Human Cutaneous Nevi Transplanted onto Nude Mice: A Model for the Study of the Lesional Steps in Tumor Progression


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ABSTRACT

Normal human cutaneous nevi were transplanted to the skin of nude mice and some of the grafts were treated topically with 7,12-dimethylbenz[a]anthracene (DMBA, 1.6 μmol weekly). Histologically proven human skin was present in 22 grafts. In the 9 untreated control grafts, the tendency of nevocells to form nests and the number of nevomelanocytes decreased with time; the melanocytic cells showed no signs of hypertrophy or atypia. In most of the 14 specimens treated with DMBA, the nevomelanocytes showed distinct signs of hypertrophy. The cells were enlarged and often dendritic and were filled with melanin granules for which the transfer to keratinocytes appeared to be blocked. The nevomelanocytes of 4 of the 9 specimens treated with DMBA for ≥82 days (9–16 DMBA applications), showed atypical enlarged nuclei with mitotic figures. Since atypia is one criterion for identifying precursors of transformed cells, the model of human nevi grafted onto nude mouse skin may be useful for studying the various steps involved in the progression of benign melanocytic nevi to malignant melanoma.

INTRODUCTION

It is widely assumed that human malignant melanoma can arise from preexistent melanocytic nevi. However, this concept is derived largely from circumstantial evidence such as a clinical history of a preexistent nevus at the site of a primary melanoma and the anatomic association of malignant melanoma with intradermal and junctional nevi (reviewed in Ref. 1). Knowledge about the various steps of tumor progression from a benign melanocytic nevus to malignant melanoma has been obtained largely from retrospective studies (reviewed in Refs. 1 and 2), from data extrapolated from animal experiments (reviewed in Ref. 3) or from human tissue in culture (4, 5). An in vivo model for the study of tumor progression in human tissues would be very useful.

The athymic nude mouse accepts human skin grafts (6–16) and can be used to study human skin on a biological support system in which it retains its ability to proliferate. Transplanted human skin retains donor characteristics as determined by histological and functional criteria (8, 10, 12, 16) in contrast to the marked modifications observed in in vitro culture of explants of human skin (17). The in vivo explant model has already been used in carcinogenesis studies on human skin (10, 13). Adult human skin grafts have been shown to undergo a proliferative response when TPA was applied topically (10). Systemic administration of urethane to the nude mice grafted with neonatal human foreskin, followed by topical TPA application, induced the formation of benign squamous papillary lesions in the grafted human skin (13).

In the present paper, we have studied the biology of normal human nevi transplanted to the skin of nude mice and have evaluated the suitability of the system for the study of chemical carcinogenesis in human nevic tissue. DMBA was used because of its carcinogenic activity on melanocytes in hamsters, guinea pigs, mice, fish, and gerbils (reviewed in Ref. 3). In most of those studies, DMBA provoked the formation of melanocytic tumors with no competence for metastasis. However, Clark et al. (18) and Pawlowksi et al. (19) describe the induction by DMBA of widespread invasive melanoma in guinea pigs. In our study, human nevi transplanted to nude mice and treated with DMBA showed signs of atypia not observed in any of the untreated specimens. This explant model may be useful for studying the early steps involved in the progression of a benign nevus to malignant melanoma.

MATERIALS AND METHODS

Mice. Nude mice (nu/nu, BALB/c background) were obtained from Harlan Sprague Dawley, Indianapolis, IN. The mice were housed in a germ-free environment provided by Maximizer Units, Thore Cages, Inc., Hazelton, PA. They were maintained on sterile bedding and given autoclaved feed and water. The mice were 6 to 10 weeks old at the time of grafting. All manipulations on the mice were performed under a laminar flow hood.

Nevus Grafting. Nevi were obtained from healthy volunteers under informed consent. Donor ages varied between 19 and 58 years. All nevi were removed from the back. Most donors had a history of sun exposure during the summer months. Nevi were taken during all seasons of the year.

A split-thickness nevus graft, ~0.3 mm thick and 2 to 4 mm in diameter, was taken from the anesthetized area by a tangential shave technique using a hand-held razor blade. Graft beds were prepared on the right dorsal thorax in anesthetized nude mice and the graft was held in place by a heavy layer of Vaseline petroleum jelly and a bandage. Mice with grafts were housed individually until the bandage was removed 10 days later. Because pigmentation of nevi tended to decrease gradually with increasing time following grafting, exact localization of the grafts initially was difficult. Therefore, in later experiments, India ink was injected intradermally into the nude mouse’s skin at the border of the grafts.

Carcinogenesis Studies. At 2 to 3 weeks after grafting, 400 μg (1.6 μmol) DMBA in 100 μl acetone were applied once weekly to the grafted area throughout the entire experiment.

Histological and Immunohistochemical Procedures. Grafted tissues

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2 To whom requests for reprints should be addressed.

3 The abbreviations used are: TPA, 12-O-tetradecanoylphorbol-13-acetate; DMBA, 7,12-dimethylbenz[a]anthracene.

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were removed from the recipient mice either at predetermined intervals after grafting or at the time of severe-wasting syndrome. Mice were killed by cervical dislocation; grafts were excised and fixed in 10% neutral-buffered formalin. The sections for light microscopy studies were stained with hematoxylin and eosin, and examined by three observers (D. E. E., W. H. C., D. H.) who had no knowledge of the treatment protocol. The sections were evaluated for the presence of human skin. The graft-mouse skin junction could be identified by the distinct differences in the thickness of the two epithelia (multilayered in human versus 2–3 layers in mouse) (Fig. 1A). In addition, the presence of melanin and fully developed hair follicles served as markers for human skin which contrasted well with the nonpigmented epidermis and underdeveloped hair follicles of nude mouse skin. Mouse and human skin was further delineated by Hoechst dye No. 33258 staining which reveals in fluorescence microscopy several small intranuclear fluorescent bodies only in the mouse cells (20). In addition, the sections were evaluated for the following features: presence of a scar or tattoo indicative of a graft site; presence of a “nevus” (nests of 5 or more nevomelanocytes in the epidermis and/or dermal nevus cells); and presence of nevomelanocytes, either nested or not, in the human epidermis and/or subjacent dermis. When nevus cells were present, they were evaluated for cytoplasmic or nuclear abnormalities. Cytoplasmic “hypertrophy” was defined as increased amount of cytoplasm with or without prominent dendrites. Cytoplasmic melanin pigmentation was often intense in hypertrophied cells. Nuclear “atypia” was defined as enlargement of nuclei with hyperchromatism and membrane irregularities. Sections were also evaluated for keratinocytic dysplasia of either mouse or human skin.

Fig. 1. Control compound nevus from group C-1 grafted onto nude mouse skin and removed after 24 days. In A, note differences between human skin (left) and mouse skin (right), presence of dermal nevus cells (arrow), and a few nevus cells in the epidermis. H & E, x 13. B, another control nevus (group C-1). Note uniform nuclei and modest cytoplasmic pigment content in the cells of this nevus. H & E, x 52.

RESULTS

A total of 171 human nevi were transplanted onto nude mice. Of those, 22 survived the predetermined time period and had histologically proven human skin present (see “Materials and Methods”). Thus, our overall rate of successful nevus transplantation was only 12%, which may be related to initial difficulties in exactly localizing the grafts (see “Materials and Methods”) and to occasional spontaneous viral infections in the nude mouse colony. During various periods of our study, conducted over a total of 2.5 years, take rates fluctuated between 8 and 66%. The higher success rates in other studies might rest in the use of normal untreated human skin grafts (6, 8, 10–12).

Histology of Untreated (Control) Grafts. Nine grafts served as untreated controls and were divided into 3 groups (Table 1, groups C-1–C-3). Grafts of control group C-1 were removed for histological examination 12 to 28 days, those of group C-2 43 days, and of group C-3 81 to 88 days following transplantation. Human skin could be clearly identified in all 9 specimens. The presence of “nevomelanocytes” [nevus cells in the grafts forming nests in the epidermis (junctional nevi) and/or single melanocytes in the epidermis and/or dermal nevus cells] was inversely correlated with the period of time from grafting to the observation. In the total of 6 specimens from groups C-1 and C-2, nevomelanocytes had survived in all 6 grafts for periods up to 43 days after grafting (Fig. 1). In contrast, none of the 3 specimens of group C-3 examined at 81 to 88 days following grafting revealed the presence of nevomelanocytes, although human skin was identified in all. Neither nevus cells nor melanocytes showed signs of hypertrophy or atypia.

Histology of DMBA-treated Grafts. In all 13 DMBA-treated specimens (Table 1, groups E-1–E-4), human skin could be clearly identified. Nevomelanocytes were present in 8 DMBA-treated specimens. In 3 specimens, these cells formed nests. Nevomelanocytes were found at 58 (group E-1), 54 (group E-2), 82 to 91 (group E-3), and 114 to 133 (group E-4) days following grafting, respectively, corresponding to 3 to 16 DMBA treatments. Thus, 6 DMBA-treated specimens contained nevomelanocytes at a time following grafting when none of the control specimens was positive for these cells (Table 1). In 8 of the 13 specimens examined, DMBA-treated melanocytes showed distinct signs of hypertrophy (Table 1; Figs. 2–4) as evidenced by hyperpigmentation of the cells with concomitant block of melanin transfer from melanocytes to keratinocytes. Enlarged nuclei and elongated dendrites were further signs of melanocytic hypertrophy (Figs. 3 and 4). The nevomelanocytes of 4 of the 9 specimens (groups E-3 and E-4) that were treated for more than 82 days (corresponding to 9 to 16 DMBA applications) showed distinct atypias as evidenced by atypical enlarged nuclei with frequent mitotic figures. The atypical nevus cells were confined to the epidermis with no signs of invasiveness into deeper layers of the skin (Fig. 4). In one of the 4 specimens, the induction of atypias by DMBA could have been in part related to previous sun exposure, since the donor of this specimen reported frequent prolonged sun exposure during summer months and the frequent use of a sun lamp during the winter.

A large proportion of DMBA-treated human grafts also revealed keratinocytic dysplasia (Table 1). Furthermore, in one specimen of group E-2 (DMBA, 5 times) and in 2 specimens of group E-4 (DMBA, 15 and 16 times, respectively), squamous cell
HUMAN NEVI GRAFTED TO NUDE MICE

Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Duration of transplant on nude mice (days)</th>
<th>Total no. of specimens/group</th>
<th>No. of specimens with</th>
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<tbody>
<tr>
<td>C-1</td>
<td>None</td>
<td>12-28</td>
<td>4</td>
<td>Nevomelanocytes present</td>
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<tr>
<td>C-2</td>
<td>None</td>
<td>43</td>
<td>2</td>
<td>Nevic nests present</td>
</tr>
<tr>
<td>C-3</td>
<td>None</td>
<td>81-88</td>
<td>3</td>
<td>Melanocytic hypertrophy</td>
</tr>
<tr>
<td>E-1</td>
<td>DMBA, 2-3 times</td>
<td>35, 59</td>
<td>2</td>
<td>Melanocytic atypia</td>
</tr>
<tr>
<td>E-2</td>
<td>DMBA, 5-6 times</td>
<td>54, 61</td>
<td>2</td>
<td>Keratinocytic dysplasia</td>
</tr>
<tr>
<td>E-3</td>
<td>DMBA, 9-11 times</td>
<td>82-91</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>E-4</td>
<td>DMBA, 14-16 times</td>
<td>107-133</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

C, control; E, experimental.

a All specimens were obtained from different donors except that in 3 cases 2 specimens were obtained from the same donor (groups C-1, E-2, and E-4). Each specimen included human skin as judged histologically.

b Nevus cells and melanocytes apparently of human origin.

c Melanocytes are enlarged, often dendritic, and filled with melanin granules, transfer of which to keratinocytes is blocked (see Fig. 3).

d Melanocytic cells express atypical nuclei with mitotic figures, often with features of hypertrophy as well. Histopathological appearance of radial growth phase of acral lentiginous melanoma (see Fig. 4).

e Nevus cells in dermis, enlarged nevomelanocytes in epidermis with abundant cytoplasm and copious pigment outlining prominent dendrites ("melanocytic hypertrophy;" Table 1 and text), but no atypia. H & E, × 850.

DISCUSSION

With increasing time following grafting of human nevi to nude mice, we have observed a decrease in the tendency of the nevic cells to form nests and a reduction in the number of nevomelanocytes despite survival of the human epithelium. Whether this...
phenomenon is due to cell migration with formation of single nevus cells or to the rejection of those cells by host-derived mechanisms is not clear. Although we detected no evidence of host cellular reaction, infiltration of the grafted areas with host cells could have occurred before the time of examination of the specimen. In studies with guinea pig cutaneous nevi transplanted to athymic nude mice (21), all nevi showed slight to advanced features of degeneration and disappearance even when guinea pig epidermis had survived. In these studies, host cellular reactions seemed to account for the disappearance of nevi from the athymic nude mouse skin.

In accordance with the findings of Duprez (22), the 9 transplanted control nevi in our study showed no signs of atypia. In contrast, 8 of 13 nevi treated topically with DMBA showed melanocytic hyperplasia with increased pigment synthesis, effects of DMBA frequently observed in other animals (reviewed in Ref. 3). Four treated nevi also showed atypical enlarged nuclei with mitotic figures.

We cannot exclude the possibility that the atypical cells observed in DMBA-treated nevus specimens represent cells preexisting in the skin prior to grafting but regard this as unlikely because only clinically normal appearing nevi from normal volunteers were included in our study. Unfortunately, the separation of a section from each specimen before grafting for control purposes was technically impossible because of the small size (2–4 mm) of the nevi. Furthermore, such manipulations would have destroyed the integrity of the tissues to be transplanted. However, the fact that the changes described here were found in high frequencies in adequately treated specimens but in none of the untreated specimens argues for a role for the chemical carcinogen in the induction of the described melanocytic atypias.

The atypical cells observed in DMBA-treated specimens were confined to the epidermis, and invasion of these cells into deeper layers of the dermis was not observed. Although atypia and mitotic figures are important criteria of precursors of transformed cells, we cannot exclude the possibility that the observed alterations might reflect the toxicity of DMBA rather than transformational changes, in the absence of firm criteria for malignancy such as invasion or tumorigenicity. Antigenic analysis of the atypical cells by monoclonal antibodies could clarify this point if antibodies were available that bind differentially to melanoma cells and not to nevic cells. However, each of the anti-melanoma monoclonal antibodies thus far produced in our laboratory also reacts with nevic cells (23).

Treatment of grafted human nevi beyond the maximum time period of 112 days in the present study might have resulted in migration of melanocytes to the dermis and in their proliferation, as described previously by others in mice treated topically with DMBA and croton oil (24). As a rule, metasasis of human melanomas does not occur until there has been invasion of the dermis, with proliferation there of the invasive cells to form a mass lesion (vertical growth phase) (25). The atypical epidermal melanocytic cells did not reach this phase of malignancy in our study, perhaps due to the relatively short exposure of the transplants to DMBA. Support for this notion comes from the studies of others in guinea pigs (18, 19), in which initial clinically recognizable melanoma appeared between 7 and 16 months after the first application of DMBA, followed several months later by metastases to lymph nodes and internal organs. The smallest number of paintings required to induce melanoma in guinea pigs was 76 with 1% DMBA (19). However, the results described in guinea pigs might not bear directly on our system in which human xenografts are transplanted to immunologically deficient nude mice.

The reduced life expectancy observed in DMBA-treated nude mice in our study could have been due to overdosage of the carcinogen, as suggested by the appearance of squamous papillomas and carcinomas in DMBA-treated nude mouse skin. Lower DMBA dosages also might be desirable in future studies in light of the frequent keratinocytic dysplasias and, in some cases, squamous cell carcinomas in DMBA-treated nevus transplants since studies in hamsters suggest that lowering the dose of DMBA reduces the number of epithelial tumors without affecting the development of melanocytic lesions (26, 27). Furthermore, two-stage carcinogenesis models with DMBA or urethane as initiators and TPA or UV as promoters (10, 13, 28, 29) may be considered in future attempts to drive the explant system described here to the biologically late steps of tumor progression, including vertical growth phase and metastasis.

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