The Growth and Metastasis of Amelanotic Melanomas in Heterologous Hosts

HARRY S. N. GREENE AND ELIZABETH K. HARVEY

Department of Pathology, Yale University School of Medicine, New Haven, Connecticut

Summary

The transformation of a hamster melanotic melanoma to an amelanotic melanoma is associated with an enhancement in heterologous transplantability and the attainment of the ability to metastasize in the alien host. Heterologous transfer is successfully accomplished with the eye, brain, testicle, muscle, or subcutaneous space utilized as a transplantation site, and metastasis is rapid and widespread.

Introduction

The occurrence in the hamster of a melanotic melanoma with a propensity for amelanotic transformation has been reported (2). The melanotic tumor and its amelanotic derivatives differ in behavior as well as in appearance. The most distinctive biologic changes associated with amelanotic transformation consist of an enhancement in heterologous transplantability and the attainment of the ability to metastasize in the alien host.

Materials and Methods

A comparative study of the 2 tumors was initiated shortly after discovery of the amelanotic variant. The altered behavior of the variant was noted, but a heating accident in the animal colony resulted in widespread fatality, including all of the hamsters bearing amelanotic tumors. Several melanotic tumor bearers survived the hyperthermia, and their growths have been carried by serial transfer to the present time. The spontaneous occurrence of amelanotic transformation, however, has not been observed.

Experimental attempts to modify the melanotic tumor or its bearer were initiated, and 2 of these proved successful. In one instance, a melanotic tumor was frozen and maintained at low temperature for a month. Transfer of fragments resulted in widespread fatality, including all of the hamsters bearing amelanotic tumors. Several melanotic tumor bearers survived the hyperthermia, and their growths have been carried by serial transfer to the present time. The spontaneous occurrence of amelanotic transformation, however, has not been observed.

The hamsters used in the experiments were of the Golden Syrian variety, and the heterologous hosts were purebred standard Dutch rabbits. Occasionally, guinea pigs of mongrel stock or mice of the DBA or BDF strain were utilized. The method depends upon the fact that the intra-aortic inoculation of cells from melanotic melanomas produces amelanotic metastases. Melanotic melanoma cells introduced i.v. or by vascular invasion from an s.c. transplant metastasize to the lung in 100% of the cases. The cells enter the systemic circulation on the 11th day in the former instances and during the 7th week in the latter, but metastases in the organs of that circulation are extremely uncommon (3). Further, all metastases are black and duplicate the primary tumor in appearance.

Results

INTRA-ARTERIAL DISSEMINATION IN HAMSTERS. Metastasis rapidly follows the intra-arterial injection of melanotic melanoma cell suspensions in the hamster, and the majority of animals die within a month. Secondary growths are present, which are

---

1 Research supported by the Jane Coffin Childs Fund for Medical Research (45), the USPHS, (CA-918), and the American Cancer Society, Inc. (P-336).

Received for publication May 26, 1965; revised September 21, 1965.
of large size, in the kidneys, liver, ovaries, adrenals, lungs, heart, and thymus, and many thoracic, abdominal, and subcutaneous lymph nodes are involved. Metastasis to the eye and brain occurs in approximately 10% of the cases. This distribution differs radically from that following invasion from a transplantation site or i.v. inoculation, for in such cases, metastases are limited to lymph nodes and lungs and are not found in organs of the systemic circulation. The metastases also differ in appearance. Intravenous metastases are uniformly black and duplicate the melanin content of the original tumor. In contrast, metastases derived from arterial-borne cells, without lung passage, range through grades of gray to uniform white (Figs. 1, 2).

Microscopic study of larger, partially pigmented metastases shows a preponderance of melanin contained in macrophages or in normal tissues of the involved organs rather than in the tumor cells themselves. The tumor cells in white metastases are identical with those found in melanotic growths, with the exception of a complete absence of melanin granules. There is no constancy in the bodily area associated with pigmented or nonpigmented growths, but the kidney, brain, eye, and inter- scalpular nodes appear to be the most consistent sites of completely amelanotic tumor tissue.

In any case, further amelanotic transformation follows additional intra-arterial passage of the cells, and a similar result attends brain transfer (Fig. 3). However, transfer to the hamster's subcutaneous space enhances rather than diminishes the ability to produce melanin.

Heterologous Transfer of the Parent Tumor. The parent melanoma is rarely transplantable in alien species. Growth has been obtained in the brain of a single DBA mouse and in the subcutaneous space of another, despite the use of 60 animals in the experiments. The mouse bearing the brain tumor was killed 2 months after transfer, and the transplanted fragment had increased by 3 diameters in size. The subcutaneous growth was detectable by palpation in 70 days and showed no increase in size during the subsequent month. Both of the growths were highly pigmented on microscopic examination, and neither survived serial transfer. No growth was obtained in the anterior chambers or brains of rabbits or guinea pigs, although attempts were made in 30 animals of each species.

The melanotic tumor is deep black in appearance. Morphologically, it contains an abundance of melanotic pigment, but nonpigmented cells comparable to those found in amelanotic metastases are invariably present (Fig. 26). The transplanting behavior of this tumor has been described (2, 3).

Transfer of Amelanotic Metastases to Rabbits. In contrast to the failure of melanotic tumor tissue to survive rabbit transfer, tissue derived from amelanotic metastases arising from the intra-arterial transfer of melanoma cells in the hamster grows readily in alien species. The amelanotic metastases represent proliferations of nonpigmented cells present in the injected suspension, and the tumors induced on heterologous transplantation are made up entirely of such elements (Figs. 15, 25). On electron microscopic examination, the cells are primitive in appearance and, on a fine structural level, cannot be differentiated from other anaplastic growths.

The original transplantation site in all instances has been the eye or the brain, but subsequent transfers have been carried out in the testicle, the thigh muscle, and the subcutaneous space, and the tumors have been maintained by serial passage in these sites.

Amelanotic tumor tissue derived from 15 different melanoma strains has been successfully transferred to rabbits. It is essential for successful passage that completely amelanotic tissue is used. Two rabbits were utilized in each of the transfers noted above, and takes occurred in all but 1 animal. In contrast, no takes whatsoever followed transfer of partially amelanotic tumors to a total of 20 rabbits.

The initial transplants in the rabbit’s eye fill the anterior chamber in approximately 2 weeks, but with continued passage, the rate increases and a growth of comparable size is found in 7 or 8 days. Rupture of the cornea occurs shortly after the chamber is filled, and the majority of experiments have been terminated before that time. However, 5 rabbits held to the 31st day showed widespread metastases involving the skin, contralateral eye, brain, pituitary, mammary tissue, skeletal muscle, liver, kidney, spleen, ovaries, adrenals, gastric and intestinal mucosa, diaphragm, lungs, heart, and thymus (Figs. 5-20).

The earliest metastases have been found on the 21st day, involving the contralateral eye and the lung. However, vascular invasion in the eye and endothelial binding in various organs can be demonstrated as early as the 7th day by the growth of tumor in tissue fragments transplanted to normal animals (5). The rapidity of vascular invasion in alien species contrasts sharply with the period of 5 weeks required by the parent tumor, the malignant melanoma, to manifest the same ability in the natural host, the hamster.

Growth in the rabbit’s brain also varies in speed in different transplant generations (Fig. 4). Early transplants require a month or more to induce sufficient intracranial pressure to cause death, but the length of this period decreases to 15 or 20 days in later passages. The transplants are large and may replace two-thirds of a cerebral hemisphere. Despite their size, the vascular supply is abundant and necrosis is uncommon. The brain tissue is actively invaded, and extension into the meninges with distant passage and re-entry into brain substance through Virchow-Robin spaces is often observed (Fig. 14). Ventricular extension occurs as a solid column of tumor or as isolated cell clumps. The latter eventually adhere to the choroid plexus and invade its substance. Secondary growths, limited to the brain, may arise in this manner, but true extracranial metastasis has been observed only in animals living for more than a month after transfer. In such cases, the distribution is as widespread as that following dissemination from the anterior chamber of the eye.

Growth in the testicle to the point of extensive metastasis usually requires 30 days. At this time, the growth is large, contains massive areas of necrosis, and extends into the abdomen along the spermatic cord. Metastasis is widespread, involving the eye and brain as well as all organs of the abdomen and thorax. Intramuscular transplants also attain a large size, and central necrosis is a dominant feature. The time and distribution of metastases are comparable to those following testicular transfer. Subcutaneous transplants are largely necrotic at the end of the month, and metastasis is long delayed and of limited distribution.

Intravenous and Intra-Arterial Transfer. The i.v.
intra-arterial injections of amelanotic tumor cell suspensions in the rabbit result in death between the 20th and 30th days. The distribution of metastases is identical, irrespective of a venous or an arterial source of tumor cells, and duplicates that obtained from the vascular invasion of transplanted tumor tissue. This behavior constitutes a pronounced deviation from that of the parent tumor in the natural host. The metastatic distribution of the melanotic melanoma in the hamster from s.c. transplants and from the i.v. injection of cellular suspensions is identical, being limited to the lymph nodes and lungs. But the results of intra-arterial transfer are entirely different, consisting of body-wide distribution. It should be emphasized that the experiments represent homologous transfers in the case of the parent tumor and heterologous transfers in the case of the derived tumor.

TRANSFER TO GUINEA PIGS AND MICE. The amelanotic tumors also survive transfer to guinea pigs and mice, and in both species, the brain is a better transplantation site than the anterior chamber of the eye (Fig. 21). Tases in the guinea pig's brain occur with twice the frequency noted in the mouse, and this species provides an economical site for long-term maintenance.

RETранFER TO HAMSTERS. Transfer of the heterologously grown tumors back to the subcutaneous space of hamsters is associated with a significant diminution in the number of takes. The loss of transplantability is incident to heterologous transfer, for if the tumor is maintained in the hamster as well as transferred to rabbits, the modification is shown only by the rabbit-grown specimen. Necrosis is a dominant feature of the successfully transplanted tumors, and growth is short-lived (Fig. 22) In addition, the i.v. or intra-arterial injection of cellular suspensions of rabbit-grown tumor fails to produce metastases in hamsters.

Similarly, transfer from the rabbit to the hamster brain results in a reduction in the frequency of takes—15% in the hamster in contrast to 100% in the rabbit. However, the incidence of takes in the hamster's brain is increased to 100% on 2nd-generation transfer, and the brain-grown tumor attains the ability to grow in the subcutaneous space and to produce metastases on i.v. or intra-arterial injection (Figs. 23–25).

Discussion

Partially amelanotic melanomas have been produced in mice by the continuous selection of paler portions of a melanotic tumor for transplantation (1, 6). A similar procedure was successfully used in early experiments with the hamster melanoma, but the accidental death of a large proportion of tumor-bearers apparently involved all of the tumor tissue capable of spontaneous transformation. The mechanisms operative in inducing transformation by the methods presently employed are not understood, but the possibility exists that the arterial circulation and the brain provide conditions favoring the growth of a scanty content of tumor cells incapable of producing pigment.

The change in behavior following amelanotic transformation is not evidenced on continued hamster transplantation. Transfer of the amelanotic tumors from the hamster's brain or bodily organs to the subcutaneous tissues of another hamster does not result in a more rapid growth rate nor is the metastatic distribution different from that noted in the case of the parent melanotic melanoma. Further, the distribution of metastases following i.v. injection is identical with that of the parent tumor, being limited to lungs and lymph nodes.

The change in behavior becomes apparent only after rabbit growth. Transfer of the original melanotic tissue to the rabbit's eye is not associated with growth. In contrast, the amelanotic variant fills the anterior chamber in less than 2 weeks, and large, widespread metastases are present in a month. Actually, vascular invasion in the anterior chamber has been demonstrated to occur on the 7th day of growth, whereas vascular invasion of the parent tumor in the subcutaneous space of the hamster requires approximately 5 weeks (3, 5).

The amelanotic melanomas also metastasize from the rabbit's brain. This is a rare property of homologous brain transplants and has not been observed in any experiments with heterologous brain transplants. Vascular invasion and endothelial binding do occur in both types of brain transfer, but the bound cells in heterologous species apparently do not permeate endothelial walls to attain stroma and grow to adequate size for microscopic recognition (4). However, the cells of rabbit brain transplant of a hamster amelanotic melanoma not only invade blood vessels and adhere to endothelium but also permeate to interstitial tissues and grow to constitute metastases.

The spontaneous amelanotic conversion of large segments of growing melanomas previously noted in this laboratory has not occurred in recent years and presumably relates to a lowered ratio of the melanotic cell content of the growths. The i.v. injection of cellular suspensions of present melanotic tumors gives rise to rare, minute pulmonary amelanotic metastases, but although tumor cells are present in the systemic circulation 2 weeks after entrance in the lung, the uncommon metastases found in organs of this circulation are always melanotic in character. A question, therefore, arises in relation to the high frequency of amelanotic metastases of the same tumor suspension following arterial, rather than venous, inoculation.

It is suggestive that, as in the case of other tumors, long retention of melanotic tumor cells in the lung before release into the systemic circulation is likewise associated with an absence of metastases in the organs of that circulation. The failure to metastasize is not related to an inability to grow in the organs concerned, for direct transfer of fragments or cellular suspensions produces large, rapidly growing tumors. On the contrary, the failure stems from the unrestrained passage of tumor cells through the blood stream without endothelial adherence, permeation, and extravascular position. The loss of binding capacity follows long retention in the lung, and in present experiments, the arterial circulation was utilized to bypass the lung and preserve the ability of injected amelanotic tumor cells to adhere to endothelium.

The procedure led to the production of amelanotic tumors, but the incidence was in excess of the estimated amelanotic cell content of the injected suspensions. Sufficient melanotic cells were present in the suspensions to produce innumerable melanotic pulmonary metastases on i.v. injection, but their occurrence on arterial injection was a rarity. Several possible explanations appear: either the amelanotic cell content of the tumors used is higher than their heavily pigmented appearance suggests, a
conversion of melanotic to amelanotic cells occurs before endothelial binding, or an affinity for amelanotic cell binding distinguishes arterial endothelium.

The loss of the ability to produce melanin and the enhancement of malignancy may be causal or fortuitous in association. The properties concerned in the production of melanin may act as deterrents in the development of full neoplastic potential of the cell, or the melanin may exist without inhibitory influence and function in this respect only as an incidental marker of the developmental stage of the tumor. In any case, the melanotic melanomas do not possess neoplastic attributes comparable to those of the amelanotic tumors, and the mechanism involved in the continuation of malignant development is the subject of present study.

References
FIG. 1. Metastases from subcutaneous transplant of melanotic melanoma in hamster. Note metastases limited to axillary lymph node and lung. X 1.

FIG. 2. Metastases from intra-arterial injection of melanoma cells in hamster. Metastases are present in lung, liver, adrenals, and kidney, and except for several growths in the ovary, all are amelanotic in character. X 2.5.

FIG. 3. Growth of amelanotic tumors in hamsters' brains 16 days after transfer from hamster kidney of metastases of intra-arterial-borne melanotic melanoma cells. X 1.5.

FIG. 4. Early growth of amelanotic melanoma in brain of rabbit killed 16 days after transfer. X 4.
FIG. 5. Metastases in lung and kidneys of rabbit from intramuscular transplant of amelanotic melanoma killed 2 month after transfer. X 1.

FIG. 6. Metastases in adrenals and ovaries in same animal. X 1.5.

FIG. 7. Metastases in gastric mucosa in same animal. Such metastases occur with great frequency in the fundus of the stomach and grossly resemble ulcers. X 1.

FIG. 8. Metastases in gastric mucosa of rabbit with testicular transplant of an amelanotic melanoma. X 1.

FIG. 9. Metastases in liver of same animal. X 0.5.

FIG. 10. Metastasis in ciliary process of eye in same animal. X 130.
Fig. 11. Section of liver metastasis. X 110.

Fig. 12. Section of ovarian metastasis. X 110.

Fig. 13. Section of kidney metastasis. X 110.

Fig. 14. Section of brain metastasis showing extension along meninges and growth down an enlarged Virchow-Robin space. X 110.

Fig. 15. High-power photograph of brain growth. X 220.

Fig. 16. Section of growth at brain junction to show brain invasion. X 110.
FIG. 17. Section of lung metastasis. X 110.
FIG. 18. Section of heart metastasis. X 110.
FIG. 19. Section of metastasis in gastric mucosa. X 55.
FIG. 20. Section of metastasis in intestinal mucosa. X 220.
FIG. 21. Section of transplant in guinea pig's brain. X 180.
FIG. 22. Section of transplant in hamster's subcutaneous space. The fragment for transfer was obtained directly from the rabbit's eye. X 220.
Fig. 23. Photograph of 8th-generation hamster bearing a subcutaneous transplant of amelanotic tumor derived from a growth in a hamster's brain of tissue obtained from a rabbit's anterior chamber. × 0.75.

Fig. 24. Metastasis in lung of tumor shown in Fig. 23. × 200.

Fig. 25. High-power view of metastasis shown in Figure 23. × 700.

Fig. 26. Section of melanotic melanoma from the subcutaneous space of a hamster. Note melanin-producing cells as well as amelanotic cells comparable to those in Figs. 15 and 25. × 700.
The Growth and Metastasis of Amelanotic Melanomas in Heterologous Hosts

Harry S. N. Greene and Elizabeth K. Harvey


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/26/4_Part_1/706

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.