Advances in Brief

Differences in Latency and Inducibility of Mouse Skin Melanomas Depending on the Age and Anatomic Site of the Skin

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Abstract

To determine whether the occurrence of skin melanoma is influenced by the age or the anatomic source of the skin in melanoma-susceptible transgenic mouse models, skin was grafted from donors of different ages or from different anatomic sites to a standard (lateral trunk) site in adult recipients of the same transgenic strain. In 27 grafts of neonatal body skin, melanomas arose with a significantly shorter latency than in 37 grafts of older body skin. The difference may reflect not only the larger number of extrafollicular melanocytes in a given area of neonatal skin but also their unusually high mitotic activity shortly after birth and the influence of other growing skin cells nearby. Each of these body-skin grafts usually developed a single tumor situated near the graft edge. Because maximal wound healing occurs at the edge of such full-thickness skin grafts, melanocytes near the edge would receive the highest exposure to growth factors and cytokines associated with wound healing. In contrast to these results, grafts of snout skin yielded many melanomas, each originating from melanocytes within a vibrissa follicle rather than at the graft edge. The relatively strong local tumorigenic stimulus may be attributable to intrafollicular growth factors normally involved in whisker growth. The above-described experiments support the conclusion that agents in the immediate skin environment of the melanocyte, in addition to the state of the melanocyte itself, contribute to melanoma formation.

Introduction

The age of the individual and the anatomic location of the skin have been suggested as possible factors in human cutaneous melanoma. For example, in relation to solar radiation as a suspected cause, migrants arriving in Australia before 10 years of age were found to have larger numbers of skin lesions prognostic for melanoma that are associated with sun exposure than migrants arriving at later ages (1). Severe sunburn episodes in childhood also have been reported to be correlated with an increased risk of eventual melanoma. These data have, however, been regarded as inconclusive (2). Other evidence (reviewed in Ref. 3) has been taken by some investigators to signify local skin differences in melanoma susceptibility.

We describe here in vivo experiments in melanoma-susceptible mice to learn whether there are age-related or anatomic site-related differences affecting melanoma occurrence in these animals that are intrinsic to the skin or its melanocytes. Any differences found in the mouse models would not be literally applicable to human melanoma but may point to underlying influences on melanoma development that may be relevant for both species. The experiments were carried out in transgenic mice whose melanoma susceptibility is due to an initiating influence of the Tyr-SV40E transgene, which is expressed in pigment cells (4). Spontaneous skin melanomas are infrequent in these animals, but an appropriate promotional stimulus due, for example, to wound healing or to UV radiation reliably results in malignant melanoma (5-7). Melanotic nevi appear, and an orderly progression follows, as in human melanoma, to melanotic and ulcerated tumors, to more rapid tumor growth, and often to a decline in melanization (6-11). In the grafting experiments, skin is taken from a transgenic donor of relatively high melanoma susceptibility and transferred to a standard site in a transgenic host of low melanoma susceptibility. The melanomas invariably arise in the grafts, and their metastases invade organs of the hosts. This was shown by the Southern blot pattern of the donor line, reflecting the donor-specific site of transgene integration (5). The graft area is fairly small (approximately 1 cm in diameter) and can be closely monitored for early lesions. Based on external examination of hundreds of such skin grafts, supplemented with histological study of examples, the occurrence of a melanoma can be reliably recognized at an early stage. Here, we have used the latency, or the interval from grafting to detection of an early melanoma, as the basis for comparing skin of different donor ages and the number and location of tumors in the graft as measures of melanoma inducibility in skin obtained from different sites.

Materials and Methods

Skin Grafts. Grafts were usually derived from Tyr-SV40E (C57BL/6 strain) transgenic hemizygotes of the high melanoma susceptibility line 8, except for nine cases (in the second series) that were homozygotes of the moderately susceptible line 9; all hosts were hemizygotes of the low susceptibility line 12 (4, 5). In the first series of experiments on the effect of differences in age of the skin, the grafts originated from the dorsal body skin of mice ranging in age from 2-70 days. (Donors over 7 days old were shaved with an electric razor.) For approximately the first 3 weeks of life, the hairs are actively growing; after an inactive period, hair growth recurs, and the cycles are repeated. To avoid the loss of melanocytes associated with grafting older skin during active hair growth (12), grafts from donors over 3 weeks of age were taken in the inactive phase of the cycle. In the second series of experiments, based on differences in the site of skin origin, grafts of shaved skin were taken from young adults (approximately 8-12 weeks old), either from dorsal body skin, from the snout region encompassing the vibrissae, or from the base of the tail (with half of the graft from body skin and the other half from tail skin). Full-thickness skin was prepared and grafted in all cases to the lateral trunk of the host as described previously (5). Snout grafts were cut in two, and the halves were juxtaposed in the graft bed to enable the skin to lie flat. Graft hosts in both series were young adults. Bandages were removed after 9 days, and grafts were examined weekly and more frequently thereafter if an early lesion anticipating melanoma was seen. A few graft-bearing animals in each experimental series were discarded for reasons unrelated to the experiments (e.g., illness or accidental damage to the graft). Almost all grafts in long-lived hosts developed melanoma.

Melanomas. Examples of malignant melanomas and earlier lesions were sectioned and stained with H&E for histological examination. Some entire grafts were removed from recipients and fixed in formalin for observation before sectioning.

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Results

Age-related Differences in Melanoma Latency. Melanoma latencies were compared in 64 grafts of dorsal body skin of different ages in which one or more tumors developed. Comparison between latencies in the 27 grafts from neonatal donors (2–7 days of age) and those in 37 grafts from older donors (15–70 days of age) revealed a markedly shorter delay in melanoma occurrence in the younger skin (Table 1). The data were examined in two latency ranges of similar duration: (a) 12–29 weeks; and (b) 30–45 weeks. Although 89% of the melanomas in neonatal grafts arose within the shorter latency span, only 27% of the melanomas in older grafts appeared during that period. Analysis with the Mann-Whitney U test for P-values of paired comparisons showed a high level of significance (P < 0.0001) of these age-related differences in melanoma latency. In the older donor group, five donors were 15 days of age, and all others were 25 days of age or older. Most of the 15-day-old cases gave borderline results, as might be expected; e.g., two cases had tumor latencies of 28 and 29 weeks, and one case had a latency of 30 weeks.

Among the 64 skin grafts, a single melanoma developed in each of 55 grafts; 2 or 3 apparently independent melanomas arose in each of 2 grafts from neonatal donors and in each of 7 grafts from older donors. The latencies of multiple tumors within a graft were similar, and each group was counted as a single case in Table 1; four of these cases were in the shorter-latency group, and five were in the longer-latency group.

Site-related Differences in Melanoma Induction. The grafts of snout skin yielded results strikingly different from those in grafts of body skin. Many melanomas arose in the snout grafts, in contrast to the usually solitary tumors in grafts of comparable size from the dorsal body region (Table 2). Each tumor in snout skin clearly originated from melanocytes situated in or near the hair bulb at the base of a vibrissa follicle (Fig. 1D), whereas tumors in the other grafts seemed to arise from extrafollicular melanocytes. No tumors were seen in the follicles of ordinary hairs included in the snout skin. The orderly spatial pattern of the whisker follicles dictated the distribution of the melanomas in snout skin; in body skin, the tumors generally developed in the periphery of the graft, as noted previously (5).

Melanomas in vibrissa follicles of a given snout-skin graft often grew at different rates (Fig. 1, A and B). The larger tumors breached the whisker shaft (Fig. 1E). With further growth, they spread beyond their original confines (Fig. 1B) and tended to become amalgamated with neighboring melanomas, forming a large ulcerated mass penetrating deeply into the dermis and subjacent tissue. Grafts from the base of the tail spanned two types of skin: the relatively thin tail skin, which is one of the few areas in the mouse with many pigmented melanocytes remaining in the epidermis; and the thicker body skin, in which pigmented melanocytes are seen chiefly in the hair bulb of the follicles and to a lesser extent in the extrafollicular dermis near the junction with the epidermis. Most of the melanomas arose in the body-skin component of the grafts (Table 2; Fig. 1C); however, the number of cases in this series was small. The tumors were found either at the edge or in the interior of the graft.

Discussion

A factor contributing to the shorter melanoma latency in neonatal than in older body skin (Table 1) may be the rapid and transient increase in the numbers of extrafollicular melanocytes shortly after birth, thereby favoring the early success of a low-frequency transformational event. According to the data of Hirobe and Takeuchi (13), the number of epidermal melanocytes in mouse body skin increases rapidly during the first 4 days of life and then gradually declines as the cells begin to appear in the base of the hair follicles, presumably by migration. Although few pigmented melanocytes are evident in the epidermis by 2 weeks of age, unpigmented ones remain there in a dormant state (14). In human skin, there is also a significant increase in the number of epidermal melanocytes soon after birth (15). We suggest that a factor more critical than melanocyte number may be the presence of large numbers of actively dividing melanocytes in young skin, especially if dividing cells are more vulnerable to transformation. The more rapid growth of neonatal than of older skin may also provide more growth-stimulating factors from cells such as keratinocytes in the immediate environment of the skin melanocytes.

That the local melanocyte environment may stimulate its proliferation in vivo is strongly implied by the fact that melanomas in mouse body-skin grafts almost always arose near the edge of the grafted skin, as shown here (Table 2) and in a previous report (5). Wound healing is greatest at the edge of a full-thickness skin graft, and the growth factors and cytokines involved in wound repair may well act as promotional stimuli in melanoma-susceptible (initiated) skin. Examples of growth factors known to be produced by participating cell types at a wound site are specific members of the transforming growth factor-β and fibroblast growth factor families (16). Wounding alone, even without grafting, can in fact elicit local melanocytic hyperplasia in Tyr-SV40E transgenic mouse skin (5).

More dramatic evidence of a local environmental role in melanoma induction is the development of premelanoma lesions and melanomas from melanocytes residing within the vibrissa follicles of transgenic snout-skin grafts (Table 2; Fig. 1, A, B, D, and E). Occasional spontaneous early melanomas, without skin grafting, have previously been seen in the whisker follicles of our transgenic mice (4). It is not surprising that growth factors such as transforming growth factor-β1, hepatocyte growth factor, keratinocyte growth factor, and insulin-like growth factor, which occur in cells of these large follicles in mice, are believed to influence vibrissa growth and development (17–20). Although there have been suggestions that some human melanomas may arise from melanocytes in ordinary hair shafts (15), there is as yet no clear evidence in either humans or mice to support this view. Additional indications of extrinsic local effects on melanoma development

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<tr>
<th>Table 1 Comparative latencies of melanomas arising in grafts of dorsal body skin from neonatal versus older transgenic donors</th>
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<td>Age of graft donor</td>
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<tr>
<td>Neonatal (2–7 days)</td>
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<td>Older (15–70 days)</td>
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- Measured in weeks from the time of grafting to the earliest external detection of a melanoma. All skin graft donors were Tyr-SV40E transgenic hemizygotes of the high melanoma susceptibility line 8; all hosts were Tyr-SV40E adult hemizygotes of the low melanoma susceptibility line 12. Melanomas arose only in the grafted skin (usually one per graft).
- Five donors in this group were 15 days old; the others were 25 days of age and older.

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<table>
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<th>Table 2 Melanoma induction in transgenic adult skin grafts from different anatomic sites</th>
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<td>Donor skin site</td>
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<tr>
<td>Snout</td>
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<td>Base of tail</td>
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<td>Dorsal body</td>
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- Snout skin included the vibrissa follicles, within which all the melanomas arose.
- Grafts from the base of the tail spanned skin (in which 14 of the 16 melanomas arose) and tail skin.
- These cases are taken from the older donor group in Table 1, excluding the five donors less than 25 days old.
are the differences in latency and growth rate of melanomas arising in separate vibrissa follicles of a given snout-skin graft (Fig. 1, A and B). Melanomas near the edge of a given body-skin graft tend to have similar latencies and growth rates, as expected in this less sequestered local environment.

These results raise the question of whether changes in the skin extrinsic to the melanocyte, e.g., after UV radiation-induced skin damage, may generate local factors with melanomagenic potential. If the primary or initiating stimulus to melanoma formation is a mutational change in the melanocyte, this may be a necessary cause but not a sufficient cause of melanoma. Perhaps a nonmutational secondary change, acting as a promotional stimulus, may be supplied by agents external to the melanocyte.

References
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