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SHORT REPORT

Ultrastructural Aspects of Melanization of Hamster Ab Amelanotic Melanoma in Primary Cell Culture

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When the hamster Ab amelanotic melanoma has been grown as subcutaneous transplants in hamsters, melanin and identifiable melanosomes have never been observed by electron microscopy. When the cells were placed in primary cell culture, melanosomes of a granular type which contained small amounts of melanin appeared during the first 24 hours of cell culture. As the cultures grew older, the number and degree of melanization of the melanosomes increased, and eventually after 3–5 days they were found in all melanoma cells. (Received June 11, 1986.)

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Melanogenesis is a manifestation of differentiation of normal and malignant melanocytes. The Ab hamster amelanotic melanoma is an anaplastic and rapidly growing tumor. During 21 years of serial passage of the Ab melanoma in Syrian hamsters, melanin has never been detected in this tumor by any method (1, 2). This neoplasm arose spontaneously by an alteration of the hamster Ma melanotic melanoma and involved: (i) a loss of melanin and melanosomes; (ii) a decrease in tyrosinase activity and (iii) an acceleration of growth rate (1, 2).

When Ab amelanotic melanoma cells were placed in primary cell culture, tyrosinase activity rapidly increased on day 1 of culture (3). Melanin was first detected by histological, histochemical and electron spin resonance (ESR) methods on day 3 of culture (4, 5). To further investigate this abrupt onset of melanization during primary cell culture a study of the ultrastructural cellular changes was undertaken.

MATERIALS AND METHODS

A non-enzymatic method was used to isolate cells from transplants of the Ab amelanotic melanoma (4). These cells were used for starting the primary cell cultures. One million cells were seeded in 80 mm glass Petri dishes, containing 10 ml of Eagle's Minimal Essential Medium, supplemented with 10% calf serum (Biomed, Poland), 100 units/ml penicillin, and 100 μg/ml streptomycin. The cultures were grown at 37C, 5%CO_2 in air and 80% humidity. Cells from solid transplants and days 1, 2, 3 and 5 of primary cell culture were used. Following mechanical scraping from the glass substratum, the cells were centrifuged at 800 × g for 5 min. The pellets were fixed in 6.25% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4 and postfixed in 1% OsO_4 in 0.1 M cacodylate buffer and embedded in Epon 812. The ultrathin sections were cut with glass knife in a Reichert OmU_2 ultramicrotome, stained with lead citrate and uranyl acetate and inspected in JEM 7A electron microscope.

RESULTS AND DISCUSSION

During in vivo growth (1, 2) and immediately following their isolation from subcutaneous transplants the Ab amelanotic melanoma cells contained neither melanin nor identifiable melanosomes (Fig. 1). On day 1 (20–24 hours after the start of the cultures), a few

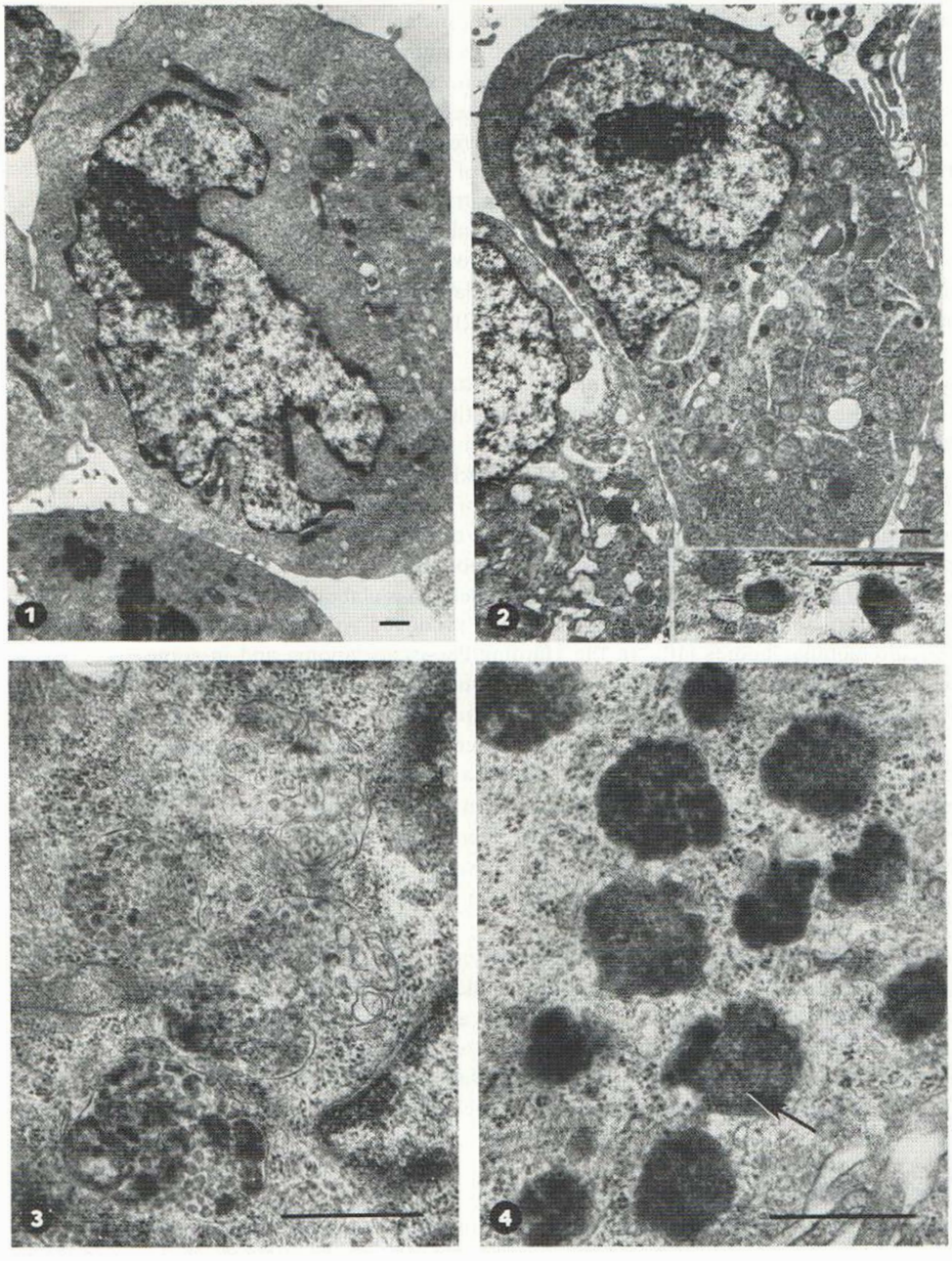

Fig. 1. Ab cells immediately after isolation from transplants. Melanin and identifiable melanosomes are lacking. Bar=0.5 μm.

Fig. 2. Ab cells from day 1 of primary cell culture. Few granular, partially melanized melanosomes are visible. Melanin is deposited within finely granular substance. Bar=0.5 μm.

Fig. 3. Ab cell from day 2 of primary cell culture. Numeorous melanosomes are visible. Melanin is deposited on the matrix composed of vesiculo-globular bodies. Bar=0.5 μm.

Fig. 4. Melanosomes in an Ab cell from day 3 of primary cell culture, containing melanin, vesiculo-globular bodies and amorphous substance. Fibrillar matrix is visible in one melanosome (arrow). Bar=0.5 μm.

premelanosomes of a granular type with small amounts of melanin appeared in some Ab cells (Fig. 2). The premelanosomes were characterized by a round or irregular shape and a disorderly deposition of melanin within a homogenous or finely-granular substance of medium electron opacity. Similar organelles has been described in the Ma melanoma (1, 2). On day 2, the number of cells with melanosomes increased as well as the degree of melanization within these melanosomes. A second type of melanosome appeared at this time, characterized by the presence of a regular inner matrix resembling vesiculo-globulr bodies (Fig. 3). At day 3, nearly every cell contained melanized melanosomes. In general, their inner structure was obscured by increased amounts of melanin and it was therefore impossible to categorize them (Fig. 4). However, some melanosomes did contain either (1) an amorphous substance and vesiculo-globular bodies or (2) a fibillar matrix admixed with scattered deposits of melanin (Fig. 4).

Tyrosinase has been found previously by means of ultrastructural tyrosine and dopa reactions to occur within some cysternae and vesicles of the smooth endoplasmic reticulum in the Ab amelanotic melanoma (2). This enzyme activity has been shown to significantly increase during day 1 of cell culture (3). The working hypothesis for the lack of pigment in this tumor line has been that there is a failure of tyrosinase to incorporate into premelanosomes due to their absence (2). The formation of premelanosomes during the first day of primary cell culture seems to support this hypothesis.

It has been reported that in the normal melanocytes eumelanin is elaborated by elliptical melanosomes with fibrillar matrix, while pheomelanin is produced within melanosomes containing vesiculo-globular bodies (10). In the Harding-Passey melanoma and in some human melanomas the granular melanosomes have been seen and found to produce eumelanin (7, 8). However, in the Ab cells cultured in vitro, two major types of melanosomes were observed: (i) granular melanosomes characterized by the lack of a regular matrix and by a disorderly deposition of melanin within a field of amorphous or finely-granular substance (Fig. 2), similar to melanosomes seen in other melanomas; and (ii) melanosomes characterized by the presence of a regular inner matrix in the form of vesiculo-globular bodies (Fig. 3), similar to pheomelanosomes seen in normal melanocytes. Further biochemical analyses are necessary to identify the type of melanin synthesis.

There are three markers of melanocyte differentiation: tyrosinase activity, melanosomes and melanin. The previous and present observations indicate that all of these markers appear readily in the cells of the Ab amelanotic melanoma after their transfer from in vivo to in vitro growth conditions. Thus the Ab melanoma is a good model for studying the process of melanosome formation, its relationship with melanogenesis and the relationship between the ultrastructure of melanosomes and type of melanin produced.

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Cis-urocanic Acid Does Not Inhibit Mitogen Induced Lymphocyte Transformation in Man

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Urocanic acid (UCA) is a photoreceptor in the epidermis which absorbs UVB. To elucidate the role of UCA as an immunomodulator in the mechanism of immune suppression which occurs after UV irradiation in human, we studied the effect trans-UCA as well as that of its UV induced cis-isomer (cis-UCA) on mitogen induced lymphocyte transformation and on delayed type responses elicited by intradermal testing of a standardized battery of microbial antigens. No inhibitory effect was seen either in vitro or in vivo. Our negative results, both in vivo and in vitro, suggest that if UCA and UCA photoproducts exert an immunomodulatory effect, their effect should be analysed by a more subtle evaluation of the immune system. *Key words: Urocanic acid (cis and trans); Immune responses; Intradermal skin tests; Topical urocanic acid.* (Received January 3, 1986.)

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The possibility that exposure to UV might influence the immune system in man and in experimental animals is now widely accepted. The mechanism of the immunosuppression after UV irradiation has been mainly investigated on antigen specific contact hypersensitivity. On the other hand, in vivo UV irradiation leads to a decrease in PHA stimulated lymphocyte proliferation in healthy individuals (1). A question is how UV irradiation that is mainly absorbed in the epidermis may lead to a decrease in PHA induced proliferation of lymphocytes and in the expression of contact hypersensitivity. Normal human skin contains a large amount (0.4% of the wet weight) of trans-urocanic acid (trans-UCA) (2). The function of this accumulation has not been established, although the fact that the