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. transplanation 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 parameters that influence the growth and metastasis of malignant melanomas.

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 finely 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-sq cm 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.

For subcultivation of cell cultures, cell monolayers were overlaid with 2 ml of 0.05% trypsin and 0.02% EDTA (Flow Laboratories) 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 minimal 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% non-skeletal amino acids, 100 IU penicillin, and 100 μg streptomycin per ml, 25 μg fungizone per ml, 30 μg 4-(2-hydroxyethyl)-1-piperazinethanesulfonic 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 overlaid 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 minimal 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).

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AUGUST 1982 3157
The tumor grew progressively for 7 weeks (Chart 1C), at which time the mouse died. Upon necropsy, numerous melanotic 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 melanomas. (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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REFERENCES


Induction of Melanomas in C57BL/6 Mice

Fig. 1. Metastasis of J.B/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.
Induction of Melanomas in C57BL/6 Mice

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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