcinomas but no melanomas. Hennings et al. (10) also obtained papillomas and carcinomas after administration of DMBA and 12-O-tetradecanoylphorbol-13-acetate to newborn and adult SENCAR mice. BALB/C mice were shown to be much less susceptible to these 2 agents.

Kripke (11) induced the K-1735 melanoma in a C3H/HeN (lacking mammary tumor virus) mouse by short-term exposure to UV, followed by twice-weekly applications of croton oil to the scapular region. In Week 92 of the experiment, a melanoma arose on one mouse. Upon necropsy, pigmented bilateral metastases were found in the superficial draining lymph nodes (8, 11).

In contrast to the reports of Epstein et al. (2) and Kripke (11), our melanomas arose in the absence of UV. In addition, the JB/MS melanoma arose just 16 weeks after initiation of treatment, and the JB/RH melanoma arose 23 weeks later. Thus, the latent periods of both melanomas were substantially shorter than the 92 weeks required for induction of the K-1735 melanoma.

We feel that the JB/MS and JB/RH melanomas have several features that make them particularly useful for studies of malignant melanoma. (a) The melanomas are syngeneic to C57BL/6 mice and therefore provide a comparative melanoma system for extensive studies done previously with the B16 melanoma. (b) The melanomas were induced in the absence of UV. This raises important questions about the role of UV in the induction of melanomas (1). To answer some of these questions, UV can now be superimposed upon this system to determine if exposure of mice to UV radiation will increase the growth or metastasis of the MS and RH melanomas.

And (c) s.c. transplantation of the JB/MS and JB/RH melanomas produced spontaneous metastases in the transplant recipients. Thus, this melanoma system affords the opportunity to investigate host and tumor cell factors that influence the extent and distribution of spontaneous metastases.

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![Chart 1. A, growth of JB/MS melanoma in the primary C57BL/6 host after treatment with DMBA and croton oil; B, growth of the JB/MS melanoma after s.c. transplantation of tumor pieces to a C57BL/6 mouse; C, growth of the JB/RH melanoma after s.c. transplantation of tumor pieces to a C57BL/6 mouse.](chart1.png)
REFERENCES


Fig. 1. Metastasis of JB/MS melanoma to cervical lymph node of original C57BL/6 host. The bleached lymph node section is almost totally effaced by metastatic melanoma. Arrow, small numbers of residual darkly staining lymphocytes. × 25.
Fig. 2. a, section of the JB/RH melanoma in the original C57BL/6 host illustrating deep dermal growth of the heavily melanized tumor. H & E, × 25. b, morphological similarities to the JB/MS melanoma. Melanin bleach, × 50.
Fig. 3. JB/MS melanoma after transplantation to a C57BL/6 mouse. a, heavily melanized melanoma cells invading adjacent skeletal muscle, × 25. b, higher magnification of a section showing spindle tumor cells cut in cross-section separated by nest of pigment-laden macrophages (PLM) with eccentric small darkly staining nuclei. Melanin bleach, × 50.
Fig. 4. a, section of a JB/MS melanoma transplant illustrating a relatively amelanotic area and highlighting the interdigitating fascicles of spindle tumor cells. Scattered melanin-containing tumor cells are apparent. H & E, x 50. b, higher magnification illustrating the varying amounts of melanin within the tumor cells. Arrows, the melanin outlining the dendritic cytoplasmic extension of the tumor cells x 100.
Fig. 5. Melanotic nodules in the lung of a C57BL/6 mouse after s.c. transplantation of the JB/RH melanoma.

Fig. 6. a, *in vitro* morphology of the JB/MS melanoma illustrating spindle cells with dendritic processes. b, *in vitro* morphology of the JB/RH melanoma illustrating small rounded cells with long thin processes. × 160.
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