Morphogenesis of Compound Melanosomes in Melanoma Cells of a Gray Horse
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ABSTRACT. A thoroughbred horse, gelding, gray color, aged 19 years old had cutaneous melanomas from the root to the middle of the tail, and throughout the connective tissues of the whole body. Histologically, the tumors were diagnosed as mature melanotic melanomas characterizedly deposited with abundant melanin pigment. Examined with an electron microscope, melanosomes were electron opaque without internal structure (stage IV), or as mature granular and lamellar types. Most of them were fused with each other, and formed compound melanosomes, which were similar to internal melanin aggregates in shape. The internal melanin aggregates gradually disintegrated, and compound melanosomes grew spherical. The compound melanosomes changed into autophagosomes. — KEY WORDS: compound melanosome, horse, melanocyte.

Melanoma is composed of melanin-producing cells arising from the neural crest of neuroectodermal origin. Normal melanocytes are ordinarily disseminated in the germinal layer of the epidermis [17]. In the horse, 6 to 15 percent of skin tumors were melanomas [18], and gray horses showed a high incidence of melanoma [4, 9, 10, 13, 17, 18, 20]. It is well known that the melanocytes go through dendritic processes in the germinal layer of normal skin, and that mature melanosomes may proceed to adjacent keratinocytes by phagocytosis through the dendritic processes [3, 5]. Melanomas frequently contain variable numbers of cells known as melanophages. These contain large bodies called compound melanosomes, filled with granules which undergo degradation [2]. Compound melanosomes neoplastic melanocytes were reported in cases of many species [3, 6, 8, 11, 12, 14, 15], but the morphogenesis was not so clear. In this report, we describe the morphogenesis of compound melanosomes in melanoma cells from a gray horse.

Case history: A 19-year-old gray color thoroughbred gelding and weighing 498 kg was examined. It had been used as a leading horse at a race track, but due to old age, it was necropsied after euthanasia with exsanguination on December 23, 1987.

Necropsy and histological examination: After detailing observation at necropsy, tissue blocks were taken from the organs and tissues. They were fixed in a 10% neutral formalin solution. Paraffin sections were cut and stained with hematoxylin and eosin (HE). Selected sections were treated with potassium permanganate for bleaching of melanin.

Electron microscopic examination: Tiny blocks of the neoplasm of the tail root were prefixed in 6.25% glutaraldehyde and postfixed in 1% Millonig's OsO4. They were dehydrated according to routine method and embedded in Epon 812. Ultra-thin sections were cut using an ultramicrotome, and double stained with uranyl acetate and lead citrate. They were observed under a Hitachi H-600 electron microscope.

Necropsy and macroscopic findings: Many melanomas, the largest being 7.5 × 7 cm in size, were found in the dermis, from the root to the middle of the tail (Fig. 1). They were smooth, homogeneous and blackish in color on the cut surface. A chicken-egg-sized and a quail-egg-sized melanomas were found in the connective tissue between the left parotid gland and the masseter muscles and between the right parotid gland and the masseter muscles respectively. A small chicken-egg-sized melanoma was found in the connective tissue near the maxillary vein. Many fine blackish tumors grew together on the root of the cranial mesenteric aorta. Several thumbs sized melanomas were found in the biceps femoris muscle, and in the connective tissue near the femoral canal. It was diagnosed as multiple melanomas and metastasis in the whole body.

Histological findings: In perianal melanoma, plump round cell (P cell) with a large amount of melanin granules in a huge cytoplasm proliferation occasionally associated with synctium formations were found in the subcutaneous and muscular tissues. Huge epithelioid cells (E cell) with a large spheroid nucleus (Fig. 2) and spindle cell (S cell) with a spindle nucleus (Fig. 3) were also located in this area. In other portion, most of the melanocytes were necrotized, where some P and S cells remained alive (Fig. 4).

In the connective or adipose tissue around the root of the cranial mesenteric aorta, P and E cells proliferated in a solidly or partially fascicular way. Focal proliferation of S cells and several bizarre cells (B cell) with a huge strange

Fig. 1. Melanomas at the ventral portion of the tail root to the center.
shaped nucleus (Fig. 5) that have a rough cytoplasmic figure, and a huge polymorphic nucleus was noted.

Electron microscopic findings: In the present case, neoplastic cells were about 10 μm in diameter, and predominantly spherical, but some were spindle-like in shape. Dendritic processes were well developed in the neoplastic cells scattered among the collagen fibers. However, they were not well developed in the densely accumulated spherical cells. Several macrophages phagocytizing melanocytes were found. Most of the nuclei were pressed by melanosomes in the cytoplasm, and some spherical nuclei with slight concavity were also found in the center of the cytoplasm. Invagination of the nuclear membrane was not evident. The heterochromatin was aggregated at the nuclear margin, and sometimes formed a large mass. The size of the melanosomes measured 0.06 to 1.7 μm and the average size was about 0.5 μm in diameter. A few lysosomes were also present in the cytoplasm. The swollen mitochondria were scattered, but other organelles were not so conspicuous. Most of the melanosomes were spherical, homogeneous, and high in electron density, that is, electron opaque without internal structure. Most of them aggregated towards each other forming compound melanosomes. There were two types of compound melanosomes. One type was like the internal melanin aggregates in shape (Figs. 6, 7 & 8A). Another was almost spherical and not associated with the internal melanin aggregates (Fig. 8B). The largest one was more than 3 μm in diameter. A melanosome was found surrounded by fine granules, which was considered lysosomal granules (Figs. 7 & 9). The compound melanosome attached on other lysosome (Fig. 9) or melanosome (Fig. 7), and then fused together (Figs. 7 & 10). The melanin aggregates disintegrated into granules in the compound melanosome (Figs. 8 & 10).

The metastatic lesions were composed of mature melanocytes in the present case, but they were found in systemic connective tissues. Accordingly, it might be reasonable to consider that they were immature in the beginning of the growth [1] and maturation and the differentiation occurred after systemic metastasis.

Electron microscopic observation showed that melanosomes corresponded to stage IV reported by Fitzpatrick et al. [3], and the granular or maturing lamellar type reported by Hunter et al. [6]. Neoplastic cells of the present case were thought to be highly differentiated.
They had many mature melanosomes, which were clustered compound melanosomes, and showed this integration.

The reason why so many compound melanosomes were found in the present case was thought to be as follows:
The excessive internal melanosomes may be the result of incomplete formation of dendritic processes in the neoplastic melanocyte by the absence of adjacent keratinocyte, and autophagocytosis for excessive melanosomes may have occurred inside the cells (Fig. 11). It is reported that formation of compound melanosomes in melanocytes occurs also in the state of hypomelanosis by blocking of
fused together and compound melanosomes resembling internal melanin aggregates were formed. 3) Activity of the internal lysosomal enzymes of melanosome became more active. 4) The internal melanin aggregates gradually disintegrated, and compound melanosomes grew spherical. 5) Compound melanosomes changed into autophagosomes.

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