The Developmental Biology of Induced Malignant Melanoma in Guinea Pigs and a Comparison with Other Neoplastic Systems

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SUMMARY

Malignant melanoma has been induced in the Weiser-Maple guinea pig by prolonged application of 7,12-dimethylbenz(a)anthracene. The tumor shows a biphasic growth pattern analogous to the radial and vertical growth phase of human cutaneous malignant melanoma. It evolves through a predictable series of cellular events classified as intraepidermal melanocytic hyperplasia, dermal melanocytosis, dermal melanocytoma, malignant melanoma without intraleisional transformation, and, finally, malignant melanoma with intraleisional transformation, which is characterized by the appearance of "new kinds of cells" and is associated with widespread metastases and massive lymph node involvement. Clinically, the lesions evolve from diffuse hyperpigmentation to brown-black macules, to nodules of increasing size, to overt malignant melanoma associated with metastases, wasting, and death. Examples of intraleional transformation analogous to that in guinea pigs are found not only in human malignant melanomas, but in other human neoplastic systems, and such analogous cellular events are discussed in this paper.

INTRODUCTION

In the past 10 years, a significant amount of information has been published that clarifies the developmental biology of primary human cutaneous malignant melanomas. For example, the most common type of melanoma, SSM³ (6, 8), evolves through a biphasic growth pattern characterized by an initial radial growth phase followed by a subsequent vertical growth phase. As a rule, metastases do not develop until the vertical growth phase has evolved (7).

The ability of the cells comprising the vertical growth phase of melanoma to penetrate various tissue interfaces in the human skin has formed the basis for levels of invasion (6, 8, 27, 28). The quantity of tumor forming the vertical growth phase has been estimated by measurements of its thickness (2, 3, 23, 41, 42). The combined use of invasion levels and thickness has been rather precisely correlated with the presence or absence of metastatic disease (2, 3, 23, 41, 42). The penetration of vertical growth phase of the disease into the deeper reaches of the skin is not the mere expansion of tumor in space and time, but such invasion is commonly associated with qualitative changes in the vertical growth phase of the neoplasm (7, 10). The changes include the appearance of new populations of tumor cells, clearly different from those of the radial growth phase, and, commonly, disappearance of the host cellular response, so that tumor cells penetrate between collagen bundles of the dermis without intervening lymphocytes, macrophages, new blood vessel formation, or fibroplasia. The new populations of tumor cells appearing within the vertical growth phase, which are usually associated with the ultimate development of metastases, have been called intraleional transformation (7, 9).

We have attempted, over the past several years, to develop an induced animal model of malignant melanoma that is analogous to the human disease. Such a model would permit one to explore, in an experimental system, the developmental biology of a primary neoplasm and to delineate the sequential cellular phenomena which precede or are associated with metastatic disease. The common animal malignant melanomas, such as Cloudman S-19 and Harding-Passey, were originally spontaneous tumors but have been carried as transplant models. They are, therefore, not analogous to the human disease. Most induced rodent tumors are said to be blue nevus-like, and even the larger nodular lesions obtained by Epstein were not associated with systemic metastases (13, 14). Only the single guinea pig reported by Berenblum (1), the 3 guinea pigs reported by Edgcomb and Mitchelich (12), and the urethan-white Syrian hamster model of Vesselinovitch et al. (40) have shown widespread metastatic disease.

We have reproduced the studies of Edgcomb and Mitchelich (12) and have shown that the induced primary tumor evolves through a predictable series of cellular events over a period of about 1.5 years, frequently culminating in the appearance of different populations of tumor cells. This final cellular event in the development of the induced guinea pig primary melanoma is analogous to the cellular phenomenon we have termed intraleional transformation in primary human melanomas. In spite of this and other analogies between this guinea pig system and human cutaneous melanomas, the 2 tumor systems are histogenetically...
different, and the guinea pig model is not identical with the human tumor. The present paper describes the details of the development of the guinea pig tumor and compares the tumor with other animal melanomas and human cutaneous melanomas. In addition, the possible biological significance of the sequential cellular phenomena occurring within the guinea pig and human cutaneous melanoma systems is explored by comparing the cellular changes with similar changes observed in other neoplastic systems.

MATERIALS AND METHODS

Animals. Random-bred tan W-M guinea pigs (Federated Medical Resources Farm, Honeybrook, Pa.) were selected because they have a uniform coat color and an active epidermal melanocytic system in both immature and mature animals (unpublished observations). Fifty-two female and 46 male W-M guinea pigs were used, along with 81 multicolored female guinea pigs (Camm Research Institute, Inc., Wayne, N. J., and Elm Hill Breeding Laboratories, Chelmsford, Mass.) A total of 128 animals of mixed sexes and colors were placed in the experimental group, and 51 animals were controls. Selected animals were serially photographed at about 4-week intervals. The animals varied from 4 to 14 weeks of age at the beginning of the experiment. They were housed in air-conditioned quarters, fed standard guinea pig food, and were given water with ascorbic acid 

or ad libitum.

Carcinogen. The carcinogen was a 0.5% solution of DMBA (Fisher Scientific Co., Philadelphia, Pa.) in benzene. Control animals were treated with benzene alone, and a smaller control group was not treated in any way. An area 5 cm in diameter on each dorsal flank was shaved, and 0.1 ml of the carcinogen solution (500 µg) was applied to each area and to the outer portion of each ear once a week, or one-half that concentration was applied twice a week. The total weekly dosage was the same in each animal. Time schedules of carcinogen application varied from 57 to 79 weeks, and animals were followed for 88 to 138 weeks from the beginning of the experiments.

Biopsy. Serial excisional biopsies were done on both flank and ear skin. The entire application site on 1 flank and the ear on the same side were excised under general anesthesia. At the time of excision of the 2nd flank and ear, the animal was sacrificed, and an entire autopsy was done. Biopsies and autopsies were at 5- to 10-week intervals from the 5th to the 130th week (Table 1). Paraffin sections of the tissue were stained with hematoxylin and eosin or by the Fontana-Masson method.

RESULTS

By the end of the experiments, 73 of the experimental animals had died or were sacrificed, and 55 were alive with various classes of pigmented lesions. Control animals painted with benzene alone showed an occasional black or brown macule, but not to a degree significantly different from that of the untreated controls. All control animals were sacrificed after the 138th week.

Sequential Development of Clinical Lesions

Flank Lesions

The 1st clinical changes were noted soon after the initial application of DMBA, appearing as areas of diffuse and variable hyperpigmentation associated with alopecia. The area of DMBA application and the immediately adjacent skin then changed slowly but continuously through a series

![Table 1: Biopsy and autopsy schedule](image-url)
of brown-black macules, small nodules, larger nodules, and, finally, ulcerated malignant melanoma. Each change was superimposed upon the previous change but never completely replaced earlier lesions, so that an obvious melanoma was still associated with all of the various kinds of lesions that preceded it. Furthermore, even while advanced nodules were growing, new small macules and other areas of hyperpigmentation were continually appearing. Below, we describe the early changes and 7 classes of lesions. An animal was always classified by its most advanced flank lesion.

Early Changes. The normal tan skin of the dorsal flank showed alopecia within 1 to 2 weeks. This change was associated with altered color that ranged from areas of pink-tan to dark brown. These pigmentary changes were subtle and diffuse, and differed sharply from the small macular lesions to be described below. Other areas in the application site often showed matted tufts of hair and crusting.

Lesion 1. The diffuse hyperpigmentation was a subtle change which spread slowly and blended imperceptibly with the surrounding normal skin. Within the hyperpigmented area, brown or black macules appeared which were much more prominent than was the background of hyperpigmentation. The macules were 1 to 5 mm in diameter, round or angulated, and not palpable. An application site with 4 or less macules was defined as Lesion 1 (Fig. 1) and was seen in some animals after 15 weeks of DMBA application.

Lesion 2. This lesion represented an increase in the number of the brown-black macules to 5 or more (Fig. 2). With the passage of time, the macules became more prominent than those of Lesion 1. Commonly, the individual macule was larger and darker than that comprising Lesion 1. Some macules were slightly scaly and elevated. An elevated macule, however, that was less than 2 mm in width, was still classified as Lesion 2.

Lesion 3. The appearance of 1 or more black elevated nodules 2 to 5 mm in diameter was called Lesion 3. Such nodules often had a surface that was smooth and without skin markings (Fig. 3).

Lesion 4. Nodules between 5 and 10 mm in diameter were called Lesion 4 (Fig. 4). Such nodules occasionally ulcerated.

Lesion 5. Any nodule larger than 10 mm in diameter was defined as Lesion 5 (Fig. 5). These lesions were usually ulcerated and were associated with bulky lymph node metastases. Lesions 4 and 5 were, clinically, malignant melanomas.

Lesion 6. A metastasis to any site was termed Lesion 6. Lymph node metastases were associated with Lesions 2, 3, and 4, but palpable lymph nodes and widespread systemic metastases were only associated with Lesion 5 (Fig. 6).

Lesion 7. This class of lesions was a miscellaneous collection of nonmelanocytic neoplasms including epidermal cysts, squamous cell carcinomas, skin appendage tumors, and an occasional fibrosarcoma.

Ear Lesions

The same general classes of lesions developed on the ear, but diffuse scaliness and hyperpigmentation made them difficult to classify. Lesions 4, 5, and 6 were not seen on the ear, but a variety of miscellaneous lesions (Lesion 7) were seen.

Sequential Development of Microscopic Lesions

Like the clinical changes, the microscopic changes formed a continuum with each new change superimposed.
upon prior alterations. Similarly, new “early” lesions continued to develop even as older ones progressed to more advanced lesions. Microscopic changes were seen in the melanocytic, keratinocytic, skin appendageal, and mesenchymal cell systems, but the concern of this paper is with the melanocytic system only. We have divided the microscopic lesions into 5 classes and have correlated them with the clinical lesions in Table 2.

Intraepidermal Melanocytic Hyperplasia. During the 1st few weeks of DMBA application, the 1st demonstrable abnormality was an increased amount of pigment in the epidermis, due, apparently, to increased pigment synthesis rather than to a significant increase in the number of melanocytes. True intraepidermal melanocytic hyperplasia was seen after about 5 weeks of DMBA application. The individual melanocytes were typical with clear cytoplasm and prominent, pigment-engorged dendrites (Fig. 7) that formed a complex meshwork that appeared as a continuous pigmented line just above the basement membrane of the epidermis. In preparations stained by the Fontana-Masson method, the dendritic arborization and hyperpigmentation were even more striking. The melanocytic hyperplasia was prominent by the 10th week, and mitotic figures in melanocytes were easily observed by this time. At about 15 weeks an occasional large epithelioid melanocyte, heavily laden with pigment, could be seen. Although “atypical,” these cells were readily recognized as melanocytes. Between the 30th and 50th weeks, they were observed in rows along the basal layer or in small clusters, characterized by some degree of dyskinesis (Fig. 8).

Dermal Melanocytosis. The earliest appearance of dermal melanocytosis was around the 15th week, and this lesion was well developed by the 30th week. The lesion called dermal melanocytosis was either intimately applied to the basement membrane or separated from it by a narrow zone of apparently uninvolved connective tissue. It was composed of elongate bipolar cells aligned roughly parallel to each other and to the epidermal surface (Fig. 9). These cells had a fibroblast-like nucleus and 2 cytoplasmic arms engorged with pigment granules of relatively uniform size. As a rule, even the earliest of these lesions also showed large (17 to 20 μm in diameter), atypical epithelioid cells, the morphology of which was completely obscured by dense accumulations of pigment (Fig. 10). The epidermis above the dermal melanocytosis usually showed intraepidermal melanocytic hyperplasia, but this was not prominent. New lesions of dermal melanocytosis continued to appear throughout the entire course of the experiments.

Dermal Melanocytoma. This lesion evolved in association with and within dermal melanocytosis. It was fully developed by 50 weeks and was occasionally seen at an earlier time. The typical lesion (Fig. 11) was dominated by the large atypical epithelioid melanocytes described above. No internal structure was visible due to intense pigmentation, although, occasionally, one could see a suggestion of a nucleus. Within some of these lesions there were a few cells in which the pigment was no longer dominant and the nucleus was easily seen. At the base of dermal melanocytomas, cells, heavily laden with pigment but frequently dendritic in form, invaded the deeper dermis (Fig. 12).

Malignant Melanoma without Intralesional Transformation. This lesion developed between the 70th and 138th week. It was characterized by the appearance, within a dermal melanocytoma, of isolated cells with greatly reduced amounts of pigment and a readily visible internal cellular structure. These cells had a large nucleus with a prominent eosinophilic nucleolus. Although the cytoplasm was relatively pigment free, it contained clumps of small pigment granules which were especially noticeable near the plasma membrane. The large, deeply pigmented epithelioid melanocytes which are characteristic of dermal melanocytomas still dominated malignant melanoma without intralesional transformation, but were now much larger. They were usually 25 to 30 μm in diameter, and some as large as 45 μm were observed. Invariably, there was invasion at the periphery of this lesion, and, frequently, lymph node metastases were noted (Fig. 13).

Malignant Melanoma with Intralesional Transformation. The final evolution of the primary tumor was characterized by the appearance within it of qualitatively different cells which occurred in clusters and were almost completely pigment free (Fig. 14). It was this appearance of “new kinds of cells” that we termed intralesional transformation. Some clusters showed striking pleomorphism, whereas others were in circumscribed nests (Fig. 15). The periphery of such nests frequently showed remnants of the large, heavily pigmented epithelioid melanocytes seen in the foregoing lesions (Fig. 15). The relatively pigment-free cells demonstrated numerous mitotic figures, some of which were abnormal. At the same time, melanocytes with well-preserved dendritic systems could still be seen. Invasion was often well into the subcutaneous tissue. The nodules of this lesion were frequently large, rapidly growing excrescences.

Metastatic Disease. Small, dermal melanocytomas and all subsequent microscopic lesions were associated with microscopic lymph node metastases. These metastases frequently showed cells with preserved dendritic processes, clearly distinguishing the pigmented cells from melanophages. Malignant melanoma with intralesional transformation was usually associated with massive nodal deposits of tumor (Fig. 13). One such resected primary with the draining inguinal nodes (the nodes forming the larger part of the total mass) weighed 98 g. In addition, malignant melanoma with intralesional transformation was frequently associated with metastases to the lungs, liver, spleen, adrenals, kidneys, gastrointestinal tract, and bone (Figs. 16 and 17). Involvement of the brain was not observed. All of the metastatic cells contained moderate amounts of pigment and, at times, a well-preserved dendritic system (Fig. 17).

DISCUSSION

The W-M Guinea Pig. Malignant melanoma with widespread metastatic disease apparently can be induced by DMBA in a variety of colored guinea pigs. We have induced such metastasizing melanomas in the W-M animal and in multicolored animals supplied by Camm and Elm Hill. Doubtless, the animals of Berenblum and of Edgcomb and Mitchelich were from an entirely different stock. The re-
results, in general, are similar in the different kinds of animals, but the evolution of the disease is more difficult to evaluate in darker animals. We believe that the W-M guinea pig has an advantage over the multicolored animals. For example, the breeding history (inbred for 7 to 8 generations subsequent as outbred stock) has resulted in a uniform tan coat which probably allows the carcinogen to be applied to the same number and kind of melanocytes. The tan color also permits careful clinical appraisal and serial color photography of the developing neoplastic system. The serial color photographs have been invaluable in documenting the evolution of these melanomas.

**Induced Melanocytic Tumors in Other Rodents as Compared with the Guinea Pig System.** The development, in rodents, of pigmented foci in response to carcinogenic hydrocarbons has been observed for over half a century (4, 25, 34, 39). With the exception of the Fortner System in hamsters (17, 18) which probably was a spontaneous tumor histogenetically similar to the human disease, most rodent melanotic tumors resemble each other. The majority of studies in rodents have been done in hamsters (11, 22, 29–31, 35, 37, 40) and in pigmented hairless mice (13, 14, 16), but Mongolian gerbils also react in a similar fashion (33).

We shall first discuss the features common to guinea pig and other rodent melanocytic tumor systems, and later we will indicate how guinea pig melanomas differ from those of other rodents. Initially, rodents develop a pigmented dermal lesion comprised of fibroblast-like melanin-containing cells which has been called dermal melanosis or blue nevus by other workers. This lesion is identical with what we have termed dermal melanocytosis, but in the guinea pig it is not the earliest response to the carcinogen, being preceded by hyperpigmentation and intraepidermal melanocytic hyperplasia. In association with or within the 1st dermal lesions, a subsequent pigmented papule develops which is composed of large, epithelioid, pigment-engorged cells. This latter lesion was termed dermal melanocytoma by Nakai (30) and by Epstein & Epstein in one of their earlier publications (13). It is identical with the lesion we call dermal melanocytoma in the guinea pig and with the one that evolves in other rodent systems. Such melanotic tumors are generally regarded as benign. The guinea pig dermal melanocytomas have some atypical cells and show invasion and even small foci of lymph node metastases, but we do not call them malignant for the following reasons. (a) Despite the fact that animals in these experiments develop several dermal melanocytomas by the 50th week and many have microscopic lymph node metastases, the animals do not show progressive clinical lymph node involvement in later months. (b) The animals that show progressive lymph node metastases do so between 80 and 110 weeks, and such lymph nodal involvement is only in association with malignant melanoma with intralesional transformation. (c) Progressive growth of dermal melanocytomas is not always observed. The lesions can become indolent. Thus, the presence of microscopic foci of melanocytes in lymph nodes, associated with the relatively early events in primary tumor development, may or may not herald inexorable malignancy. Since there is virtually no ethically acceptable method that would allow extensive studies of lymph nodes draining the earlier developmental stages of human epithelial neoplasms, it is not known whether dysplastic epithelial lesions shed tumor cells into the lymphatic system. For example, dysplastic breast lesions may be so disturbing as to require extensive local resection or even simple mastectomy, but such lesions do not warrant lymph node resection; therefore, it is not known whether nodes draining markedly atypical breast lesions contain isolated neoplastic cells. Such nodes might well contain scattered “metastatic” cells that are not necessarily going to progress locally or disseminate.

Progression beyond the dermal melanocytoma stage does not occur or is exceedingly rare in animals other than the guinea pig. A likely major exception to the foregoing statement is the interesting tumor system induced in Syrian white hamsters by Vesselinovitch et al. (40). These workers administered urethan to newborn hamsters and gave 4 subsequent injections at 3-day intervals. The majority of the animals developed tumors, most of which were melanomas. Metastasis developed in 82% of the males and 25% of the females. The authors did not attempt to describe the developmental biology of the primary tumors, but stated that, “Histological studies showed that the skin was the primary site, while the other tumors were metastases of malignant melanomas.” Vesselinovitch et al. illustrate areas of 2 primary tumors. One of these illustrations is similar to what we have termed malignant melanoma without intralesional transformation, whereas the other figure is similar to areas we have referred to as intralesional transformation. In addition to the Vesselinovitch tumors, lesions similar to what we have called malignant melanoma without intralesional transformation have been produced in pigmented mice by chronic UV-irradiation of dermal melanocytomas induced by a single dose of DMBA. These tumors involved lymph nodes but did not produce systemic metastases (14).

We use the term malignant melanoma for 2 classes of microscopic lesions; those without and those with intralesional transformation. The latter is associated with widespread metastases, and, therefore, it is not necessary to justify the use of the word malignant. It is used for lesions without intralesional transformation for the following reasons. (a) The primary tumors are invariably invasive. (b) Individual cells appear which have prominent nucleoli and other cytological features commonly associated with malignancy. (c) The cells forming the bulk of the lesion are startlingly larger than those of previous lesions. (d) Lymph node metastases are routine. (e) The lesion usually gets inexorably and progressively larger.

The guinea pig cutaneous melanoma induced by DMBA is therefore similar to other induced rodent systems with 3 important differences. (a) The initial response to DMBA is intraepidermal melanocytic hyperplasia. Although this hyperplasia is not truly comparable to the radial growth phase of the human disease, it is a chronological precursor to the remainder of the tumor system. (b) In a few guinea pigs, the tumor progresses through a series of cellular developmental phenomena, finally forming an obvious malignant melanoma associated with widespread metastatic disease. (c) The carcinogen dosage used by us and by Edgcomb and Mitchelich (12) is much greater than that used by other workers. Others have applied carcinogen only once or for

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**Research.**
short periods, whereas we have applied it for 57 to 79 weeks. Studies under way in our laboratory suggest that such long application periods may not be necessary to produce the entire neoplastic melanoma system, but 30 to 50 weeks may well be required. Other rodents may never develop the complete system following topical application, but in all likelihood some of them will with prolonged carcinogen treatment.

Comparison between Guinea Pig and Human Melanoma Systems. Edgcomb and Mitchell (12) have stated that the application of DMBA produced numerous lentigines and junctional nevi in addition to the metastasizing malignant melanomas. This implies that guinea pig malignant melanomas have a histogenetic mechanism similar to that of humans. However, they did not report histological studies of the clinically observed lentigines and junctional nevi. The diagnosis of these lesions was presumably based upon the observation of flat brown or black macules of varying size. Our histological studies of such macules induced by methods identical with those of Edgcomb and Mitchell (12) show that the macules fall into 2 classes: (a) intraepidermal melanocytic hyperplasia, and (b) dermal melanocytic hyperplasia, manifested as dermal melanocytosis or dermal melanocytoma. The histology of both kinds of macules is, therefore, not similar to junctional nevi or lentigines. Junctional nevi and lentigines, in the conventional sense that these terms are used, cannot be regarded, in the guinea pig system, as histogenetic precursors of malignant melanoma.

The vast majority (85 to 90%) of all human cutaneous malignant melanomas evolve through a growth pattern that is at least biphasic (6-9), with the tumors showing radial, then vertical growth. The latter phase may show intraleisonal transformation. Biphasic or multiphasic growth is quite characteristic for indirect tumor progression (7,9) and is seen with variations on the same theme in SSM, lentigo maligna type, and acral lentiginous melanoma (mucous membrane, subungual, and volar melanomas). The biology of acral lentiginous melanoma has been delineated by Arrington et al.*

The most common of all human cutaneous melanomas (65 to 70%) is SSM. The earliest change is intraepidermal hyperplasia of large, atypical melanocytes. This change probably does not persist for a significant period of time, since most lesions of SSM show invasion of the papillary dermis. The combination of intraepidermal and papillary dermal involvement forms the radial or "initial" growth phase of the disease which may persist without evident metastases for some years. As a rule, there is a well-developed host response of lymphocytes and macrophages to the radial growth phase. All of the histological features of malignant melanoma are present, but metastatic disease is rare. The radial growth phase of SSM is therefore analogous to the guinea pig malignant melanoma without intraleisonal transformation, but it is, obviously, quite different histologically. It should be emphasized that the human and guinea pig systems are quite different with respect to the developmental biology of the early stages of the primary lesions. The human system (using SSM as the model) shows extensive hyperplasia of intraepidermal melanocytes with early invasion of the dermis and radial extension of this epidermal-papillary dermal stage of the primary tumor. This phase of the disease is the Level II disease or disease <0.76 mm thick, which is not associated, with rare exceptions, with metastasis (2, 3, 8). There is no guinea pig lesion histologically similar to the radial growth phase of SSM, but the guinea pig lesion we have termed malignant melanoma without intraleisonal transformation does exist for a long time with only a rare metastasis. In this sense, the guinea pig lesion is analogous to radial growth phase SSM.

Intraepidermal melanocytic hyperplasia and atypical intraepidermal melanocytes are a routine feature of the guinea pig system but are not prominent, and there is no evidence that the intraepidermal melanocytic changes are a necessary precursor of the prominent dermal lesions. It is entirely reasonable to suggest that different (perhaps smaller) dosages of DMBA could so differentially affect the epidermal melanocytes of the guinea pig that a melanoma system histogenetically identical with that of the human would result. Our methods reported here have failed to achieve this goal.

The vertical growth phase of SSM is characterized by the development of a focal area of deep invasion within the radial growth phase. Within the area of vertical growth, "new kinds of cells," mostly amelanotic, frequently appear (7, 9, 15). The combination of deep invasion and "new kinds of cells," which we call intraleisonal transformation, is commonly associated with the development of metastatic disease (7, 9) and is precisely analogous to malignant melanoma with intraleisonal transformation in the guinea pig. The phenomenon of intraleisonal transformation is not only biologically analogous in the human and the guinea pig, but is also histologically similar.

Intralesional Transformation in Human Neoplasia and in Guinea Pig Malignant Melanoma. Intralesional transformation with the appearance of "new kinds of cells" within the primary lesion is probably the event that heralds, in the guinea pig, inexorable progression to dissemination, clinical wasting, and death over a period of about 3 months. It is but a special example of what the late Dr. Leslie Foulds called tumor progression (19). Through the years, tumor progression has come to encompass all of the sequential cellular events in a given neoplastic system. Indeed, Foulds himself has used the term in this broad sense (21). In a more restricted way, Foulds has said "... progression is not the mere extension of a lesion in space and time but a revolutionary change in a portion of the old lesion establishing a new tumor having properties not formerly manifest." (20). The foregoing definition by Foulds is virtually identical with our concept of intraleisonal transformation.

There are human neoplastic systems other than malignant melanomas which readily come to mind as examples of intraleisonal transformation. It is not the purpose of this paper to attempt a lengthy discussion of the phenomenon in human neoplasia, but only to select a few well-documented examples which emphasize the importance of

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intralesional transformation as a “final” developmental event in a primary neoplastic system. One of the most interesting human systems with cytogenetic documentation of what we would term intralesional transformation is CML. Two recent publications with complete references to the progressive cellular abnormalities in CML are those of Nowell (32) and Sakurai et al. (36). In the latter, the authors state, “Furthermore, Ph'-positive CML consists of 2 distinct stages, i.e., a chronic stage and a blastic phase. The chronic stage is considered by many investigators to be preleukemic rather than a truly leukemic one, and the blastic phase very similar to AML, but more resistant to chemotherapy. In addition, the chronic form of CML is chiefly associated with a Ph'-positive but an otherwise normal karyotype, whereas the blastic phase, even though it can also be associated with such a karyotype, is often accompanied by additional cytogenetic abnormalities other than Ph’. Thus, the blastic phase of CML is associated with 2 events: additional cytogenetic abnormalities and a poor prognosis. In other words, it is characterized by “new kinds of cells” having different properties. Progression from CML to the blastic phase is therefore analogous to progression from malignant melanoma without intralesional transformation to malignant melanoma with intralesional transformation in the guinea pig system. The “revolutionary change” results in newly acquired properties which, in the case of the human myelogenous leukemia model and the animal model of malignant melanoma, have the routine ability to cause the death of the tumor-bearing host.

Without presenting the cellular details, exactly similar analogies may be seen in Hodgkin’s disease and human breast carcinoma. Progression from lymphocytic predomiance Hodgkin’s disease to the pleomorphic reticular variant [original classification of Lukes and Butler (26)] of the lymphocytic depletion type is associated with the appearance of “new kinds of cells” and an exceedingly poor prognosis (5, 26, 38). The overt breast mass, commonly regarded as “cancer” of the breast and which is associated with a relatively poor prognosis, may be regarded as an example of intralesional transformation. The beautiful work of Hutter and Kim (24), who studied sections of the entire carcinoma-bearing breast, clearly shows the “cancer” to be a “new” cellular event emerging from a variety of precursor cellular phenomena, the “cancer” having properties not manifest by the precursor lesions.

Two aspects of intralesional transformation should be emphasized. (a) Of all of the events in the development of neoplasia, only intralesional transformation is seemingly inexorable; all other phenomena can become indolent or even regress. (b) Intralesional transformation is seemingly a rare event in neoplasia. As judged from the work of Edgcomb and Mitchelich (12) and from observations of our own experiments, intralesional transformation does not affect more than 4 to 5% of animals bearing induced melanotic lesions.

Intralesional transformation could well be regarded as the most important event in the developmental biology of primary neoplasia. Without it, neoplasia would be a relatively innocuous, poorly understood form of pathological hyperplasia.

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REFERENCES

25. Lipschütz, B. Untersuchungen über Experimentelle Pigmenterzeugung
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Fig. 1. Lesion 1. Four brown or black macules form the earliest class of clinical lesion. × 0.8.
Fig. 2. Lesion 2. Five or more brown or black macules. × 0.8.
Fig. 3. Lesion 3. Brown and black macules with 1 or more black, elevated nodules 2 to 5 mm in diameter (arrows). × 0.8.
Fig. 4. Lesion 4. Previous kinds of lesions plus 1 or more nodules (arrow) 5 to 10 mm in diameter. × 0.8.
Fig. 5. Lesion 5. Previous kinds of lesions plus 1 or more nodules larger than 10 mm in diameter. The large black nodule is ulcerated. The mass in the lower left is a lymph node with massive metastasis. × 0.8.

Fig. 6. Lungs and trachea. The lung parenchyma and paratracheal lymph nodes are studded with metastatic melanoma. × 1.4.

Fig. 7. Intraepidermal melanocytic hyperplasia. Single arrows, representative melanocytes. Apposed arrows, pigment-engorged dendrites. × 700.

Fig. 8. Atypical intraepidermal melanocytic hyperplasia. The large, deeply pigmented cells in the basal layer are atypical melanocytes. × 380.
Fig. 9. Dermal melanocytosis. Apposed arrows, elongate, pigment-containing, fibroblast-like cells in the dermis which comprise this lesion. × 152.

Fig. 10. Dermal melanocytosis. A larger lesion than is shown in Fig. 9. The black zone around the pilar unit in the right half of the field is an area where atypical, larger melanocytes have become confluent. × 95.

Fig. 11. Dermal melanocytoma. The lesion is composed entirely of large atypical melanocytes, uniformly engorged with melanin. × 38.

Fig. 12. Dermal melanocytoma. Higher magnification of base of lesion illustrated in Fig. 11. There is invasion between collagen bundles in the dermis. × 152.
Fig. 13. Lymph node, metastatic melanoma. The upper half of the node is completely replaced by metastatic melanoma. The black semicircular line is the pigment cell-engorged peripheral sinus of the node. × 20.

Fig. 14. Malignant melanoma with intralesional transformation. The relatively pigment-free cells in the lower half of the picture are the "new kinds of cells" which are associated with widespread metastases. × 20.

Fig. 15. Malignant melanoma with intralesional transformation. The nests of relatively pigment-free cells first appear as small clusters within the pigment-engorged melanocytes. × 243.

Fig. 16. Lung with metastatic melanoma. × 45.
Fig. 17. Liver with isolated metastatic melanoma cells still showing preservation of dendrites. × 900.
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