MALIGNANT MELANOMA IN A BDF₁ (C57BL/6NCrj×DBA/2NCrj) MOUSE

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Abstract: A melanocytic tumor was found in the skin or subcutaneous tissue in a BDF₁ mouse maintained up to 109 weeks. The tumor was solid and well-demarcated from the surrounding tissue, and showed an extensive metastasis to lymph node, liver, and lung. The tumor was composed of both oval or polygonal cells with abundant melanin pigments, and spindle cells mixed with the oval or polygonal cells. These blackish pigments in the tumor cells disappeared by bleaching and more blackened by Fontana-Masson stain. The tumor cell showed poor cellular pleomorphism and rare mitosis. Numerous mature, stage IV-melanosomes were observed in the cytoplasm of the tumor cells by electron-microscopical examination. It was suggested that the origin of the present tumor was a dermal melanocyte by its histological features, though no definite conclusion was obtainable because of ulceration in the central area of the skin covering the nodule. (*J Toxicol Pathol* 2: 111~116, 1989)

Key words: Malignant melanoma, Mouse

Introduction

The tumors of melanogenic system have a large difference in their occurrence between animal species. These tumors are relatively common in human, dogs, and old gray horses, but they are extremely rare in mice. A few spontaneous melanomas including B-16 melanoma from C57BL/6J mouse^{2-4,10,11,13-15}, Cloudman S-91 melanoma of DBA/2 mouse^{1,3,10}, and Harding-Passey melanoma^{2,9,14} have been reported. The common characteristics of these tumors are that they are all malignant, heavily pigmented, and origins of the transplantable tumor lines and culture cells. As for the induced melanogenic tumors in mice and hamsters, there are a few studies on melanocytic tumor of the skin which was induced

by chemical carcinogen, such as 9, 10-dimethyl-1, 2-benzanthracene (DMBA)^{2,5-8,12} or 12-*o*-tetradecanoylphorbol-13-acetate (TPA)¹².

Case Report

The present case was found in a BDF₁ (C57BL/6NCrj×DBA/2NCrj) mouse*¹ of the low-dose treated group in an oncogenic test which was carried out in our laboratory. Results of the examination showed no effects likely to be caused by the test substance. Four hundred mice were maintained under the specific pathogen free (SPF) environment controlled at 23±1°C, 55±5% in humidity, 225±75 lux 13-hrs in light. They were housed individually in each suspended stainless wire-mesh cages. Free access to the basal diet, which was manufactured following modified NIH Open Formula for Rat and Mouse and sterilized by gamma radiation (Oriental yeast Co., LTD.) and tap water were provided. They were

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*¹Four-weeks-old BDF₁ mice were purchased from Nippon Charles River Co. Ltd., Kanagawa, Japan.

sacrificed at 109 weeks of age. The tissue specimens were fixed in 10% neutral phosphate-buffered formalin solution and embedded in paraffin and cut into 4 μ m sections. Sections were stained with hematoxylin and eosin (H.E.), Fontana-Masson stain or bleached with potassium permanganate oxalic acid and peracetic acid for histological examination. The bleached sections were stained with H.E. or by Azan method. The formalin fixed tissues of melanoma and liver were examined by electron-microscopy after refixation in phosphatebuffered 1% glutaraldehyde fixatives with postossification in 1% OsO₄ solution.

Gross findings :

A black mass was seen developed in the left inguinal region of the mouse since 98 weeks old. It measured 3 cm in diameter at autopsy. At sacrifice the body weight of the animal was 47.5 g. A part of the skin covering the mass was ulcerated. The other gross findings were blackish colored liver, enlargement and blackish coloration of the axillary and inguinal lymph nodes, slight splenomegaly, blackish contents in the large intestine, and a cyst of the kidney.

Histological findings :

The tumor was solid and well-demarcated from the surrounding tissue. The center of the skin covering the tumor was ulcerated (Fig. 1). Many neutrophils infiltrated into the necrotic tissue. Although the tumor was located adjacent to the basal layer of epidermal squamous epithelium, no invasion into the epithelium was found (Fig. 2). Many of the tumor cells contained abundant melanin pigments in their large oval or polygonal cytoplasm. These cells were arranged in cords or in a medullary pattern. Occasionally, the spindle cells were seen between the oval or polygonal cells, or formed a nest in several areas. These blackish pigments in the tumor cells disappeared by bleaching, and more blackened by Fontana-Masson stain. On the section stained by H.E. or Azan after bleaching, the tumor was seen to be composed of cells with a relatively uniform nucleus, which was located at the marginal area of their cytoplasm (Fig. 3). Mitosis was almost absent.

Metastasis of the tumor cells was seen in the

marginal, intermediate and medullary sinus of lymph nodes (Fig. 4). These tumor cells were oval in shape, similar to the cells in the primary tumor, and occasionally, formed a small nodule. Sporadic metastasis composing of a few oval-shaped tumor cells containing abundant melanotic pigments was seen in an emboli-form in the sinusoids of all lobes of the liver (Fig. 5). The Kupffer cells phagocytizing melanotic pigments were also mobilized in their sinusoids. A few tumor cells with black granules were seen sporadically in the alveolar wall of the lung. The cells with a small number of black granules and vacuoles found in the surrounding zone of white pulp of the spleen, seem not to be tumor cells but macrophages phagocytizing melanosomes. No abnormal finding was seen in the pituitary gland or other tissue.

Electron-microscopical findings :

Most of the tumor cells had an abundant mass of stage IV-melanosome, and consequently, the nucleus was compressed to the marginal area of cytoplasm (Fig. 6). Nuclear invagination was also evident. Many melanosomes were found in the vascular endothelial cells. Many melanosomes were also seen in the lysosome of Kupffer cells. The tumor cells with melanosomes infiltrated in the sinusoid of liver.

Discussion and Conclusion

The present tumor is characterized with the following features including 1) a compressive growth to the adjacent tissue in the primary region, 2) abundant melanotic pigment in their cytoplasm, 3) rare mitosis, 4) poor cellular pleomorphism, and 5) extensive metastasis to lymph nodes, liver, and lung, as in leukemia without showing nodule formation in the metastasized organs.

It is presumed that the source of the pigment-forming cell is the neural crest seen during embryonic period. Pigment forming cells are seen to migrate into the dermis, and mature there to a melanocyte in the fetal dermis. Some of the melanocytes in dermis migrate into the epidermis, but the others remain in the dermis. The following four types of melanocyte were observed in the

Fig. 1. Solid and well-demarcated tumor in the skin and subcutaneous tissue. H.E., $\times 5$.

Fig. 2. Tumor growth is seen just beneath the epidermis. H.E., $\times 220$.

Fig. 3. Melanotic tumor cells bleached with potassium permanganate oxalic acid. Oval or polygonal tumor cells with a small nucleus are seen. Bleach and Azan staining, $\times 300$.

Fig. 4. Metastasis of the melanocytic tumor cells in lymph node. H.E., $\times 180$.

Fig. 5. Sporadic metastasis in the sinusoids of liver. H.E. $\times 80$.

Fig. 6. Electron micrograph of the tumor cell with abundant stage IV-melanosome and a nucleus showing invagination. $\times 3,560$.

skin of BDF₁ mice: epidermal melanocyte, hair-follicular melanocyte, perifollicular melanocytic network (PFM), and intradermal melanocyte¹². Melanoma may originate from a melanocyte in the epidermis or dermis. Junctional and/or intraepithelial tumor growth is an important feature in melanoma arising from epidermal melanocytes. Melanoma originating from the dermal melanocytes lacks the feature of junctional tumor growth, and grows in the dermis being apart from the epidermis.

Currently, no classification of melanoma in mice has been established because of the rare occurrence of this tumor. The present tumor having many oval cells with abundant melanin granules in the cytoplasm, and also spindle cells between these oval cells is similar to the malignant melanoma of dendritic and whorled type just as seen in dogs^{16,17}. Malignant melanoma of the dendritic and whorled type usually shows no junctional and/or intraepithelial tumor growth, which is an important feature of melanoma arising from epidermal melanocyte. It is different from other types of malignant melanoma in dogs.

Recently, experimental induction of melanotic tumor of skin in mice and hamsters was tried^{5-8,12}. An accumulation of dermal melanocyte (blue nevus-like spots) was seen in haired, hairless and rhino mice by carcinogen treatment⁵. Melanocytic tumor in BDF₁ mice was induced by application of DMBA and TPA, and their tumor cells in the deep layer of the dermis were oval or polygonal in shape¹². Melanotic tumors induced by DMBA and TPA application were suggested to be probably originated in dermal melanocyte forming PFM¹². This histological and electron-microscopic features of the present tumor resemble these induced melanotic tumors. Consequently, it was suggested that the origin of the present tumor was a dermal melanocyte, though no definite conclusion was obtainable because of ulceration in the central area of the skin covering the nodule.

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