TWO CASES OF MÉLANOSE NEUROCUTANÉE WITH DEVELOPMENT OF MALIGNANT MELANOMA: A MICROSPECTROPHOTOMETRIC AND ELECTRON MICROSCOPIC STUDY

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Two cases of neurocutaneous melanosis with development of malignant melanoma in the Japanese are presented. The first case was a 4-year-old boy in whom a retroperitoneal melanoma appeared with giant nevi, and cerebral and spinal melanosis. The second case was a 39-year-old man, in whom a primary leptomeningeal melanoma developed with leptomeningeal melanosis and smaller pigmented nevi. Microspectrophotometric and electron microscopic studies were made on the neoplastic and non-neoplastic melanotic tissues to elucidate the histogenesis of this rare disorder. Two different patterns of nuclear DNA histograms, corresponding to melanosis and melanoma, were obtained by microspectrophotometry. Considerable variation in the ultrastructure of the melanocytes was seen by electron microscopy.

Neurocutaneous melanosis3,8,9,10,11) is a congenital abnormality, characterized by excessive proliferation of melanotic tissue, affecting the central nervous system as well as the skin. In the basic type of this condition, melanosis of the leptomeninges or brain substance is usually associated with nevus cell nevus of the skin. Furthermore, malignant melanomas frequently develop in this disorder, especially at the base of the leptomeningeal melanosis.

This paper reports two autopsy cases of the Japanese showing secondary development of malignant melanoma, and microspectrophotometric and electron microscopic observations on the neoplastic and non-neoplastic melanotic tissues.

Case Report

Case No. 1
Clinical History: A 3-year-old Japanese boy with a noncontributory family history exhibited an extensive hairy nevus at birth in July 1967. Multiple smaller nevi with similar characteristics were scattered over the face, abdomen, and upper extremities. The child was healthy in the neonatal period but at 1 month of age he developed recurrent attacks of convulsion, which responded to anticonvulsant.

Resection and electrocoagulation of the facial and brachial nevi were attempted between the 3rd and 6th month after birth. By July 1968, episodes of convulsions became less frequent. However, a swelling of the size...
of hen’s egg in the right inguinal region and a palpable abdominal mass were noticed in March 1970. On May 13, laparotomy revealed a large brownish black tumor infiltrating the right lower retroperitoneum and a biopsy specimen showed this to be a malignant melanoma.

Radiotherapy with a total dose of 4000 R of 60Co to the abdominal tumor and treatment with anticancer agents (Daunomycin, cyclophosphamide, and vincristine) were only temporarily effective. From August 1970 multiple metastases were observed in the bony skull, ribs, lymph nodes, and skin. The child died on November 2, 1970. Autopsy was carried out 3 hr after death.

Autopsy Findings (Autopsy No. TUH-26229)

Skin: A huge knickerbocker-like hairy nevus was seen with multiple smaller nevi with similar characteristics in other parts of the skin (Photo 1). Histologically the epidermis was uneven, showing slight papillary projections in some parts, but there were no lesions suggestive of cutaneous melanoma. Dense accumulation of nevus cells was found in the dermal layer (Photo 10).

The nevus cells in the upper part of the dermis were round or polyhedral with a considerable amount of melanin pigments in the cytoplasm. The nuclei were generally small and uniform in size. Junctional activity was rarely encountered.

Central Nervous System: The brain was edematous, weighing 1,140 g. There were several patchy brown spots in the leptomeninges of the frontal lobes. Thick villous proliferations were also noticed on the epi- dural surface. Histologically these foci appeared to be accumulation of melanotic cells with scanty cytoplasm and relatively uniform-sized round to oval nuclei.

In cut section, numerous dark areas were observed in the grey matter of the brain and in the white matter of the frontal lobes, right amygdala (Photo 3A), right cerebellar hemisphere, and pons (Photo 3B) without any gross alteration of the architecture. Histologically these foci consisted of accumulations of cells heavily laden with melanin (Photo 8). The cells had relatively abundant cytoplasm and small inconspicuous nuclei.

The leptomeninges covering the spinal cord were jet black (Photo 4). Section of the cord at various levels showed accumulation of black material in the subarachnoid space. The cells were round, showing moderate pleomorphism. These cells were found in clusters around the roots of the spinal nerves but there was no evidence of infiltration of the nerve sheath (Photo 9).

Abdominal Tumor: A soft, brownish black tumor, measuring $19 \times 20 \times 9$ cm, was found attached to the right lower retroperitoneum. The right kidney, ureters, both adrenals, and great vessels were embedded in this tumor (Photo 2). Numerous disseminants were also noticed on the parietovisceral peritoneum and mesentery. Unlike melanotic cells in the central nervous system and skin, the tumor cells in this area had a compact arrangement with very little connective tissue (Photo 7). Round to polyhedral cells had scanty cytoplasm and oval nuclei with prominent nucleoli and they showed marked pleomorphism with a few giant cells. Numerous mitotic figures were observed. Tumor cells showed little pigmentation and appeared amelanotic in some parts.

Case No. 2

Clinical History: A 39-year-old Japanese male was admitted to the Mitsui Memorial Hospital, Tokyo, in November 1971 with complaints of persistent frontal headache and xanthochromic cerebrospinal fluid. Examination revealed a tumor in the frontotemporal lobe. At operation, the right sylvian fissure was found to be infiltrated by a black tumorous mass and multiple miliary-sized black deposits were noted over the leptomeninges.

Biopsy material indicated a malignant melanoma. Treatment with N,N-bis(2-chloroethyl)-N-nitrosourea (BCNU) and vin-
crisine was ineffective. Right visual field disturbance and deafness ensued, and the patient died in a comatous state 3 months later in February 1972. Autopsy was performed 2 hr after death.

Autopsy Findings (Autopsy No. TUH-26735)

Skin: There were two slightly elevated hairy nevi in the lumbar region and on the left arm, measuring 4×5 cm and 6×4 cm, respectively. Brownish fleck-like pigmentation, measuring up to 2×0.5 cm, were scattered all over the skin. Most of these lesions were due simply to hyperpigmentation of the basal layer of the epidermis.

In two pigmented nevi, the upper dermis contained nests and cords of nevus cells, which had small, dark, oval nuclei and indistinct cytoplasm, and sometimes melanin pigments. Histological diagnosis of the lesions was consistent with intradermal nevus (Photo 12). No junctional activity was found.

Central Nervous System: The dura was not involved, but pigmented cells were clustered together at the site of granulationes arachnoideales. The brain weighed 1,320 g and was markedly swollen. The inferior medial portion of the right temporal lobe was infiltrated by a large tumor mass, measuring 7×4×3.5 cm (Photo 5). The leptomeninges covering the brain and spinal cord (Photo 6) were jet black. Black discoloration was marked bilaterally on the inferior surface of the temporal lobes, the cerebellum, medulla oblongata, and all regions where the subarachnoid space was wider. Deposits were relatively scanty over convexities. Involvement of the roots of the cranial nerves was observed. Continuous infiltration along the optic canal, foramen ovale, and porus acusticus internus was also noted.

Section of the spinal cord at various levels showed a large accumulation of black material in the subarachnoid space. No infiltration of the cord was found.

The histological features of the biopsy specimen and autopsy material did not vary widely. However, considerable variation was seen from section to section. The tumor cells were relatively loosely arranged, sometimes giving an alveolar appearance, but supporting connective tissue was scarce. Oval or polyhedral cells had pale, plump cytoplasm and hyperchromatic nuclei showing marked pleomorphism (Photo 11). Pigmentation of the cytoplasm was irregular and uneven. Nuclei had relatively coarse chromatin which tended to condense on the nuclear membrane and there were one or two prominent nucleoli. Giant or multinucleated cells were numerous. Mitotic figures and atypical mitoses were frequently found.

Some tumor cells had a perivascular rosette-like arrangement. Leptomeninges of the brain and spinal cord were extensively infiltrated by tumor cells, but direct invasion of the brain substance was only found at the primary site. The two optic nerves were very severely involved by tumor cells. Permission could not be obtained to examine the eyes, but the two optic tracts were removed and examined with the optic canal close to the eye balls. No tumor cell infiltration was found at the distal ends of the optic tracts. The acoustic, vestibular, and trigeminal nerves were also infiltrated bilaterally. The hypophysis was intact with only perinfundibular infiltration. Other findings of note were latent carcinoma of the thyroid, bronchopneumonia, and a tiny cavernous hemangioma of the liver. No melanotic tumor tissue was found in any of the other viscera.

Microspectrophotometric Study

The lesions examined by microspectrophotometry were the retroperitoneal melanoma, skin nevus, spinal leptomeningeal melanosis, and cerebral epidural melanosis from Case 1, and the primary leptomeningeal melanoma, skin nevus, and melanosis at the site of the granulationes arachnoideales from Case 2. As controls, neuroglial cells (Case 1) and nerve cells (Case 2) were used.
Sections of 7 μm thickness were cut from the paraffin blocks used for histological examination. Sections were treated with 0.25% K-MnO₄ at room temperature for 45 min (Case 1) or 30 min (Case 2) to bleach the melanin and then stained with Feulgen’s method for DNA (hydrolysis in 1N HCl at 60° for 10 min (Case 1) or 15 min (Case 2), and then treatment with Schiff’s reagent). Sections were examined with a Zeiss scanning microscopemphotometer. Before scanning, we focussed nuclei at various levels to ensure that the cells were completely within the tissue; partially sectioned nuclei were discarded. The total absorption of nuclei was measured with a monochromatic light (560 nm). More than 50 cells from different lesions were examined.

Melanosis of the pons and amygdala was not examined because the time required to bleach melanin in these lesions was much longer, resulting in impairment of Feulgen’s reaction. The ratio of the DNA content of the tumor and melanotic tissue to the mean value of that of control cells is expressed in arbitrary units in the histograms in Figs. 1 and 2.

The histogram of the retroperitoneal malignant melanoma in Case 1 (Fig. 1) showed a tetraploid peak and a wide range of hyperploid distribution. Other histograms from the spinal leptomeningeal, epidural, and dermal melanosis in Case 1 (Fig. 1) had a diploid peak with a slightly hyperdiploid distribution in the spinal leptomeningeal melanosis. The pattern of the histogram from
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The lesions examined by an electron microscope were the retroperitoneal melanoma, skin nevus, spinal leptomeningeal melanosis, and melanosis of the pons from Case 1, and the primary leptomeningeal melanoma, skin nevus, and melanosis at the site of granulationes arachnoideales from Case 2.

Small pieces of tissues were cut from autopsy materials, fixed in 10% Formalin, and postfixed in 1% OsO4 in phosphate buffer (pH 7.3). Tissues were dehydrated by passage through a graded series of ethanol and embedded in Epon-Araldite. Thin sections were stained with uranyl acetate and lead hydroxide, and examined with a Japan Electron Optics Laboratory JEM 100B electron microscope.

Although tissues for electron microscopy were obtained from autopsy materials, details of the cells were relatively well preserved, especially the fine structure of the melanosomes. The tumor cells in Case 1 had large nuclei with indentations and there were a number of round melanosomes showing a granular and striated inner structure (Photo 13). Melanophages were sometimes found among them. Tumor cells in Case 2 had large, irregular-shaped nuclei and abundant cytoplasm containing a number of large mitochondria with vesicular-shaped cristae. A few melanosomes showed coarse granularity (Photo 17). Autophagic melanosomes were also seen. In the spinal leptomeningeal melanosis in Case 1, the cells had euchromatic nuclei and relatively abundant round cytoplasm, containing numerous melanosomes at various stages of maturation. The melanosomes were elipsoid and premelanosomes showed distinct longitudinal striation (Photo 14).

In the melanosis of the pons in Case 1, the melanocytes also contained a number of melanosomes.
melanosomes with a homogeneous electron-dense inner structure (Photo 15). In the dermal melanosis of both cases, melanocytes were smaller in size and they were separated from the surrounding collagen by a basement membrane (Photos 16 and 18). Some cells contained a considerable number of melanosomes which were very electron-dense. They mostly had a finely granular inner structure and sometimes they were aggregated into a complex form. Fine filaments were also found in the cytoplasm.

**DISCUSSION**

The present two cases can definitely be diagnosed as cases of mélanose neurocutanée, although Case 2 showed little cutaneous manifestation of this condition.

The question of malignancy is more difficult to evaluate from histological observations alone because invasiveness, cellular atypism, and irregular proliferation of the cells are sometimes difficult to interpret in this disorder.

Bleaching of the melanin pigment is essential for histological examination. In this study, this was done by treatment with 0.25% potassium permanganate for 30~45 min, which did not affect the stainability with Feulgen's reagent. Thus, we could measure nuclear DNA by microspectrophotometry, which as far as we know has not been done previously on a melanotic tumor. Scanning microspectrophotometry of various lesions in the two cases showed two different patterns, probably corresponding to two conditions, namely melanosis and melanoma.

The histogram of the spinal leptomeningeal melanosis in Case 1 showed only a slightly hyperdiploid distribution compared to that of the other melanosis. However, this histogram was quite different from that of the retroperitoneal melanoma in Case 1.

The origin of this retroperitoneal malignant melanoma is obscure. Analysis of clinical data and very detailed autopsy failed to disclose any other possibilities, and it seems likely that the primary retroperitoneal tumor was the primary deposit. However, the presence of melanocytes in the peritoneum or the retroperitoneum in man is not recorded as a normal finding. Thus, it seems likely that the melanoma arose from spinal or sympathetic ganglionic cells which have melanin pigments.

It seems probable that Case 2 was of primary leptomeningeal origin. With regard to the histogenesis of the malignant melanoma of the central nervous system, there is a general agreement that pigmented cells normally present in the meninges may multiply, forming a meningeal melanosis, and that meningeal melanomas frequently arise from the latter.

The exact distribution of preexisting melanosis was hard to determine in Case 2 due to diffuse infiltration of malignant cells into the meninges but microspectrophotometric study revealed that melanosis remained at the site of the granulationes arachnoideales. Malignant change can occur elsewhere along the neuraxis which may have been the seat of widespread melanosis but, in this case, widespread subarachnoid aggregates were due to dissemination from a right temporal tumor, which showed distinct local invasion.

Electron microscopy of the melanosis and melanomas in both cases showed considerable variation in ultrastructure of the melanocytes in different lesions, but the dermal melanosis had a very similar ultrastructure in both cases, showing the characteristics of nevus cells.

Hirone et al. suggested that malignant melanomas which arise from normal skin, from Hutchinson's melanotic freckle, and from junctional nevus can be distinguished by the ultrastructure of their premelanosomes. However, in the case of neurocutaneous melanosis, our observations suggest that the ultrastructure of the melanin-containing granules shows no such correlation...
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between melanomas and the preexisting condition, namely melanosis.

In some cases of melanosis of the central nervous system melanosis in brain substance other than meninges has been reported. In Case 1, melanosis of the brain substance was also observed. The inner structure of melanosomes of the pons melanosis was very electron-dense and homogeneous, with a glass-like appearance. These melanosomes were thought to be identical to those of the pigments normally found in the nerve cells in gray and white matters, including the medulla oblongata, the pons, locus ceruleus, and substantia nigra. The melanin granules in this region may be different from those in other regions because they took longer to bleach. These observations suggest that these lesions are formed by multiplication of melanin-laden cells normally existing in the brain substance, and that they are not due to secondary deposits from leptomeningeal melanosis.

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EXPLANATION OF PLATES

Photo 1. Giant knickerbocker-like nevus and multiple nevi in Case 1.
Photo 2. Posterior view of large abdominal tumor, embedding the retroperitoneal organs in Case 1.
Photo 3. Melanosis of the right amygdala (3A) and pons (3B) in Case 1.
Photo 4. Spinal leptomeningeal melanosis in Case 1.
Photo 5. Melanoma arising from the right temporal lobe in Case 2.
Photo 6. Melanoma infiltrating to the subarachnoid space of the spinal cord in Case 2.
Photo 13. Electron micrograph of the retroperitoneal melanoma in Case 1. Note large indented nuclei and round melanosomes (inset) showing both a granular and a striated inner structure. ×8,000.
Photo 14. Spinal leptomeningeal melanosis in Case 1. The cells have abundant cytoplasm containing numerous melanosomes at various stages of maturation. Melanosomes are ellipsoid and premelanosomes show distinct longitudinal striation. ×17,000.
Photo 15. Melanosis of the pons in Case 1. Melanosomes had a homogeneous, electron dense inner structure (inset). ×4,800.
Photo 16. Melanosis of the skin in Case 1. Note electron dense melanosomes showing a granular inner structure. ×19,000.
Photo 17. Leptomeningeal melanoma in Case 2. Note the presence of few melanosomes and large mitochondria with vesicular-shaped cristae. ×28,000.
Photo 18. Skin melanosis in Case 2. The fine structure was similar to that in Case 1. ×14,000. H-E=Hematoxylin and Eosin stain.
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