CHANGES IN DISTRIBUTION PATTERN OF CYTOPLASMIC FILAMENTS IN HUMAN MELANOCYTES DURING ULTRAVIOLET-MEDIATED MELANIN PIGMENTATION

The Role of the 100-Å Filaments in the Elongation of Melanocytic Dendrites and in the Movement and Transfer of Melanosomes

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Filaments of 100-Å diameter have been identified in a variety of cells (e.g., fibroblasts, macrophages, neurons) (2, 4, 5, 7, 13, 15, 18) by an ultrastructure and distribution pattern that distinguishes them from microtubules (250 Å), thick (myosin-like) filaments (150-220 Å), and thin (actin-like) filaments (50-70 Å). These filaments, of still unknown function and chemical composition, are located usually in the center of cells rather than near the plasma membrane (4, 5, 13), do not react with cytochalasin (13) or heavy meromyosin (7, 14), and, in neurons, lack colchicine- and nucleotide-binding capacity (6, 16). In the present study, these 100-Å filaments were studied in the melanocytes of human skin. To examine the role of these filaments in melanin pigmentation, the skin was exposed to ultraviolet (UV) light.

MATERIALS AND METHODS

Investigation of the nature and function of 100-Å filaments was carried out by exposing the melanocytes in human skin (21 adult volunteers: 9 caucasoids, 6 mongoloids, and 6 negroids) to two types of UV light and to two chemicals. Least-exposed (buttock) skin and habitually exposed (forearm) skin were irradiated with either long UV plus visible light (340-600 nm) for 20-30 min or middle UV (280-340 nm) for 3 min to induce immediate (IT) and delayed (DT) tanning. IT occurs within a matter of minutes after exposure and fades within 3-24 h; it is related to the translocation of the pre-existing melanosomes in the skin (8).

In vitro studies were carried out by incubating sliced human skin with cytochalasin-B (cyto-B) and vincristine sulfate (VCR) (5-10 μg/ml) separately for 3 h at 37°C (10). For histochemical visualization of melanocytes, the specimen was prepared by splitting epidermal tissues with NaBr and incubating them in 3,4-dihydroxy-phenylalanine (DOPA) solutions (8). Electron microscope studies were done on tissues fixed with Karnovsky's solution (9).

RESULTS

Distribution Pattern of 100-Å Filaments before and after UV Irradiation

BEFORE IRRADIATION: The "split" epidermal sheets showed that the human melanocytes of nonexposed skin contain poorly developed dendritic processes (Fig. 1a). These melanocytes contained 100-Å filaments (114 ± 27 Å in diameter). The 100-Å filaments were abundantly and characteristically located around the nuclei, together with melanosomes (Fig. 2a-c). In the buttock skin, these two organelles were not observable along the periphery of the perikaryon or in the dendrites. In the habitually exposed skin, however, they were present in other parts of the perikaryon, as well as in the perinuclear area, but were hardly seen in the dendrites. The
FIGURE 1 Melanocytes in split-DOPA preparations before and after UV irradiation. Biopsies were obtained from the buttock skin of the same caucasoid subject. (a) Before irradiation. × 350. (b) An immediate tanning reaction biopsied immediately after irradiation by long UV plus visible light (340-600 nm) for 20 min. Note a marked elongation and elaboration of the dendritic processes of the melanocytes. × 350. (c) An immediate tanning reaction biopsied at 24 h after irradiation by long UV plus visible light (340-600 nm). Note that the dendritic processes, which had been markedly elongated at 0 min after irradiation, became less developed and probably contracted. × 350. (d) A delayed tanning reaction. Biopsied at day 5 after irradiation of long plus middle UV (290-340 nm). Note an increase in the size of the perikaryon and a marked elongation of the dendritic processes. × 350. (e) A delayed tanning reaction. Biopsied at day 10 after irradiation of long plus middle UV (290-340 nm). Compared with Fig. 1 d, the elongation of the dendritic processes became less than at day 5, but is still more than before irradiation. (See Fig. 1 a.) × 350.

100-Å filaments were so densely aggregated that they formed bundles containing 20–30 filaments and, rarely, as many as 200 (Fig. 2 b). The filaments did not run a long course and were not parallel with each other (Fig. 2 c).

The microtubules, which were much less prominent in comparison with the 100-Å filaments, were also found around the perinuclear area (Fig. 2 b), and did not have any direct contact with the 100-Å filaments.

FIGURE 2 Normal unirradiated buttock skin. (a) A caucasoid. There are four melanocytes (MC). Note that each melanocyte contains dense aggregates of filamentous organelles (arrowhead) around the nucleus. Only a few dendritic processes (DP) can be seen. Keratinocyte (KC). × 4,100. (b) A high power view of the portion of the melanocyte shown in Fig. 2 a. Note that the dense perinuclear filamentous organelles are composed of 100-Å filaments (F) and microtubules (MT) that intermingle closely with each other. Around these organelles are scattered melanosomes (MS) in unmelanized stages, mitochondria, and ribosomes. × 36,000. (c) A negroid. Note the clusters or bundles of 100-Å filaments (F) present in the center of the perikaryon. In contrast to a caucasoid subject (Fig. 2 b), the melanosomes (MS) are numerous. × 15,000.
FIGURE 3. A high power view of the dendritic processes of melanocytes, immediately after IT. (a) A dark-skinned caucasoid subject. The dendrite is aggregated with a bundle of 100-Å filaments (arrows) that runs a long course in the center of the dendritic process (DP). Note an absence of 100-Å filaments along the plasma membrane. There are a few filaments around the nucleus (N) × 29,000. (b) A negroid subject. Note the 100-Å filaments and melanosomes that intermingle closely. The melanosomes in the center of the dendrite are embedded in the network of the 100-Å filaments; the melanosomes (MS) close to the plasma membrane are not related to the bundles or network of 100-Å filaments. Keratinocyte (KC), desmosome (DS). × 49,000.
FIGURE 4 UV-irradiated melanocytes during a delayed tanning reaction. Biopsies were obtained from caucasoid buttock skin. (a) At day 10 after irradiation. Note the two melanocytes (MC) in the basal layer of the epidermis. One melanocyte (left side) contains many melanosomes, 100-Å filaments (F), and microtubules that are intricately intermingled in the perinuclear area and endoplasmic regions. The other melanocyte (right side), however, does not contain many melanosomes. The 100-Å filaments and microtubules in this melanocyte are densely aggregated in the perinuclear area. Dendritic process (DP). × 7,900. (b) A high power view of Fig. 4 a. Note an abundance of 100-Å filaments scattered diffusely in both the perinuclear area and cytoplasm. × 22,000.

AFTER EXPOSURE FOR THE IMMEDIATE TANNING REACTION: At time 0 min, immediately after IT, the split-DOPA preparation showed that the dendritic processes of the melanocytes were markedly extended (Fig. 1 b). A most notable change was observed in the distribution pattern of the 100-Å filaments and melanosomes. The 100-Å filaments were rarely seen around the nuclei after irradiation (Fig. 3 a). The aggregates, or bundles, of intermingling 100-Å filaments were invariably seen shifting from the perinuclear area toward the dendrites (Fig. 3 a). Melanosomes were prominent in the dendrites and were closely associated with the bundles of 100-Å filaments. Occasionally, the melanosomes appeared to be encircled by a “network” of 100-Å filaments. In the tip of the dendrites, the melanosomes were occasionally located outside the network of interlocking 100-Å filaments (Fig. 3 b). The 100-Å filaments were never closely associated with plasma membrane, even after an irradiation.

The microtubules were seen in the periphery of the perikaryon and, very rarely, in the dendrites (Fig. 3 a, b). They were located beyond the 100-Å filament bundles and close to the plasma membrane of the dendrites.

At 24 h after IT, the elongated dendrites became less extended (Fig. 1 c). The 100-Å filaments became densely aggregated in the perinuclear area. The melanosomes in the melanocytes became less visible.

AFTER EXPOSURE FOR THE DELAYED TANNING REACTION: At day 5, the split-DOPA preparation showed marked elongation and arborization of the dendrites and hypertrophy of the perikaryon of the melanocytes (Fig. 1 d). The 100-Å filaments and melanosomes showed again a distinct distribution pattern. In contrast to
the findings after IT, the 100-Å filaments and melanosomes were present in the dendrites and in the perikaryon. In the perikaryon, the 100-Å filaments were found either forming bundles or scattered randomly around the melanosomes. In the dendrites, the microtubules were still very rarely seen. Again, as after IT, melanosomes were scattered among the aggregates of 100-Å filaments and some of them appeared to be encircled by these filaments.

At day 10, the perikaryon and dendritic processes of the melanocytes became less prominent than they were at day 5 (Fig. 1 e). Throughout the entire perikaryon, most of the melanocytes contained diffusely scattered 100-Å filaments, microtubules, and melanosomes (Fig. 4 a and b).

Changes in the Number of Melanosomes in Epithelial Cells after UV Irradiation

Before and after irradiation for IT, the melanosomes per cell were counted, under the electron microscope, in 50 basal epithelial cells in 10 subjects of three races. After IT, a significant increase in the number of melanosomes was seen in the epithelial cells (to which melanosomes are normally transferred). Because the biopsies were taken immediately after 20-min UV irradiation, and new synthesis of melanosomes takes place at 24-48 h after exposure (8), we suggest that the significant increase of melanosome densities is related to translocation of the 100-Å filaments, followed by actual transfer of the melanosomes from the melanocytes into the epithelial cells.

Effect of Cytochalasin-B and Vincristine Sulfate on the Melanocytic Filaments

In the tissue incubated with cyto-B, there were no obvious changes in the distribution pattern and ultrastructure of the 100-Å filaments (Fig. 5 a), whereas in other tissues taken from the same donors and incubated with VCR in the same manner, the melanocytes contained aggregates of lattice (5 μg/ml) or crystalloid (10 μg/ml) forms (Fig. 5 b). These aggregates, however, did not have filaments run a long course in the center of the dendritic process (arrows). × 10,000. (b) Unirradiated caucasoid buttock skin treated with VCR (5 μg/ml) for 3 h. Note the bundles of 100-Å filaments (F) and the aggregates of a lattice pattern (LP). × 43,500.
TABLE 1

Comparison of the Number of Melanosomes per Basal Epithelial Cell before and after UV Irradiation*

| Case  | Buttock Before | Buttock After | t test | Forearm Before | Forearm After | t test |
|-------|----------------|---------------|--------|----------------|---------------|--------|
| 1 (cau) | 13 ± 7         | 23 ± 13       | P < 0.005 |
| 2 (cau) | 82 ± 31        | 124 ± 52      | P < 0.005 |
| 3 (cau) | 54 ± 17        | 80 ± 39       | P < 0.05  |
| 4 (mon) | 61 ± 28        | 91 ± 33       | P < 0.005 |
| 5 (cau) | 44 ± 20        | 53 ± 29       | P < 0.1   |
| 6 (neg) | 111 ± 43       | 141 ± 53      | P < 0.05  |

* Melanosomes per cell were calculated randomly, in 50 epithelial cells containing a nucleus, before and after an immediate tanning (IT) reaction.

Degree of freedom = 98 in all cases.

Each value is presented as Mean ± standard deviation.

Cau, caucasoid.

Neg, negroid.

Mon, mongoloid.

any direct continuities with the bundles of 100-Å filaments.

DISCUSSION

The present study clearly indicates that human melanocytes in vivo contain 100-Å filaments but no thin (i.e. 50–70 Å) filaments. It may, however, be possible to find these thin filaments in melanocytes in vitro, in which some kind of organelle may be necessary to maintain the attachment of melanocytes to the surface of the culture glass and also, probably, to cause the locomotion of the melanocytes. Wikswo and Szabo (17) found that cultured guinea pig melanocytes contain thin (30–70 Å) and thick (60–110 Å) cytoplasmic filaments. Jimbow and Davison, however, found that these guinea pig melanocytes in vivo, like human melanocytes, do not contain any of these thin filaments, (K. Jimbow, and P. F. Davison, unpublished data). They also found two types of cytoplasmic filaments (intermediate [100-Å], and thin [50–70 Å] filaments) in cultured retinal pigment epithelium, which contains another type of melanosome-synthesizing cell and is derived from the optic nerve cup. The 100-Å filaments in the cells of these tissues were located primarily in the perinuclear area and endoplasmic regions, whereas the 50–70-Å filaments were just beneath the plasma membrane, exhibiting periodic densities, or Z bands (18).

We presume that 100-Å filaments in human melanocytes are involved in the elongation of the dendrites as well as in the movement and transfer of melanosomes. We do not think that microtubules are directly involved in the melanosome movement, although they may be involved in the elongation of the dendrites. This assumption is based on our findings that (a) melanocytic filaments changed their location while the dendrites changed their shape after the UV irradiation; (b) the melanosomes changed their location, shifting from the perinuclear region to the tip of the dendrites, and thus more of them were transferred to the epithelial cells during this reaction (Table I); (c) melanosomes in the dendrites were embedded in bundles of the 100-Å filaments and were intimately encircled by some of them; (d) there was no morphologic interconnection between microtubules and melanosomes whatsoever; and (e) microtubules were very rarely seen in the dendrites.

Our present study greatly differs from previously reported studies indicating that intramelanocytic movement of melanosomes is mediated through microtubules or cytochalasin-sensitive thin filaments (1, 11, 12). Robison and Charlton, however, recently found that cytochalasin and vincristine do not inhibit the movement of pigment granules in chromatophores (15), thus correlating with our assumption that the cytochalasin-insensitive and vincristine-insensitive 100-Å filaments, not the microtubules, promote the translocation of melanosomes. Their findings that cytochalasin does, however, inhibit the development and elongation of chromatophore dendrites may be explained by the data of Everhart and Rubin (3), who
found that the surface of the plasma membrane, rather than the 50–70-Å filaments, is the primary site for the effect of cytochalasin. The melanocytic filaments appear also to be involved in the transfer of melanosomes from the melanocytes to the epithelial cells, inasmuch as there is an increase in the number of melanosomes transferred to the epithelial cells during the rapid translocation of the 100-Å filaments (Table I).

SUMMARY

Human melanocytes characteristically contain 100-Å filaments. These 100-Å filaments shift from the perinuclear area to the center of the dendritic processes and are in close association with melanosomes during the different stages of UV-mediated melanin pigmentation. We suggest that these 100-Å filaments in human melanocytes participate in the elongation of the dendrites and in the transfer of melanosomes.

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REFERENCES

1. Bille, D., L. G. Tilney, and K. R. Porter. 1966. Microtubules and pigment migration in the melanophores of Fundulus heteroclitus L. Protoplasma. 61:322.
2. Daniels, M. P. 1973. Fine structural changes in neurons and nerve fibers associated with colchicine inhibition of nerve fiber formation in vitro. J. Cell Biol. 58:463.
3. Everhart, L. P., and R. W. Rabin. 1974. Cyclic changes in the cell surface. II. The effect of cytochalasin B on the surface morphology of synchronized Chinese hamster ovary cells. J. Cell Biol. 60:442.
4. Goldman, R. D., and E. A. C. Follett. 1969. The structure of the major cell processes of isolated BHK-21 fibroblasts. Exp. Cell Res. 57:263.
5. HOLTROP, M. E., L. G. RAISZ, and H. A. SIMMONS. 1974. The effects of parathyroid hormone, colchicine, and calcitonin on the ultrastructure of osteoclasts in organ culture. J. Cell Biol. 60:346.
6. HUNEUE, F. C., and P. F. DAVISON. 1970. Fibrillar proteins from squid axons. I. Neurofilament protein. J. Mol. Biol. 52:415.
7. Ishikawa, H., R. Bischoff, and H. Houtzer. 1969. Formation of arrowhead complexes with heavy meromyosin in a variety of cell types. J. Cell Biol. 43:312.
8. JIMBOW, K., M. A. PATHAK, G. SZABO, and T. B. FITZPATRICK. 1974. Ultrastructural changes in human melanocytes after ultraviolet irradiation. In Sunlight and Man. M. A. Pathak, T. B. Fitzpatrick, L. C. Harber, M. Seiji, and A. Kukita, editors. Tokyo University Press. Tokyo. 195.
9. KARNOVSKY, M. J. 1965. Formaldehyde-glutaraldehyde fixation of high osmolality for use in electron microscopy. J. Cell Biol. 27(2, Pt. 2):137 a. (Abstr.).
10. Krishan, A., and D. Hsu. 1969. Observations on the association of helical polyribosomes and filaments with vincristine-induced crystals in Earle’s L-cell fibroblasts. J. Cell Biol. 43:553.
11. MALAWISTA, S. E. 1971. The melanocyte model: colchicine-like effects of other antimitotic agents. J. Cell Biol. 49:848.
12. McGUire, J., and G. Moellmann. 1972. Cytochalasin B: effects on microfilaments and movement of melanin granules within melanocytes. Science (Wash. D.C.) 175:642.
13. McNutt, N. S., L. A. Culp, and P. H. Black. 1973. Contact-inhibited revertant cell lines isolated from SV40-transformed cells. IV. Microfilament distribution and cell shape in untransformed, transformed, and revertant Balb/c 3T3 cells. J. Cell Biol. 56:412.
14. Pollard, T. D., S. Shelton, R. R. Weihing, and E. D. Horn. 1970. Ultrastructural characterization of F-actin isolated from Acanthamoeba castellani and identification of cytoplasmic filaments as F-actin by reaction with rabbit heavy meromyosin. J. Mol. Biol. 50:91.
15. ROBISON, W. G., and J. S. CHARLTON. 1973. Microtubules, microfilaments, and pigment movement in the chromatophores of Palaemonetes vulgaris (Crustacea). J. Exp. Zool. 186:279.
16. SHELANSKI, M. L., S. ALBERT, G. H. Devries, and W. T. Norton. 1972. Isolation of filaments from brain. Science (Wash. D.C.). 174:1242.
17. WIKSOW, M. A., and G. SZABO. 1972. Effects of cytochalasin B on mammalian melanocytes and keratinocytes. J. Invest. Dermatol. 59:163.
18. Yamada, K. M., B. S. Spooner, and N. F. Wessells. 1971. Ultrastructure and function of growth cones and axons of cultured nerve cells. J. Cell Biol. 49:614.