The Effects of Cytoskeletons on the Cultured Human Melanocytes

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***Background*: Cytoskeletons, the complex set of protein fibers found in the cytoplasm, have important roles in the movement of cells and subcellular structures and the generation of shapes. Melanocytes have numerous dendritic processes which are in direct contact with many keratinocytes and transfer the melanosomes into the neighboring keratinocytes. Little information is available on the structure and function of cytoskeletons, and the effects of ultraviolet light on the cytoskeletons of the melanocytes.**

***Objective*: The purpose of this study was to investigate the general cytoskeletal system of cultured melanocytes and to find out the effects of the cytoskeletal antagonists and UVB on the cytoskeletal system of the cultured melanocytes.**

***Methods*: Melanocytes were cultured from adult foreskin and then exposed to various cytoskeletal antagonists and UVB radiation. The changes of the cultured melanocytes were evaluated by using phase contrast microscopy, immunofluorescence staining methods and electron microscopic examinations.**

***Results*: Colchicine produced shortening of dendrites, stellate cellular contour and granular fluorescence of the tubulin. Cytochalasin D produced round cellular contour and granular flulrescence of the actin. Acrylamide produced disorganization of cytoplasmic constituents, but no specific fluorescent change was observed. Colchicine also had inhibitory effects on the vimentin. Cellular responses induced by these agents were reversible. UVB caused morphological changes of the melanocytes, but their effects on the organization of the cytoskeletal system could not be detected in this method.**

***Conclusion*: Microtubules are related to the dendritic movement of the melanocytes. Vimentin may be involved in the transfer of cellular organelles, probably including the melanosomes. Cytoskeletal antagonists produce their characteristic morphological changes to cultured melanocytes. (Ann Dermatol 6:(2) 162-173, 1994)**

Key Words: Cytoskeleton, Melanocyte, Ultrastructure

Cytoskeletons have important roles in the movements of cells and subcellular structures and the generation of shapes in eukaryotic cells. Eukaryotic cells contain three major classes of cytoskeletal fibers; 7-nm-diameter actin microfilaments, 24-nm-diameter microtubules, and 10-nm-diameter intermediate filaments.[1] Melanocytes, the most important components of the epidermal melanin unit, are located in the basal cell layer and have numerous dendritic processes which are in direct contact with many keratinocytes and transfer the melanosomes into the neighboring keratinocytes.[2] Several mechanisms for the melanosome move-

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ment within the melanocytes and the melanosome transfer into the keratinocytes have been suggested based on studies of the chromatophores and the melanocytes of the lower vertebrate and mammals[3-6] Ultraviolet irrandiation induces morphological and functional changes of the melanocytes in vivo and vitro[17-13] Ultraviolet light can cause damage to the microtubules of the calf brain[14] and the human fibroblast,[15-16] and the keratin intermediate filaments of the cultured human keratinocytes.[17] However little information is available on the effects of ultraviolet light on the cytoskeletons of the melanocytes.[4-5],[18-19] with regard to the melanocyte system, the researches on the structure and function of the cytoskeletons are limited in the literatures.[20-22] The purposc of this study was to establish an in vitro cell culture model to examine pigment translocation and transfer in human melanocytes, and also to localize the cytoskeletal structures in human melanocytes and to assess the effects of the cytoskeletal blocking agents and the UVB on the morphology and the cytoskeletal system of cultured melanocytes.

MATERIALS AND METHODS

Culture of melanocytes

During the culture procedures, Melanocyte Growth Medium(MGM) (Clonetics Corporation, CA) was used. This modified serum-free MCDB-153 formulation is supplemented with basic fibroblast growth factor(bFGF)(1ng/mL), bovine pituitary extract(BPE) (0.4% v/v), bovine insulin(5mg/mL), hydrocortisone(0.5mg/mL), phorbol 12-myristate 13-acetate(PMA)(10ng/mL) and antimicrobial agents. Heatinactivated human AB serum was added to the media with the concentration of 10%(v/v). All culture procedures were performed as described by Im et al.[23] Melanocytes were obtained from the normal adult foreskin which did not have any other autoimmune and pigmentary disorders. The foreskin was obtained by circumcision and the subcutaneous fat and deep dermis were carefully removed. Each specimen was placed in trypsin/EDTA(0.25% / 0.1%) solution at 4°C overnight, and then the epidermis was gently separated from the dermis. Each epidermal sheet was incubated in trypsin/EDTA(0.25% / 0.1%) solution at 37°C for 30 minutes. After incubation the epidermal sheet suspension was pipetted 20 times. The epidermal single cell suspensions were plated on tissue culture flask or plate at a concentration of 2×10^5 cells/cm^2. They were incubated at 37°C in a humidified atmosphere containint 5% CO_2. The media were changed after 2 days and thereafter every 3 days. Upon reaching the near-confluency of the melanocytes, they were subcultured using the trypsin/EDTA (0.25% / 0.01%) solution at a density of approximately 5×10^3 cells/cm^2. All the experiments in this study only used the melanocytes in their 2nd and 3rd passages.

Pretreatment of the cultured melanocytes

Melanocytes were seeded into 24-well culture plates at a density of $5x10^3$ cells/cm^2 for the morphological observation and into Lab-Tek chamber slides (Nunc Inc., IL) at a density of 5×10^3 cells/well for indirect immunofluorescent staining, respectively. Several drugs that were thought to influence the cytoplasmic filamentous structures were used. They were added directly to the culture medium and incubated at 37°C. Colchicine(Sigma Chemical Co., MO) was prepared as concentrated stocks (10mM) in PBS. Colchicine(50μM) was added with minimal exposure to lights. Cytochalasin D(Sigma Chemical Co., MO) was dissolved in dimethylsulfoxide (DMSO) and prepared as concentrated stocks (5mM) in PBS, and then used at a concentration of 20μM, Stock solution of acrylamide(500mM in PBS, Sigma Chemical Co., MO) was prepared and stored at -20°C and used with 5mM aliquots. Cultures were incubated with the drugs for 1, 2, or 3 hours at 37C.

UVB irradiation

Melanocytes were seeded into 6-well culture plates at a density of 5×10^3 cells.cm^3 for the morphological observation and into Lab-Tek chamber slides precoated with 1% gelatin at a density of 5×10^3 cells/well for indirect immunofluorescent staining. The source of ultraviolet B(UVB) was FS72T12-UVB-HO lamp(Elder Pharmaceuticals, OH) which emits mostly 290-325 nm of radiation, ir peak wavelength was between 310-312 nm, ir does not emit biologically significant amount of UVC. As Bruls et al.[24] reported in their previous paper that about 5-10% of the UVB which strikes the skin surface can penetrate to the melanocyte-containing basal cell layer, we chose the dose of

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5mJ/cm^2. The medium was replaced with phosphate buffered saline before irradiation, to avoid the formation of medium-derived toxic photoproducts. The cover of the plate was removed and the plate was placed under the radiation source. lmmediately after irradiation, the PBS was removed and replaced with MGM. Cultures were irradiated daily and the responses were measured 24 hours after the last exposure. The gross morphological and cytoskeletal changes of the cultured melanocytes were evaluated at day 0 and 6 in response to daily 5mJ/cm^2 of UVB irradiation.

Immnofluorescence staining

Melanocytes were seeded into Lab-Tek chamber slides at a density of 5×10^3 cells/well and incubated for 3 days. Cells were rinsed once with warm PBS at room temperature. They were treated with methanol at -20°C for 10 minutes. and washed with PBS-1% BSA and then incubated with cold aceton at -20°C for 10 minutes. The fixatives were washed with PBS-1%BSA and then rinesd with PBS and incubated at 37°C for 15 minutes with 10% normal goat serum to block non-specific binding of primary antibodies. Primary antibodies were added and the cells were incubated at 37°C for 45 minutes in a moist chamber. Mouse anti-actin monoclonal antibody(ICN Biomedicals lnc., CA) was used at 1:20 dilution in PBS. Mouse anti-β tubulin monoclonal antibody(Biodesign international, ME) was used at 1:50 dilution in PBS. Mouse anti-βvimentin monoclonal antibody (Biomeda, CA) was used at the original concentration. The cells were then rinesd with PBS 3 times and incubated with 1:40 dilution of fluorescein-conjugated goat polyclonal antibody to mouse lgG(lmmunotech SA, France)at 37°C for 30 minutes in a moist chamber. After further thorough rinsing with PBS for 15 minutes, the cells were dipped with ethanol and briefly dipped in distilled water to remove salt. They were then dried and mounted on microscopic slides with a mixture of PBS-glycerol and observed immediately under a Olympus fluorescence microscope. Photographs were taken using Kodak Ektachrome 400 Film.

Transmission electron microscopy

Cultured melanocytes before and after the treatment of 5mJ/cm^2 of UVB irradiation were collected in a cornical tube and fixed with Karnovsky's fixatives[25] for 2 hours at room temperature and then were washed in 0.1 M sodium cacodylate buffer 3 times. The cells were treated with 1% osmium tetroxide for 1 hour and washed in buffer 3 times and then stained with 0.5% aqueous uranyl acetate for 30 minutes. They were dehydrated with the graded ethanols and embedded in Epon, cut into several ultrathin sections. Ultrathin sections were stained with uranyl acetate followed by lead citrate and then examined in a Philips CM-10 electron microscope.

Scanning electron microscopy

Cultured melanocytes were seeded into Lab-Tek chamber slides precoated with 1% gelatin at a concentration of $5x10^3$ cells/cm^2 and maintained for 3 days. After the treatment of 5mJ/cm^2 of UVB irradiation, they were washed, prefixed with 1% glutataldehyde and 1% paraformaldehyde, and postfixed with 1% osmium tetroxide. They were dehydrated with the graded ethanols, transferred to isoamyl acetate and dried in a critical point drier(Hitachi ICP2, Japan). The cells were coated with gold in sputter coater, and then examined with a Hitachi S-450 scanning electron microscope

RESULTS

Melanocytes were successfully kept on long-term maintenance. Human foreskin melanocytes maintained in our culture conditions showed ellipsoidal cellular contour, transluscent cytoplasm and mostly bipolar and occasionally multipolar, enlongated dendritic processes in phase contrast micropscopic examination (Fig. 1-A). Colchicine-treated melanocytes lost their characteristic morphology and produced a marked shortening of dendrites, and the cellular contour became darkened, irregular and stellate within 1 hour of exposure (Fig. 1-B). Upon removal of colchicine by washing after 2 hours of exposure, the changed cellular rnorphology reversed to the normal untreated state within 1 hour. The melanooytes exposured to cytochalasin D for 2 hours became round in their outer cellular contour but their dendrites were not altered by this treatment (Fig. 1-C). These changes were also reversible after removal of cytochalasin D within 2 hours of e posure. But melanocytes exposured to cytochalasin D for more than 3 hours became round and detached from

the culture vessels. Acrylamide produced disorganization of cytoplasmic constituents, thus the dendrites became irregularly beaded due to clulmping of the intracellular components along the dendrites, but the dendrites were not shortened(Fig. 1-D). Vimentin organization returned to normal within 24 hours of the removal of the drugs. Repeated doses of 5 mJ/cm^2 of UVB for 6 days increased the number of dendritic processes, increased the perinuclear area and resulted in the darkening of the melanocytes(Fig. 1-E). These changes were apparent 4 days after irradiation. The number of UVB-responded melanocytes increased with the long exposure.

By immunofluorescent microscopic examination, microtubules normally extended through the long axis of the melanocytes showing a fibrous appearances, but they minimally covered the nuclear area and condensed at perinuclear area and dendritic process(Fig. 2-A). Actins stained throughout the whole cytoplasm and dendrites, but in slender cytoplasmic areas actin bundles could not be observed(Fih. 2-B). Vimentin filament system extended from the nucleus to the periphery and these filaments ran irregularly in the melanocyte(Fig. 2-C). However, it was difficult to determine the exact distribution of the cytoskeletal system, for the cultured melanocytes were small in the nucleus-containing area and had a highly elongated morphology. Within 1 hour of colchicine treatment, there was a marked reduction in the appearance of fibrous tubulin staining in comparison with the control cultures, and by 2 hours few cells retained an intact microtubular array. Granular immunofluorescence were deposited in the cytoplasm(Fig. 2-D). Upon removal of colchicine by washing, the microtubular fibers reappeared. Actin staining in cytochalasin D-treated melanocytes showed granular deposition of fluorescence in the cytoplasm within 1 hour(Fig. 2-E). Acrylamide-treated melanocytes showed irregular clumping and collapse of the vimentin fibers in some areas in 2 hours(Fig. 2-F). All cytoskeletal antagonists had their specific inhibitory effects to their unique cytoskeleton except for the colchicine to vimentin(Table 1, 2). Vimentin organization collapsed toward the cell center in response to clochicine, the microtubule-depolymerizing agent. Repeated exposures of 5 mJ/cm^2 of UVB for 6 days gave hypertrophy in the perikaryou, thus detailed fibrous structures of each cytoskeleton could be easily observed. But no singificant changes in their organization could be observed(Fig. 2-G,H,I).

Transmission electron microscopic examination showed that melanocytes had numerous mitochondria, vesicles, smooth endoplasmic reticulum, rough endoplasmic reticulum, Golgi apparatus and abundant melanosomes(Fig. 3-A). In the UVB-irradiated melanocytes no other structural abnormality and degradation process were observed except for the increased number of melanosomes(Fig. 3-B). Scanning electron microscopic examination gave detailed surface morphology of the melanocytes in culture. Most cells possessed bi-or tri-polar dendritic processes and their surfaces were smooth or covered with microvilli(Fig. 4-A,B). Anchoring filaments were observed only in the microvilli-covered cells. The tip of the dendritic process also posessed two distinct findings; one was clubbed and smooth, and the other resembled the microspikes. All the above findings were intermingled within the same culture slide. No significant changes were observed after UVB exposure.

DISCUSSION

The development of a human melanocyte culture system is a prerequisite to revealing the biological characteristics of melanocytes and to investigating pigmentary dermatoses. With the advent of techniques for growing melanocytes in culture, research for elucidating the mechanisms underlying pigmentary disorders became active. By using selective growth and long-term maintenance conditions we can selectively culture melanocytes derived from normal adult foreskin.

Melanocytes have a round to oval nucleus, numerous mitochondria and vesicles, microfilaments, intermediate filaments and microtubules in the cytoplasm. There are abundant smooth and rough endoplasmic reticulum and Golgi apparatus, all of which are microanatomic features of cells actively involved in the synthesis of proteins. Dendrites from the pigment cells extend between surrounding keratinocytes. They extend and retract to the different areas surrounding the melanocytic cell body.[26] Melanosomes, the specialized ellipsoidal organelles that contain melanin, are synthesized only in the melanocytes and transferred to the keratinocytes through phagocytosis

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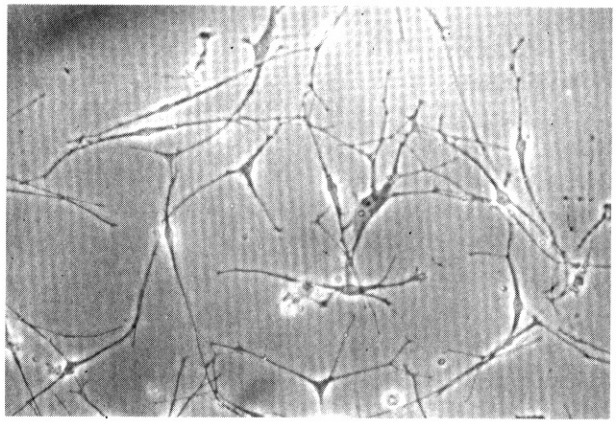

Fig. 1-A. Normal cultured melanocytes showing ellipsoidal cellular contour and enlongated dendrites(Phase contrast microscopy, × 200).

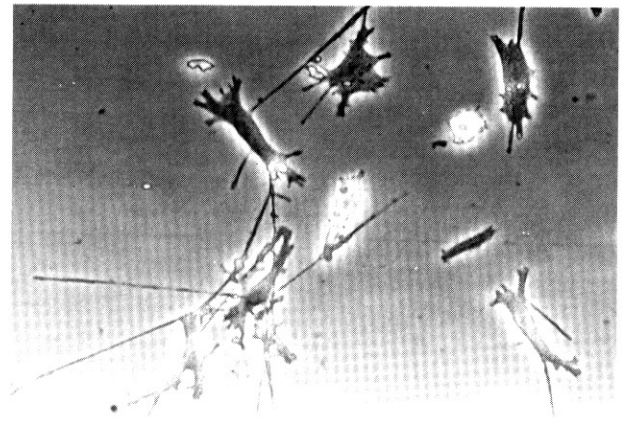

Fig. 1-B. Colchicine-treated cultured melanocytes showing stellate cellular contour and shortened dendrites (Phase contrast microscopy, ×200).

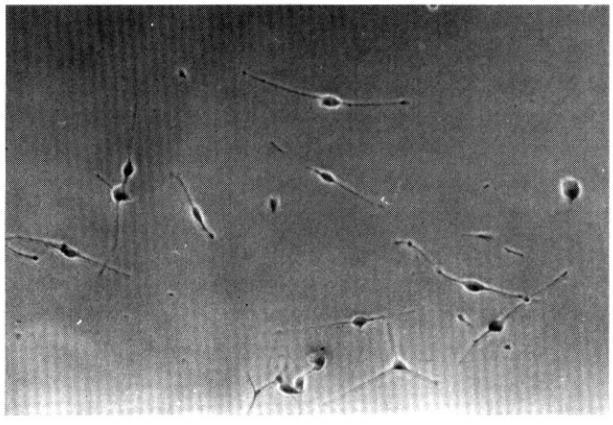

Fig. 1-C. Cytochalasin D-treated cultured melanocytes showing round cellular contour(Phase contrast microscopy, × 200).

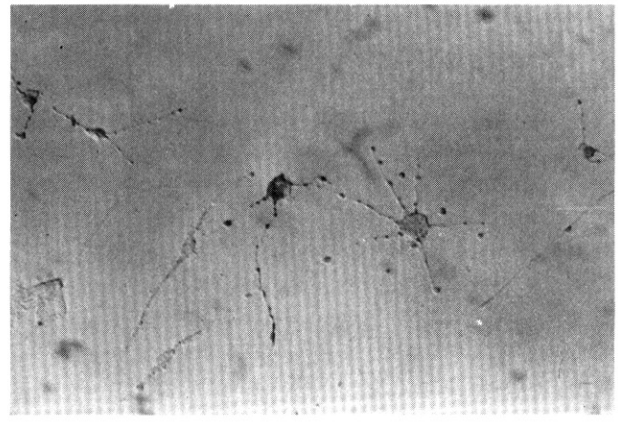

Fig. 1-D. Acrylamide-treated cultured melanocytes showing beaded accumulation of cellular components in the dendrites(Phase contrast microscopy, ×200).

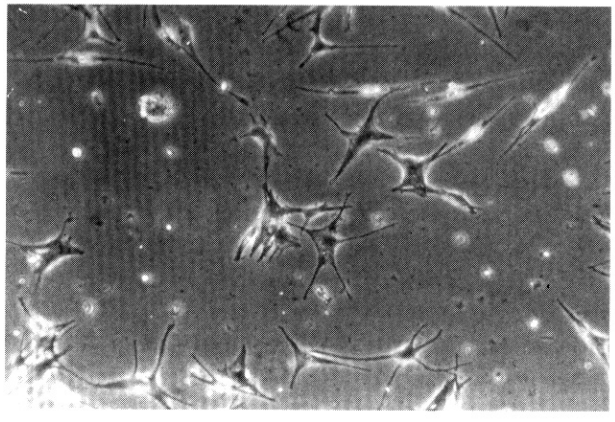

Fig. 1-E. UVB-irradiated cultured melanocytes showing increased number of dendritic processes and perinuclear area, darkened cytoplasm(Phase contrast microscopy, × 200).

of the dendritic tips. In the keratinocytes, the melansomes are degraded forming the secondary lysosomes and the melanin is dispersed evenly in the keratinocytes forming epidermal density filter.

Cytoskeletons have important roles in the movements of cells and subcellular structures and in the generation of shape of cells. There are three major classes of cytoskeletal fibers in eukaryotic cells; microtubules (24nm in diameter), actin microfilaments (7nm in diameter), intermediate filaments (10nm in diameter). Microtubules are involved in th mitotic spindle and chromosome mo e-ment and cell separation during mitosiz. They are also involved in the movement of cilia and flagella and provide tracks for transport of small vesicles. Actin microfilaments have a structural role in

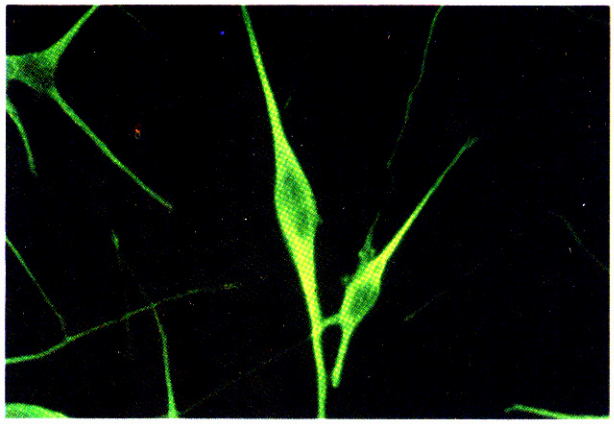

Fig. 2-A. Tubulin staining in the normal cultured melanocytes showing fibrous appearance through the long axis of the cytoplasm(IF, × 400).

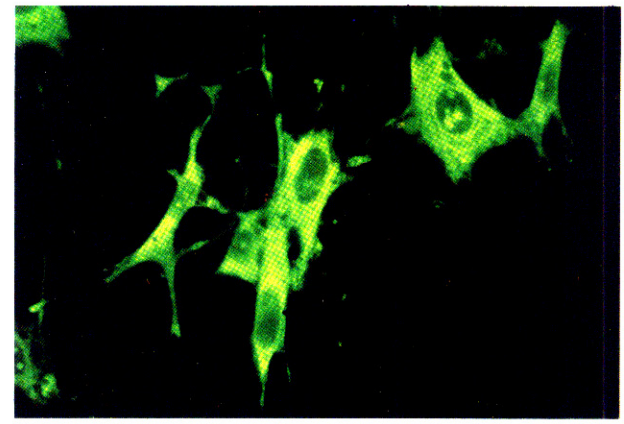

Fig. 2-B. Tubulin staining in colchicine-treated cultured melanocytes showing granular appearance in the cytoplasm(IF, × 400).

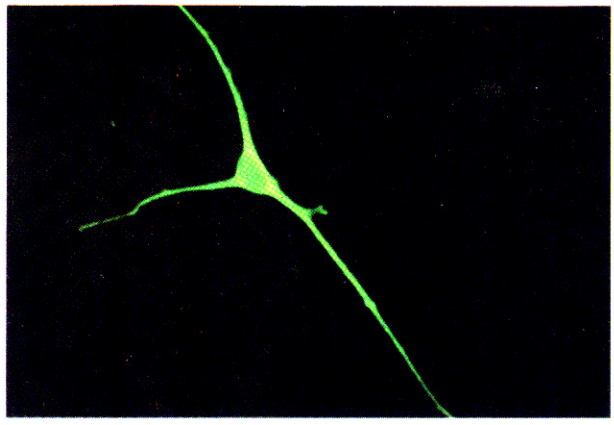

Fig. 2-C. Actin staining in the normal cultured melanocytes showing fibrous appearance in the cytoplasm(IF, × 400).

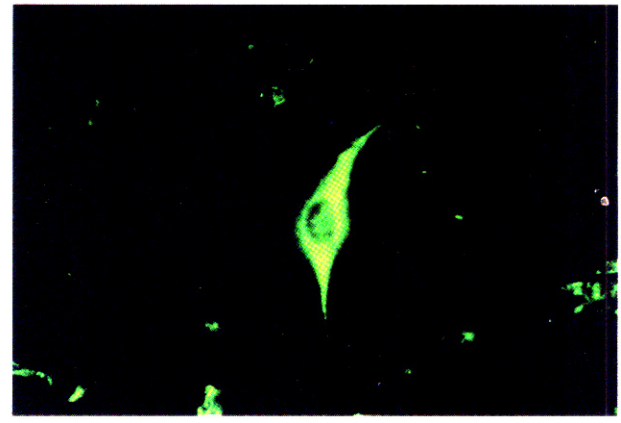

Fig. 2-D. Actin staining in cytochalasin D-treated cultured melanocytes showing granular appearance in the cytoplasm(IF, × 400).

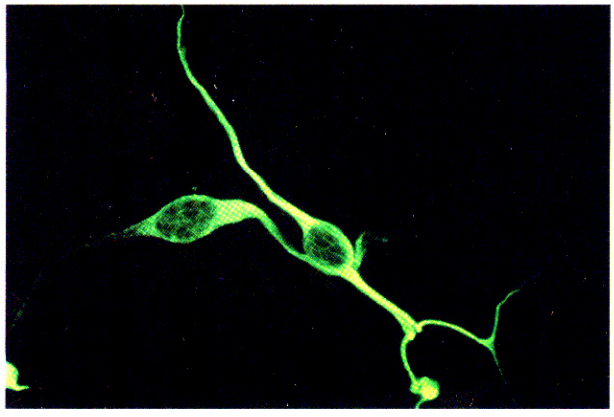

Fig. 2-E. Vimentin staining in the normal cultured melanocytes extended from the nucleus to the periphery(IF, × 400).

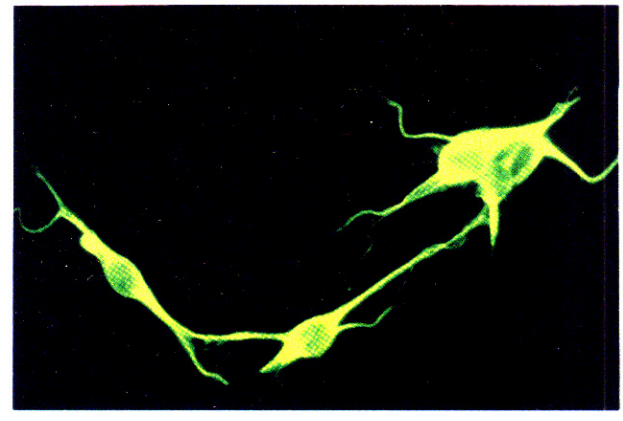

Fig. 2-F. Vimentin staining in acrylamide-trated cultured melanocytes showing irregular clamping and collapse of of the filaments(IF, × 400).

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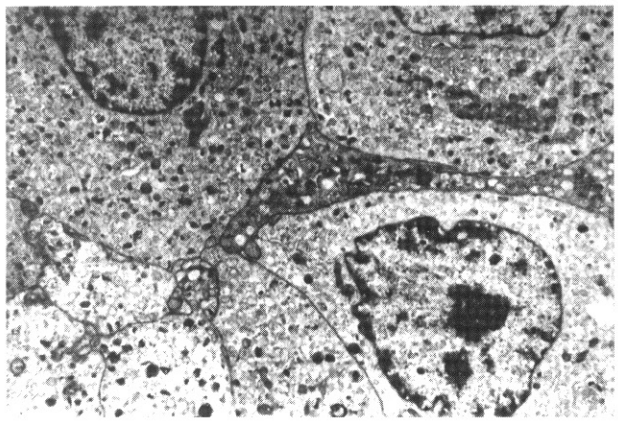

Fig. 3-A. Cultured melanocytes showed numerous mitochondria, vesicles, endoplasimic reticulum, Golgi apparatus and melanosomes(TEM, × 4,720).

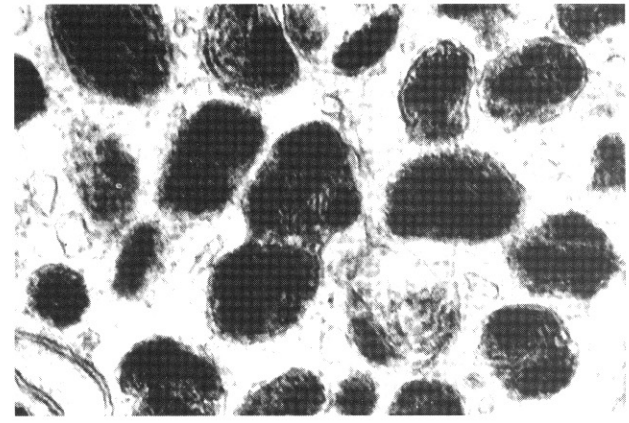

Fig. 3-B. UVB-irradiated cultured melanocytes showed increased melanosomes in the cytoplasm(TEM, × 45,600).

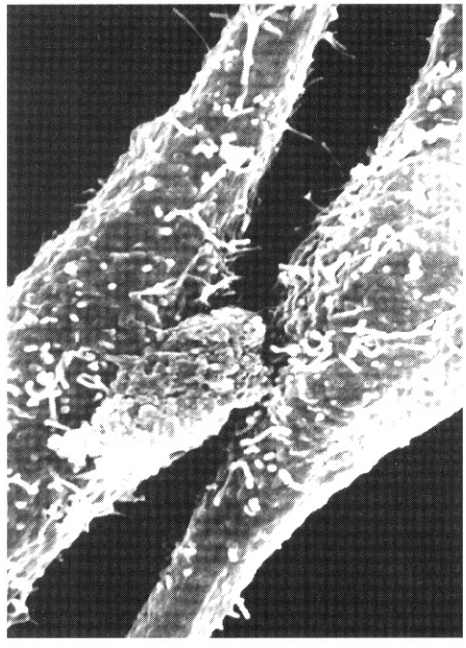

Fig. 4-A. Surface topography of the cultured melanocytes covered with numerous microvilli(SEM, × 5,500).

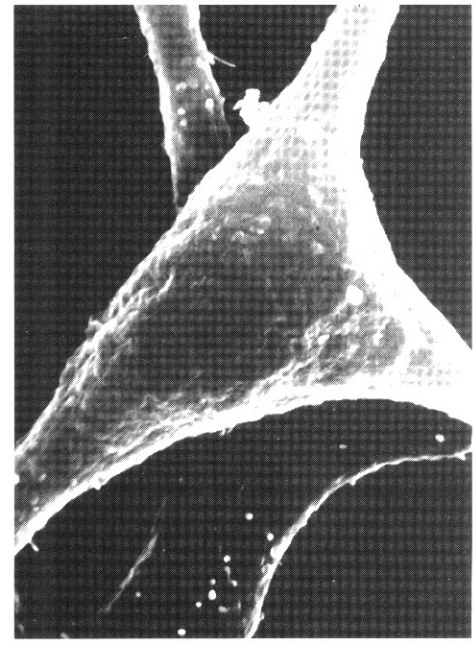

Fig. 4-B. Surface topography of the cultured melanocytes with smooth surface(SEM, × 5,500).

maintaining the shape of microvilli, the movement of endoplasmic reticulum and the attachment of cultured cells to substratum via stress fibers. Intermediate filaments keep the nucleus or other organelles in a defined place within the cell and are useful in identifying the cell type.

Microtubules are permanant and/or transient. For example, in a confluent, nondividing monolayer of cultured fibroblasts, microtubules have a predominantly radial orientation. Microtubules in growing animal cells are in a dynamic state of assembly and disassembly. The microtubules extend outward from a microtubule-organizing center(M-TOC). The distal ends of the microtubules are near the plasma membrane. Using the deep-etching technique, the cytoskeleton lattice of the microtubules and intermediate filaments that extends along the axons and dendrites of neuron revealed the connection between adjacent microtubules and between micrortubules and microfilaments.

Table 1. Patterns of cytoskeletal inhibition

Structure	Antagonist	Effect of antagonist	
		Phase contrast	Immunofluorescence
Tubulin	Colchicine	Shortened dendrites Stellate cellular contour	Shortned dendrites Stellate cellular contour Granular fluorscence
Actin	Cytochalasin D	Round cellular contour	Round cellular contour Granular Fluorescence
Vimentin	Acrylamide	Beaded cellular contents especially in dendrites	Irregular clumping of fluorescence

Table 2. The effects of antagonists on each cytoskeleton in immunofluorescent examination

Structure	Colchicine	Cytochalasin D	Acrylamide
Tubulin	+	–	–
Actin	–	+	–
Vimentin	–*	+	

+: charateristic changes, -: no effect, *: clumping of immunofluorescence

These microtubules act as guides or tracks along which protein particles and organelles move up and down the axon.[27] Melanophores founded in amphibians contain the granules of pigment. Under nervous and hormonal control, these pigment granules can be transported inward and outward to change their color. During this movement, the microtubules act as guides along which pigment granules and associated materials can move in both directions.[3] Microtubules contain two kinds of proteins: α-tubulin, β-tubulin. They form a.b dimer and these dimers assemble the microtubules. The function of microtubules result from their capacity to assemble and dissemble reversibly

Colchicine, a plant alkaloid, basically binds the tubulin and prevents the polymerization of the new tubulins into microtubules and results in net loss of microtubules and an accumulation of free tubulin. Microtubules synthesis is reinduced after removal of colchicine. Microtubules exposced to low temperature or high pressure also undergo disassembly. Within cells, vesicles and protein particles and Golgi apparatus are frequently transported to a particular cellular regions along the t act of microtubules. Energy released during ATP hydrolysis is used as a "molecular motor" to power the movement of vesicles and particles along the tract of microtubules.[1] The fact that colchicine partly inhibited the development of dendrites supported the theory that melanocyftes filaments are also involved in the development of dendrites.

The globular protein actin is the major subunit of microfilaments and all eukaryotic cells contain actin. Microfilaments, sometimes called fibrous actin or F actin, are polymers of a globular protein subunit called globular actin or G actin. The microfilaments inside the cells are expected to be much more stable than microtubules. Actin has major role in muscle cell contraction in concert with myosin. In contrast to orderly filamentous arrangement of actin in striated muscle cells, the spatial organization of actin in nonmuscular cells in highly variable and complex. In most nonmuscular cells,[1] these preteins may serve both structural and contractile filaments. Actin microfilament occur in a well-ordered pattern in microvilli number of motile events in the nonmuscle cells. Actin fibers are thick and straight like a cable, often extending right across the cell periphery and stained intensely at the cell boundary. The movement of endoplasmic reticulum is powered by myosin-like motors that move along stationary bundles of actin filaments. The movements of many animal cells involve rapid extensions and retractions of regions of the plasma membrane that contain bundles of actin filaments. During

the fibroblast movement, lamellipodia that are rich in actin filaments have a key role. In cultured fibroblasts and endothelial cells, much actin occurs in long bundles of paralell filaments termed stress fibers which often extend the length of the cells. Stress fibers are believed to be involved in the attachment of the cultured cells to a substratum and also in the generation of the stress or tension that detemine the shape of the flattened cultured cells.[1] Actin in seen as stress fibers or cables, as staining around the the cell edges, presumably as as submembranous sheath,[28] and as diffuse cytoplasmic fluorescence which expresses the soluble actin or actin arranged in structures with dimensions below the resolution of the immunofluorescence technique.

Cytochalasins, the fungal products specifically block the polymerization of actin filaments by binding to the end of a growing filament. Cytochalasins cause the melanocytes to round up and also inhibit the projections of cell surface membrane. These results showed that the primary site of cytochalasin D is the surface of the plasma membrane. Gross alterations in cell surface morphology as well as disruption of actin microfilamen

Intermediate filaments are unique to multicellular organisms, and different types of differentiated cells usually contain specific types of intermediate filaments. They are similar in structure and function but composed of different types of subunit protein. Intermediate filaments are now thought to stabilize the epithelium, from the major structural proteins of skin and hair. Intermediate filaments are not dynamic polymer and insoluble. Vimentin is expressed typically in mesemchymal cells such as fibroblasts, endothelial cells and melanocytes. Vimentin fibers are typically organized in an extended system that stretches from the nuclear envelop to the plasma membrane and often terminate at desmosome or adhesion plaques on the plasma membrane. They are closely associated with microtubules. They may function to keep the nucleus or other organelles in s defined place within the cells or form a cage, probably preventing cell organelles from themselves and cell membrane. Intermdiate filaments carry out specific functions of differentiated cells such as nucleus and other organelles in their proper positions. In this sense, intermediate filaments have a more stable role in the regulation of cell processes than do the microtubules and actin.

Acrylamide causes the collapse of vimentin organization while leaving the microtubule organization apparently unaffected. The normal vimentin organization depends primarily upon an interaction with the microtubule array and to a lesser degree on an interaction with the plasma membrane. These results suggest that the organization of vimentin filaments in determined in some way by the lattice of microtubules possibly by protein connectors between the two types of filaments. The disruption of microtubules organization causes the disruption of normal vimentin organization but the disruption of vimentin organization has no apparent effect on microtubule organization.[29] Our experiment showed that acrylamide had little effect on the gross morphology of the melanocyte, and this might suggest that vimentin functions as an organelle-moving matrix only in the cytoplasm rather than as a structural skeleton. In most nonmuscular cells, microfilaments and intermediate filaments are organized in a seemingly random array. Along with the microtubules, these filaments organize the cytoplasm. They provide a structure to which proteins and organelles can bind, so that different cytoplasmic proteins are localized to different regions of the cells. Often these filaments are associated with the plasma membrane, thus playing key roles in determination of cell shape and motility.

During the past two decades, fluorescence microscopy has provided important clues to the mechanisms of various motile activities and the functional roles of many cytoskeletal systems. On small cells and cells with a highly elongated morphology, it is difficult to determine whether the cytoskeletal system was extended or collapsed at the area of the cell containing the nucleus. Thus little is known about the molecular details of how the organization of the melanocyte cytoskeleton is controlled.

The movement of satge III and IV melanosomes from the perinuclear region is probably mediated by intermediate filaments. The microtubules provide the microskeletal sturctures for the formation of dendrites. Melanosomes are transferred from their sites of synthesis in the perikaryon of the melanocyte to the tips of dendrites through the motive action of cytoskeletal elements.[4-5] The selective recognition factors on the melanosoal membrane. Two experimental systems for the

melanosomal movement within melanocytes, an immediate pigment darkenning(IPD) reaction[19] and dark and light adaptation of the eye,[5] indicated that 10-nm intermediate filaments are probably involved in the movement of melanosomes. Jimbow and Fitzpatrick.[5] reported that the movement of melanosomes in the melanocyte is related with 10-nm filaments observing the IPD response. It has been tentatively concluded that stimuli which affect the microtubular organization might influence the vimentin organization and thus might be involved in melansome movement. UV light is the best-known stimulant of melanogenesis. These indicate that melanocytec filaments change their location after exposure to UV light. UVB is the most efficient and effective tanning specturum Direct exposure of melanocytes or melanoma cells in culture to UVB induces pigment formation.[11] This suggests either that the cell has an internal photoreceptor resopnsible for activation of the tyrosinase system or that UVB generates by-products, like oxygen radicals that cause the cells to respond by forming melanin. In response to UVB, epidermal cells produce prostaglandin E and D and vitamin D, interleukin-1, 6 and tumor necrosis factor-α,α-MSH which modulate the melanin synthesis.[32] All these cytokines have been shown to affect pigment production. Melanocytes exposed to dither UVA and UVB should be activated to remove the by-products. Physilolgic does of UVB induces the differentiation of the melanocytes by increasing the tyrosinase activity and melanin contents in a c-AMP-independent mechanism. UVB is toxic to the melanocytes when irradiated in large doses.[20] Melanocytes showed different morphological, proliferative and melanogenic characteristics according to the culture condition.[21-22] Zaremba et al.[14] reported that isolated microtubular proteins irradiated by UVB and UVA light, composed of environmentally relevant wavelengths, was found to disrupt the cytoplasmic microtubule complex of the cultured human fibroblasts in a dose-dependent manner and not of the microfilaments, but the induction of microtubular disassembly did not correlate with the cytotoxicity of ultraviolet light of varying composition. But their actual dose of UVB was 500ml/cm^2, which was nonphysiologic and toxic to the melanocytes, and the source of cells was not the UV-protective melanocytes Although UVB irradiation in our experimental doses produced the morphological changes in the melanocytes, we could not observe any significant alteration in the cytoskeletal organization using the immunofluorescent microscopic examination. More investigations on the effects of the larger dose of UVB which can induce erythema, cellular inactivation, mutagenesis, transformation, and other bilolgic effects are necessary. In this experiment, we only observed the cytoskeletons of cultured melanocytes. But to further elucidate the changes of the dendricity and the mechanism of melanosomal movement co-culture of human melanocytes and keratinocytes will need to be undertaken.

Ultrastructural studies of the melanocytes demonstrated only a limited amount of information. The increase in the number of melanosomes after UVB irradiation might be caused by the newly synthesized melanosomes as in the delayed tanning(DT) reaction. To determine the effects of UVB on the cytoskeletons of the melanocytes, more detailed parameters are necessary and high resolution electron microscope and immunoelectron microscopic approaches should be applied. Scanning electron microscopy extends the knowledge of the overall shape, surface topography, and the membrane configurtion of the cultured cells beyond the scope of the light microscopy, for it provides a vivid three-dimensional picture.[23] Cells in the monolayer culture examined by scanning electron microscopy might, exhibit a variation in the surface topography depending upon the individual cell lines, the nature of the medium, fixation and other processing steps, and contamination with cellular debris or mycoplasma. Because in the monolayer cultures the cell shapes such as round or enlongated, and the cell surface configurations such as smooth or covered with microvilli were determined by the stage in mitotic cycle,[24] various surface topographic findings might be found in the same specimen. Little information is available on the scanning electron microscopic analysis of melanocytes, and more effort to establish the basic scanning electron microscopic findings of the cultured melanocytes is essential. Although our data do not confirm the precise roles of cytoskeletons in the melanocytes, these results indicate that cytoskeletions may be related to the dendricity and the movement of melanosomes in the melanocytes. However the relative roles and the relationship between melanocytic cytoskeletons in the transfer of

melanosomes are still not clearly understood and remain to be investigated.

REFERENCES

1. Darnell J, Lodish H, Baltimore D: Microtubules and cellular mevements. Actionm myosin, and intermediate filaments:Cell movements and cell shape. In Darnell J, Lodish H, Baltimore D(eds): Molecular Cell Bilolgy, 2nd ed. Scientific American Books, New York: 1990, pp815-902.
2. Jimbow K, Quevedo Jr. WC, Fitzpatrick TB, Szabo G: Biology of melanocytes In Fitz TB, Eisen AZ, Wolff K, Freedberg IM, Austin KF (eds): Dermatology in General Medicine, 4th ed. McGraw-Hill New York: 1993, pp261-289.
3. Schliwa M: Stereo high voltage electron microscopy of melanophores: Matrix transformation during pigment movements and the effects of cold and colchicine. Exp Cell Res 118: 323-340, 1979.
4. Jimbow K, Pathak MA, Fitzpatrick TB: Effect of ultraviolet on the distribution pattern of microfilaments and microtubules and on the nucleus in human melanocytes. Yale J Biol Med 46: 411-426, 1973.
5. Jimbow K, Fitzpatrick TB: Changes in distribution patterns of cytoplasmic filaments in human melanocytes during ultraviolet-mediated melanin pigmentation J Cell Biol 65: 481-488, 1975.
6. Okazaki K, Uzuka M, Morikawa F, Toda K, Seiji M: Transfer mechanism of melanosomes in epidermal cell culture. J Invest Dermatol 67: 541-547, 1976.
7. Rosdahl IK, Szabo G: Mitotic activity of epidermal melanocytes in UV-irradiated mouse skin. J Invest Dermatol 70: 143-148, 1978.
8. Gilchrest BA, Blog FB, Szabo G: Effects of aging and chronic sun exposure on melanocytes in human skin, J Invest Dermatol 73: 141-143, 1979.
9. Nordlund JJ, Ackles AE, Traynor FF: The proliferative and toxic effects of ultraviolet light and inflammation on epidermal pigment cells. J Invest Dermatol 77: 361-368, 1981.
10. Stierner U, Rosdahl I, Augustsson A, Kagedal B: UVB irradiation induces melanocyte incease in both exposed and shieled human skin. J Invest Dermatol 92: 561-564, 1989.
11. Friedmann PS, Gilchrest BA: Ultraviolet radiation directly induces pigment production by cultured human melanocytes. J Cell Physiol 133: 88-94, 1987.
12. Yohn JJ, Lyons MB, Norris DA: Cultured human melanocytes from black and white donors have different sunlight and ultraviolet A radiation sensitivities. J Invest Dermatol 99: 454-459, 1992.
13. Ramirez-Bosca Am Bernd A, Dold K, Holzmann H: Effect of the dose of ultraviolet radiation on the pigment formation by human melanocytes vitro. Arch Dermatol Res 284: 358-362, 1992.
14. Zaremba TG, Lebon TR, Millar DB, Smejkal RM, Hawley RJ: Effects of ultraviolet light on the in vitro assembly of microtubules. Biochemistry 23: 1073-1080. 1984.
15. Zamansky GB, Chou IN: Environmental wavelenghts of ultraviolet light induce cytoskeletal damage. J Invest Dermatol 89: 603-606, 1987.
16. Zamansky GB, Perrino BA, Chou IN: Disruption of cytoplasmic microtubules by ultraviolet radiation. Exp Cell Res 195: 269-273, 1991.
17. Zamansky GB, Chou IN: Disruption of keratin intermediate filaments by ultraviolet radiation in cultured human keratinocytes. Photochem Photobiol 52: 903-906, 1990.
18. Mayerson PL, Brumbaugh JA: Lavender, a chick melanocyte mutant with defective melanosome translocation: A possible role for 10 nm filaments and microfilaments but not microtubules. J Cell Sci 51: 25-51, 1981.
19. Honigsmann H, Schuler G, Aberer W, Romani N, Wolff K: Immediate pigment darkening phenomenon. A reevaluation of its mechanisms. J Invest Dermatol 87: 648-652, 1986.
20. Chao-Hsing KAO, Hsin-Su YU: A study of the effects of phorbol 12-myristate-13-acetate on cell differentiation of pure human melanocytes in vitro. Arch Dermatol Res 283: 119-124, 1991.
21. Lacour JP, Gorden PR, Eller M, Bhawan J, Gilchrest BA: Cytoskeletal events underlying dendrtie formation by cultured pigment cells. J Cell Physiol 151: 287-299, 1992.
22. Si SP, Tsou HC, Lee X, Peacocke M: Cultured human melanocytes express the intermediate filament vimenitin. J Invest Dermatol 101: 383-386, 1993.
23. Im S, Hann SK, Park YK, Kim HI: Culture of melanocytes obtained from normal and vitiligo subjects. Yousei Med J 33: 344-350, 1992.
24. Bruls WAG, van Weeiden H, van der Leun JC: Transmission of UV-radiation through human epidermal layers as a factor influencing the minimal

erythema dose. Photochem Photobiol 39: 63-67, 1984.
25. Karnovsky MJ: A formaldehyde-glutaraldehyde fixative of high osmolarity for use in electronmicroscopy. J Cell Biol 27: 137A, 1965.
26. Rosdahl I, Bagge U: Vital microscopy of epidermal melanocytes. Acta Derm Venereol (stockh) 61: 55-59, 1981.
27. Okabe S, Hirokawa N: Microtubule dynamics in nerve cells: analysis using microinjection of biotinylated tubulin into PC 12 cells. J Cell Biol 107: 651-664, 1988.
28. McNutt NS, Culp LA, Black PH: Contact inhibited revertant cell lines isolated from SV-40 transformed cells. IV. Microfilament distribution and cell shape. J Cell Blol 50: 691-708, 1973.
29. Klymkowsky MW: Metabolic inhibitors and intermediate filament organization in human fibroblast. Acta Derm Venereol(Stockh): 282-290, 1988.
30. McGuire J, Moellmann G: Cytochalasin B; effects on the microfilaments and movement of melanin granules within melanocytes. Science 175: 642-644, 1972.
31. Moellmann Gm McGuire J, Lerner AB: Ultrastructure and cell biology of pigment cells. Intracellular dynamics and the fine structure of melanocytes. Yale J Biol MedLee 46: 337-369, 1973.
32. Morelli JG, Kincannon J, Yohn JJ, Zekman T, Weston WL, Norris DA: Leukotriene C4 and TGF-alpha are stimulators of human melanocyte migration in vitro. J invest Dermatol 98: 290-295, 1992.