Skin Melanoma Induced by 7,12-Dimethylbenzanthracene in Albino Guinea Pigs and Its Similarities to Skin Melanoma of Humans

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ABSTRACT

A model for a metastasizing melanoma was developed, and its characteristics were established. Sixty-five albino guinea pigs were painted with 7,12-dimethylbenzanthracene in acetone. There was evidence that, after 18 months, 40% of the animals developed melanomas. Melanomas arose by a malignant transformation of junctional nevus cells and/or by transformation of amelanotic melanocytes. Metastases to the skin and internal organs were multiple and eventually fatal for the animals. Histology and electron microscopy of induced melanomas were reviewed in detail. Clinical and histological events leading to development of melanoma in albino guinea pigs were found to be similar to human melanomas in a number of aspects. Fragments of melanomas were successfully transplanted to "nude" mice and healthy albino guinea pigs. The described model could be used for study of the various cellular and tissue events which precede nevus, lentigo maligna, and melanoma formation. It could also be useful in studying carcinogenic potential, for studying development of metastases, and presumably for trials of treatment.

INTRODUCTION

Cutaneous melanomas have been already observed in laboratory animals. Some of them developed from carcinogen-induced blue nevi (8, 14, 21, 22); others developed from the intraepidermal melanocytic hyperplasia (4, 5). However, intrallesional transformation to melanoma in a latter group did not affect more than 4 to 5% of the animals (4). Spontaneously appearing junctional nevi and melanomas in Sinclair swine had a tendency toward spontaneous regression, and metastases were uncommon (15). Development of melanomas in Sinclair swines was not fully predictable, and the householding was expensive and difficult. There is also a trend to reproduce the original tumor morphology in animals after heterotransplantation of human tumor (13).

This paper presents results which demonstrate many resemblances between the albino guinea pig melanoma model and human melanoma.

MATERIALS AND METHODS

Seventy-seven 2-month-old male Hartley albino guinea pigs (Bio-Breeding Laboratories of Canada, Ltd., Ottawa, Ontario, Canada) were used. Five animals were kept per cage. "Nude" mice were obtained from the University of Guelph, where they were bred on a genetic background of the strain C57BL. Mice were kept in individual sterile plastic cages with metal wire lids. The lids were covered with nonwoven spun polyester filter that allows passage of air but not of bacteria and particulates. The bottoms of the cages were covered with usual wood granulate. Seven nude mice, 8 to 12 weeks old, were used in the experiment. All animals were fed commercially prepared spital pellets and water ad libitum. The room (5.74 x 3.35 x 2.87 m) was windowless and was lit from the top by 16 F30 30-watt white lamps. The guinea pigs were divided into 2 groups. One group of 25 guinea pigs was used for studying the effects of varying concentrations of DMBA. These were divided into 5 subgroups of 5 guinea pigs each. The following concentrations of DMBA were used: 0.1%, 0.25%, 0.5%, 1.0%, and 2.0%. The remaining 40 guinea pigs were painted with 1.0% DMBA.

Sixty-five guinea pigs were treated with DMBA in acetone. An area 5 cm in diameter on both dorsal flanks was shaved, and 0.3 ml of the carcinogen solution was applied twice a week to each field. Serial color photographs of the painted areas were taken throughout the experiment.

The guinea pigs were kept for 18 months, during which shaved areas were painted. After this time, animals were sacrificed. Samples of skin from the pigmented spots and all nodules were taken for light and electron microscopy. Other details have been described elsewhere (20).

Twelve additional guinea pigs, as well as 7 nude mice, served as hosts for transplants for fragments of induced nodules. Implantation s.c. of 2-cm^3 blocks of tissue in the area of the neck (guinea pigs) or dorsal flank (nude mice) (19) were done. The animals transplanted with tumor were kept for 3 months.

RESULTS

The results of the preliminary experiment with varying concentrations of DMBA showed that all the concentrations of DMBA used produced nevi in a large number of guinea pigs. There was no statistically significant difference in the number of nevi in the guinea pigs in the various groups. The number of animals in each group was not sufficient to determine whether there was any difference in the number of melanomas in the guinea pigs of each group. In view of these findings, 1.0% DMBA was used for the major experiment described in this paper.

Initially, there were inflammation and desquamation that subsequently subsided, leaving the hair thin and scanty. The initial brown and black macules were observed 196 days after the first painting. Small and large nodules and finally ulcerated lesions appeared subsequently. The initial clinically recognized melanoma appeared 214 days after the first application of DMBA (Figs. 1 and 2).

1 The abbreviations used are: DMBA, 7,12-dimethylbenzanthracene; LM, lentigo maligna (melanosis of Dubreuilh); DOPA, 3,4-L-dihydroxyphenylalanine.

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Twenty-six guinea pigs died or appeared so ill that they were killed before the end of the experiment. The cause of death was pneumonia in 21 cases or spread of the tumor with progressive cachexia in 5 cases. Of the 10 animals dying in under 400 days, only one had melanoma.

By the time of the end of the experiment, 52 of 65 guinea pigs had 6853 dark lesions that were macroscopically recognized as nevi. They were classified accordingly to the color (black, brown, or light brown), diameter, and elevation (flat or raised). Forty-eight of these lesions were black, 818 were dark brown, and the rest were light brown. Fifty-two lesions had a diameter above 0.5 cm, and 101 lesions were raised. All black, raised, and large (above 0.5 cm in diameter) lesions, as well as a random selection of other lesions as described above, were histologically investigated.

Histology done on 277 preselected nevi showed 52 junction-type, 80 compound, and 100 mainly dermal nevi (11 were blue nevi). Sixteen lesions showed changes resembling LM, 8 were angiomas, and 10 were melanomas. Histology of the nevi showed structures similar or even identical to human nevi (20). Typical LM lesions showed proliferation of melanocytes at the epidermodermal junction. Individual cells exhibited a "honeycombed" appearance due to the vacuolation of cytoplasm and shrinkage of the nuclei. There was no nesting of the cells. DOPA reaction was positive.

Twenty-six of 65 guinea pigs (40%) developed histologically confirmed invasive melanoma. The smallest number of paintings required to induce melanoma was 76 with 1% DMBA. All together, 54 invasive skin melanomas were found in the 26 guinea pigs. Thirty-eight of them were classified as primaries. Of the 38 primaries, 27 (71%) arose in areas of black macules, and 3 were amelanotic and started as an amelanotic tumor. Features differentiating primary skin melanoma from metastases are presented in Table 1.

Metastases to lymph nodes and internal organs were amelanotic, usually multiple, in the form of well-circumscribed nodules in lungs (Fig. 3), kidneys (Fig. 4), and adrenals or of infiltrated and necrotic areas in lymph nodes, spleen, and liver.

Light Microscopy. Serial sections of primary melanomas revealed different ways of development, from nevi and from LM.

In the primary melanomas arising in nevi, well-circumscribed tightly packed nevus cell nests were found apposed to not so well-circumscribed loosely packed abnormal cells. Intraepidermal nests showed a tendency to press against the epidermodermal junction as well as upper epidermal layers. Spindle and granular cells showed considerable thinning and flattening. Increased number of mitoses (more than one per nest), cellular shape and size irregularities, loosing of the nest, lack of cohesion between cells in the nest, and separation of the cells from the surrounding "capsule" were often found in early cancers (Figs. 7 and 8). In primary melanomas developed from nevi or LM, proliferation of blood vessels and congestion were found adjacent to abnormal nests or groups of abnormal cells (Figs. 8 and 9). Dermal cancer, without major epidermal involvement, was not found in primary tumors.

The "dropping off" cells showed either nevus or melanoma cell characteristics (3, 6, 23). There was a decrease of donor oxidase activity of the cells with depth of invasion. The deeper cells were also more dispersed. Polymorphism of cells in primary melanomas varied not only in melanin content and enzymatic activity but also in differences in size, shape, and nucleus/cytoplasm ratio (Fig. 10). Large epithelioid cells or spindle cells, small round cells, or mixtures of the different cells were noticed (Figs. 8 and 10).

Although definite regression phenomena (2) were not observed, patches of fibroplasia were marked on a few sections from 6 primary melanomas.

In primary invasive melanomas with predominance of LM features at the periphery of the lesions (Table 1), nesting did not exist. The epidermis showed increased amount of melanocytes. Some were normal, others larger than normal, and others atypical to bizarre (Fig. 9). These types of melanocytes were found in the external sheaths of hair roots. In the melanomas arising from LM, atypical melanocytes invaded the dermis without formation of the nests.

Skin metastases were characterized by amelanotic large bizarre cells (Table 1; Fig. 11), spreading from the deep dermis and s.c. tissue towards the epidermis and/or muscle layer. Staging of histological skin invasion (28) showed that most of melanomas were in Stage V of development (Table 2).

The number of metastases and the organs involved are shown in Table 3. The relationship between the number of organs with metastases to the stage of histological invasion is shown in Chart 1.

Nodular metastatic lesions in the internal organs showed malignant amelanotic cells very similar to those already described for skin metastases. With nodular metastases, the normal structure of the involved organ was obscured by the tumor and lost. In the lymph nodes, spleen, and liver, cells characteristic for the individual organ intermingled with tumor cells and necrotic patches.

In addition to melanoma, guinea pigs used in the experiment developed angiogenic tumors (5 animals), lymphomas (2 ani-

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**Table 1**

| Differential characteristics between primary and metastatic skin melanoma |
|---------------------------------|----------------|----------------|
| **Clinical characteristics**    | **Primary**   | **Metastatic** |
| Growth                          | Slow          | Fast           |
| Color                           | Black, 36.8%; brown, 22%; spotty, 26.1%; pink, 15.1% | Spotty, 43.1%; pink, 56.9% |
| Ulceration                      | Shallow, slowly increasing | Deep, appears early in tumor development (93.8%) |
| DOPA reaction                   | Positive, at least in an upper part of the lesion (82%) | In the epidermis and/or in a junction area |
| Abnormal cells (polymorphic, multinucleated, giant, bizarre) | In the epidermis and/or in a junction area | Mainly in the dermis |
| Histology and histochemistry     |                |                |
| Presence of nesting             | In 73.4%      | In 25%         |
| Subepidermal vascular dilatation and/or proliferation | In 39.8% | In 6.2% |
| Malignant cells in the lumen of the blood vessels and/or lymphatics | Sporadic | Common |
| Periphery of the lesion         | Junction-type nevi, 28.9%; compound, 31.6%; dermal, 13.1%; blue nevi, 52%; LM, 15.8%; inconclusive, 5.4% | Inconclusive |
| Response of host cells          | In 39.8%      | In 50%         |
dominated in 2 cases. Relatively "normal" looking melanosomes with evenly dispersed melanin granules and granular (dark) components (Fig. 13). Three of 7 primary melanomas, with mainly junctional or compound nevi components at the periphery. There were only small metastases of one melanoma studied (Fig. 12).

In the epidermis, keratinocytes were pushed aside by melanin-producing cells. They sporadically contained conglomerates of melanosomes but not single melanosomes. Intraepidermal melanin-producing cells were described elsewhere (20). The epidermodermal junction was well marked only at the edge of one melanoma studied (Fig. 12).

In the dermis, individual melanoma cells differed in size, presence or partial absence of the cell membrane, amount and extent of ramifications, and internal structure. More closely packed cells had fewer ramifications than those that were more dispersed; their membranes were also better preserved. Nuclei of melanoma cells were large and irregular, with nucleoplasm densely packed at the edges (Fig. 12). Multiple and large nucleoli showed distinct segregation into their fibrillar (light) and granular (dark) components (Fig. 13). Three of 7 primary melanomas had prominent Golgi, whereas 2 had extensive endoplasmic reticulum (Fig. 14).

Melanosomes were located close to both Golgi and endoplasmic reticulum. They were pleomorphic with disordered internal structure. Spheroidal organelles with filaments oriented in haphazard manner with no cross-linking, and irregular pigmented depositions dominated in 3 of 7 melanomas (Figs. 12 and 15). These were "abortive" melanosomes (11). "Granular" melanosomes with evenly dispersed melanin granules dominated in 2 cases. Relatively "normal" looking melanosomes in Stages II and III of development (3) were seen in one case only (Fig. 16). "Vacuolar" structures dominated in another case. Melanization progressing to complete obliteration of melanosomes was not observed. In many malignant cells, melanin was found not only in melanosomes but also in lysosomes. In a few instances, it was difficult to differentiate between melanophages and melanoma cells showing autophagocytosis of melanin (Fig. 6); however, some melanosomes were disposed individually.

Fibroblasts, macrophages, and lymphocytes occurred between groups of abnormal melanocytes.

Electron microscopy of one skin metastatic nodule showed a similar picture to primary melanoma in a dermal location. The presence of dominating vacuolar melanosomes (empty spheroidal organelles limited by the unit membrane) in the cytoplasm, with absence of relatively normal and granular melanosomes, was the only marked difference.

**Implants.** Nude mice, implanted with fragments of skin derived from melanotic nodules, showed local malignant growth in 5 of 7 implants (Fig. 5). There were no metastases. Of 12 guinea pigs given transplants, 2 demonstrated local growth at the place of implantation (Fig. 6) and metastases to local (axillary) lymph nodes. Tumor growth was observed from the second week onwards after the implantation. After 3 months, the sizes of the 2 nodules were 8 x 5 and 2.5 x 2.5 cm. Histology of the nodules implanted in nude mice and guinea pigs was compatible with the original lesions; however, all of them were amelanotic. DOPA reaction and electron microscopy of the implanted tumors were not done.

**DISCUSSION**

It would appear that DMBA produces 2 distinct effects in the albino guinea pig: (a) it produces some change whereby amelanotic melanocytes become capable of making melanin; and (b) it stimulates them to production of nevi and melanoma. There are many possible ways for DMBA to work; e.g., it either stops some inhibitor of melanin synthesis from functioning or activates an enzyme system which is normally inactive in the albino guinea pig and/or causes a cell mutation.

The model described in this paper enables one to induce skin invasive melanoma from nevi or from LM. These 2 biologically and clinically different malignant melanomas are found in humans (18). According to several papers, at least one-half of the human malignant melanomas may arise from precursor nevi
(9, 27). This type of melanoma is very common in our animal model. The incidence and type of the guinea pig makes this a very interesting, useful, and relevant model. In the Mapple Veisel guinea pig model described by Clark (4), the tumor progress through a series of cellular developmental phenomena did not include formation of junction-type nevi. In contrast to our model, histology of pigmented macules was not similar to junctional nevi or lentigines. That primary melanomas developed mainly from the pigmented lesions was supported by evidence from serial color photographs, constant observations of the animals, and serial histological sections. This suggests that melanomas arose by malignant transformation of junctional nevus cells or melanocytes involved in LM.

There were only a few cancers other than melanoma. Neither they nor the small number of papillomas hindered the observations of melanotic lesions and final results of the experiment. Histogenetic classification of human malignant melanoma is usually based on 3 main forms, reflecting the biological behavior of the growth (3). In the case of the guinea pigs involved in the experiment, this classification was difficult to use, because most melanomas were nodular, 2 or more kinds of cells were observed, and ulceration was often present. However, serial sections of melanomas that were clinically misrecognized as nevi and the edges of nodular melanomas showed biphasic-radial and vertical growth.

Staging of histological skin invasion was easier to use than histogenetic classification. Metastases to internal organs appeared only when the skin melanoma was in Stage V, except in one case, in which the skin lesion was in Stage IV. Metastases to the lymph nodes, however, could already be found in Stage III of skin melanoma.

In differentiation between the primary and secondary melanomas, color and type of ulceration were the most striking clinical differences whereas, in histology, presence of nesting, dilatation of the superficial blood vessels, and an organized periphery of the lesion were characteristic for the primary tumor.

In contrast to development in humans (3, 6, 18), there was no difference in time in the development of melanoma from nevi and LM. Melanoma developing from LM has been shown to be slowly growing and relatively benign in humans (3, 10), whereas in guinea pigs contrasting biological behavior with melanoma arising from nevi did not exist. This can suggest that the mode of carcinogenesis in LM in albino guinea pigs is not different from other types of melanoma. On the other hand, the percentage (15.8% Table 1) of melanomas that were developed from LM is comparable to the data presented for humans (2). The pattern of migration for LM and the melanoma arising from it were similar to that for humans, with characteristic irregular spreading and small islands of normal skin within the confines of the lesion (Ref. 2; Figs. 1 and 2). Histology, however, did not confirm regression of the malignant changes.

Conversion from melanotic to partly melanotic or amelanotic lesions appeared among nodules, as did conversion from DOPA-positive to DOPA-negative melanocytes. The reason for the decrease in melanogenesis in deeply invading primary melanomas and metastases is unknown. Although the synthesis of melanin is a specialized function of the pigment cell, tumorigenicity and melanization are not necessarily intimately related in melanoma.

Guinea pigs melanomas were also vascularized. Multiple and large blood vessels, found next to developing melanoma, increased the possibility of intravasation of tumor cells, leading to the development of tumor emboli and metastases in distant organs. This way of spreading was recently confirmed in hamster amelanotic melanoma (26).

Other histological characteristics of nodular melanomas in the guinea pig also did not differ from those in humans (23, 24).

Electron microscopical observations in the guinea pig melanoma seemed to be of limited value. Differences in sizes and shapes between melanosomes that are not fully melanized were difficult to classify. This remains in agreement with some authors (12) and in disagreement with others (18) regarding human melanomas. Starting amounts of abnormal melanosomal aggregates in the keratinocytes adjacent to the melanocytes, characteristic for human melanomas (3), were not found in the guinea pigs. It is possible that highly decreased numbers of aggregates in keratinocytes depended on the albino characteristics of the animals.

Melanoma cells contained both single and aggregated melanosomes. Degradation of melanosomes in aggregates was pronounced to such an extent that one could consider its lysosomal activity. This phenomenon is typical but not characteristic for melanoma cells (20). There is a possibility that abnormal melanosomes could increase lysosomal and proteolytic activities of the malignant cells. Disorientation of melanosomal fibers, resulting in the formation of mainly abortive and granular melanosomes, was probably caused by a genetic mutation (10). Probably in a similar way, the carcinogen affected the development of melanosomal membranes, producing organelles of the vacular and lamellar type.

In the normal melanocytes from albino guinea pig, endoplasmic reticulum is rather scant but, in the guinea pig melanoma cells, large cysternae and ribosomes were well pronounced. This was suggestive of a high metabolic activity apart from melanin production.

Segregated nucleoli were characteristic for developed melanomas, but this is not a specific feature (16, 17). Some data suggest that segregated nucleoli predominate when there is some inhibition of RNA synthesis or some disturbance in maturation or transport of ribosomal precursors (25).

It is interesting that melanoma induced with chemical carcinogen can be easily transplanted not only to nude mice but also to other guinea pigs.

Nevi and melanotic lesions developing in the albino guinea pigs can be regarded as histogenetic precursors of melanoma. This new model for a metastasizing and transplantable skin melanoma can serve in further understanding of the cellular and tissue phenomena which lead to nevus, precancerous melanosis, and melanoma formation. It can also be useful for studying development of metastases for evaluating biochemical tests for melanoma as well as presumably for trials of treatment.

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Fig. 8 shows histology of the edge of the largest nodule. Part of this nodule was implanted under the skin of the nude mouse in Fig. 5. x 1.5.

Fig. 9. Melanoma developed from LM. Epidermodermal border has vanished. V, superficial dilated blood vessels. Arrow, abnormal mitotic figure. H & E. x 760.

Fig. 10. Giant and bizarre cells of the guinea pig melanoma. H & E. x 3,000.

Fig. 11. Y-shaped mitotic figure. Polymorphic and fragmented cells of secondary amelanotic skin melanoma. H & E. x 3,000.

Fig. 12. Abnormal melanocyte in the epidermis flanked by a keratinocyte (K). Well-preserved epidermodermal junction. Arrows, abortive melanosomes. x 25.000.

Fig. 13. Segregated nucleoli in melanoma cells, x 18,000.

Fig. 14. Extensive endoplasmic reticulum in a malignant cell, x 10,000.

Fig. 15. Mainly abortive (arrows) and one granular (R) melanosomes. x 22,000.

Fig. 16. A branch of a malignant cell with relatively normal-looking melanosomes and degraded melanosomes packed in a lysosome. x 25,000.

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Fig. 1 and 2. Areas of the guinea pig skin at which DMBA was applied. Short arrows, primary melanomas; long arrows, skin secondaries; D, precancerous LM.
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