Electron Microscopic Analysis of Melanosomes in Oral Pigmentation and Malignant Melanoma

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Introduction

Eumelanin and pheomelanin can be distinguished by their melanosome morphology, stereologic images, specific alkali dissolution properties and presence of sulfur. The detection of 5-S-CD, an intermediate product of pheomelanin suggests an increase in the pheomelanin content of melanosome. The purpose of the study was to elucidate the characteristics of melanin in normal oral mucosa, oral melanosis and malignant melanoma by examining the morphology and sulfur content of melanosomes with conventional and energy-filtering transmission electron microscopy (TEM).

Materials and Methods

Paraffin embedded blocks from human tissues comprising of 3 normal mucosa, 3 melanosis, 1 *in situ* and 4 invasive melanoma were used. Tissues were prepared for conventional TEM (H800, Hitachi Co., Japan). Alkali elution was performed by treating ultrathin sections with 0.5N sodium hydroxide. An energy-filtering TEM (LEO912Bio, Carl Zeiss) was used to observe elemental distribution of sulfur. Sulfur elemental map was constructed using two-window method. The elemental sulfur distribution was obtained by subtracting scaled pre-edge image from post-edge image with a computer. The sulfur distribution image was colored orange and superimposed on high-contrast energy loss image after binary treatment.

Results

The normal mucosa contained ovoid melanocytes with melanosomes measuring 0.1 to 0.3 mm. In oral melanosis, ovoid and ellipsoid melanocytes were observed. Most of the melanosomes retained the melanin after alkali elution (Fig.1). In *in situ* melanoma, atypical melanocytes contained pleomorphic, ellipsoid and spheroidal melanosomes of varying density. There was partial or complete digestion after alkali treatment (Fig.2). In invasive melanoma, atypical melanocytes contained spheroid melanosomes of different sizes. Many spheroid and ovoid melanosomes were digested after alkali treatment (Fig.3). Mapping of elemental sulfur varied among oral melanosis, *in situ* melanoma and invasive melanoma. In oral melanosis, sulfur was only mapped in intracellular filaments and desmosomes in keratinocytes and rarely in melanocytes. Little sulfur was detected after color treatment. In *in situ* melanoma, spheroid and ellipsoid melanosomes of varying degrees of brightness were observed in atypical melanocytes. Sulfur distribution was seen in many melanosomes. In invasive melanoma, compound melanosomes

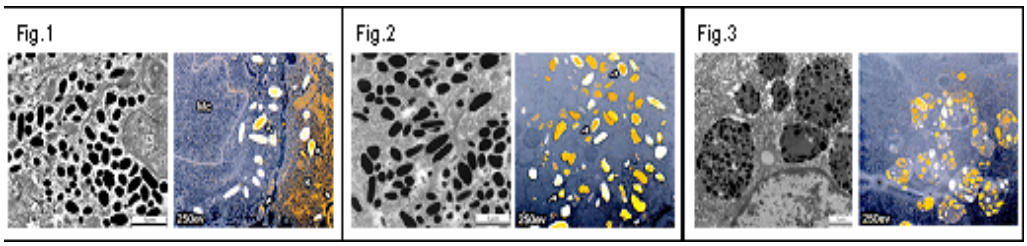
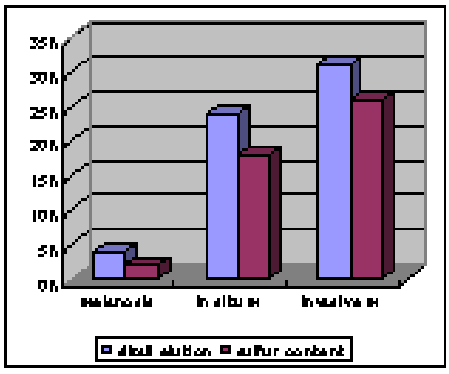


Fig.1 Ovoid and ellipsoid melanosomes in melanocyte were observed in oral melanosis. Most melanosomes retained the melanin after alkali elution. Little sulfur was detected after color treatment.
Fig.2 In *in situ* melanoma, atypical melanocytes contained pleomorphic, ellipsoid and spheroid melanosomes of varying density. There was partial or complete digestion after alkali elution. An increase in the amount of sulfur was detected in pleomorphic melanosomes. Sulfur was localized in most melanosomes after color treatment.
Fig.3 In invasive melanoma, atypical melanocytes contained spheroid melanosomes of different sizes. Many spheroid and ovoid melanosomes were digested after alkali elution. An increase in the amount of sulfur was detected in compound melanosomes. Sulfur was localized at the periphery and center after color treatment.
Graph. The alkali elution rate and sulfur content in oral melanosis, *in situ* and invasive melanoma.



showed many sulfur-containing pleomorphic, spheroid and ellipsoid melanosomes. Sulfur was localized in the interior or along the circumference of the melanosomes.

Discussion

The study demonstrated the dual melanosome formation in melanocyte in oral melanosis and atypical melanocytes in malignant melanoma by energy-filtering TEM. The study also showed the differential analysis of eumelanin and pheomelanin and detected a shift in favor of pheomelanogenesis from benign oral melanosis to *in situ* melanoma and invasive melanoma. In oral melanosis, most of the melanins were eumelanin since they were ellipsoid without pleomorphism and the alkali elution rate and sulfur amount were low. An increase in the alkali elution rate and sulfur amount was observed in atypical melanocytes *in situ* melanoma. This shift became more evident in invasive melanoma in which further increase in alkali elution rate and sulfur amount were observed. The gradual increase of pheomelanin and sulfur amount from oral melanosis to invasive melanoma suggests a direct relationship between pheomelanin content and grade of malignancy. In conclusion, the pheomelanin amount may be used as a predictive factor for malignancy risk and progression as well as a marker for differential diagnosis.