Original

Characteristics of Melanosomes in Melanotic and Amelanotic Melanomas

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Abstract: The morphology and characteristics of melanosomes are important features for differentiating melanocyte-derived melanotic lesions such as benign and malignant melanoma. There are two types of melanosome; eumelanin and pheomelanin. In this study, we attempted to elucidate the characteristics of melanosomes in malignant melanomas (melanotic type and amelanotic type) by ultrastructural analysis. In the melanotic type malignant melanoma *in situ*, pleomorphic and ellipsoid abnormal melanosomes, so-called eumelanin melanosomes, were detected, together with an increase in alkali elution rate. In the melanotic type invasive malignant melanoma, irregular ellipsoid and spheroid melanosomes were found either as discrete bodies or compound melanosomes, with further increase in alkali elution. In the amelanotic type malignant melanoma *in situ*, there were no melanosomes in atypical melanocytes and keratinocytes. In the amelanotic type invasive malignant melanoma, a few round melanosomes with low density matrix, so-called pheomelanin melanosomes, distributed in the cytoplasm. Our findings suggest that abnormal melanosome morphology and characteristics are predictive differential markers for the melanotic type or amelanotic type of oral malignant melanomas.

Key words: melanosome, pheomelanin, eumelanin, melanotic melanoma, amelanotic melanoma.

Introduction

Melanins can be classified into two distinct types; eumelanin and pheomelanin¹⁾. These two pigments are distinguished by the morphology of the melanosomes, stereologic images, specific alkali dissolution properties, and presence or absence of sulphur in the melanin structure²⁻⁵⁾. Recent studies have demonstrated that eumelanin and pheomelanin are synthesized concurrently in the same human melanocyte²⁾. Although atypical melanosomes have been reported in malignant melanomas⁶⁾, no study has documented the differential analysis of eumelanin and pheomelanin in melanotic and amelanotic melanomas. In the present study, we attempted to elucidate the characteristics of melanosomes in different types of malignant melanomas (melanotic type and amelanotic type) by examining the morphology and characteristics of melanosomes by conventional electron microscopy.

Materials and Methods

Specimens

Surgically removed tissues stored as paraffin-embedded blocks were obtained from the Department of Oral Pathology, Okayama University Dental School. All data presented were derived from the clinic-based melanoma registry of the Department of Oral Pathology and Medicine, Okayama University, Japan. The histological materials used in this study were obtained from 4 patients (age range: 58–79 years) who underwent surgery for primary invasive oral malignant melanoma. The patient and tumor characteristics are listed in Table 1. Tumors were staged according to the Western Society of Teachers of Oral Pathology⁷⁾.

Conventional transmission electron microscopy (TEM)

Tissues preserved in 10% buffered formalin were fixed in a mixture of 2% glutaraldehyde and 2% paraformaldehyde, and post-fixed with 1% osmium tetroxide. Then, epon-embedded ultrathin sections were prepared, double-stained, and examined under a type H800 transmission electron microscope (Hitachi Co., Japan).

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Table 1.Characteristics of patients and tumors

Age/Gender	Location	Clinical highlights	Histologic pattern	Outcome
75, M	Palatal mucosa	40 x 25mm Exophytic tumor. Duration from detection to operation; 6 months	Invasive with <i>in situ</i> amelanotic and melanotic tumors. No infiltration Depth 8mm	Palate bone resection and neck Lymph node metastasis Oral recurrence after 1year
79, F	Left gingiva, palate	25 x 15mm Exophytic tumor. Duration from detection to operation; 6 months	Invasive with <i>in situ</i> amelanotic tumor. No infiltration Depth 5mm	Chin bone resection Lymph node metastasis Relapse not confirmed
58, M	Right palate	20 x 13mm Exophytic amelanotic tumor. Duration from detection to operation ; 6 months	Invasive with <i>in situ</i> melanotic tumor. Bone infiltration Depth 10mm	Chin bone resection No neck dissection Lymph node metastasis
61, F	Left anterior palate	30 x 20 mm size 20x10mm (anterior) Multiple tumors	Invasive with <i>in situ</i> melanotic tumors Bone infiltration Depth 7mm	Chin bone resection No neck dissection Lip relapse

Alkali elution was performed by treating the ultrathin sections with 0.5 N sodium hydroxide as reported previously⁸⁾.

Results

Malignant melanomas *in situ*

In the melanotic type malignant melanoma *in situ*, atypical melanocytes were identified as irregularly shaped, dendritic cells containing numerous ellipsoid and spheroid melanosomes ranging in size from 0.1 to 0.7 μ m along their major axis. A number of these melanosomes migrated to the keratinocytes (Fig. 1A). Atypical binucleated melanocytes were also identified containing large numbers of pleomorphic melanosomes. After alkali elution, partial digestion was observed in a number of the ellipsoid

melanosomes (Fig. 1B).

However, in the amelanotic type malignant melanoma *in situ*, many atypical melanocytes in the shape of irregular dendritic cells demonstrated no small round melanosomes (Fig. 2A-2B).

Invasive malignant melanomas

In the melanotic type invasive malignant melanoma, irregularly shaped tumor cells were observed. Variably sized immature spheroid melanosomes with dense matrix were found in the vicinity of the Golgi apparatus in atypical melanocytes (Fig. 3A). Polymorphic and variably sized melanosomes (0.1 to 0.5 μ m along major axis) existed either as discrete bodies or as complex melanosomes within lysosomes (Fig. 3B). The isolated

Fig. 1. Transmission electron micrographs of a malignant melanoma *in situ* (melanotic type). (A) Atypical melanocytes are seen within the basal layer and a number of melanosomes migrate to the keratinocytes (2000 $\times$). (B) Digestion is observed in a few ellipsoid melanosomes after alkali treatment (5000 $\times$).

Fig. 2. Transmission electron micrographs of a malignant melanoma *in situ* (amelanotic type). (A) Many atypical melanocytes are seen in the basal layer and spicle layer in the epithelium (1500 $\times$). (B) There are no melanosomes in atypical melanocytes and keratinocytes (3000 $\times$).

Fig. 3. Transmission electron micrographs of an invasive malignant melanoma (melanotic type). (A) Variable size spheroid melanosomes are dispersed in an atypical melanocyte cytoplasm($\times 6000$). (B) Compound melanosomes contain polymorphic and variably sized spheroid and ellipsoid melanosomes in an atypical melanocyte ($\times 4000$). (C) A few spheroid and ovoid melanosomes are digested after alkali treatment ($\times 6000$). (D) In compound melanosome, most of the internal melanosomes are eluted by alkali treatment but some melanosomes with high density are not digested ($\times 6000$).

melanosomes were irregular, small, spheroid or ovoid structures, while compound melanosomes were a collection of irregular, ellipsoid, mature and immature melanosomes containing a high density material. When the melanosomes were treated with alkali solution, some isolated spheroid and ovoid melanosomes were eluted by alkali (Fig. 3C). Many internal melanosomes within compound melanosomes were also eluted after alkali treatment (Fig. 3D) but some melanosomes with high matrix density were not eluted by alkali.

In the amelanotic type invasive malignant melanoma, irregular tumor cells were observed. A few small round melanosomes were found in the cytoplasm. Round melanosomes showed a low density matrix (Fig. 4A). When the round melanosomes were treated with alkali solution, the matrix of round melanosomes was completely eluted by alkali solution (Fig. 4B).

Fig. 4. Transmission electron micrographs of an invasive malignant melanoma (amelanotic type). (A) Variable round melanosomes with low density matrix are dispersed in cytoplasm($\times 4000$). (B) Round melanosomes are digested completely after alkali treatment ($\times 5000$).

Discussion

The coexistence of the two types of melanosomes in human melanocytes has remained uncertain for a long time³. Increase of 5-S-CD, an intermediate product of pheomelanin, demonstrated in the serum of malignant melanoma patients also suggests an increase in pheomelanin content of melanosomes^{9,11}. Quantitative analysis of 5-S-CD and the touch-fluorescence method with the cut surface of lesions were used for differential diagnosis of early malignant melanomas^{8,11}. We demonstrated dual melanosome formation (eumelanin and pheomelanin melanosomes) within individual melanocyte in oral malignant melanoma by energy filtering transmission electron microscopy⁵. Increases in both alkali elution rate and sulphur content in the melanotic type malignant melanoma *in situ* as well as the appearance of atypical pleomorphic melanosomes suggested a switch in favor of pheomelanogenesis⁵. This shift became more evident in the melanotic type invasive malignant melanoma, in which further increase of dissolution after alkali treatment and sulphur content in melanosomes were detected. Histopathologically, amelanotic melanoma appears non-pigmented, but electron microscopic examination of the secondary resected tissue showed few premelanosomes¹².

In the amelanotic type invasive melanoma, a few small round melanosomes showing low density were completely eluted by alkali, suggesting that these melanosomes contain typical pheomelanin. Our data shows the coexistence pheomelanin and eumelanin melanosomes in the melanotic type invasive melanoma, whereas only pheomelanin melanosomes exist in the amelanotic type melanoma without premelanosomes¹². Our data suggest that abnormal melanosome morphology and characteristics may be differential diagnostic markers for the melanotic or amelanotic type melanoma.

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