Brief Report

DOI: 10.5582/ddt.2022.01075

Membrane translocation of vinculin after UVA exposure facilitates melanosome trafficking

Hiroyuki Yamamoto[1,*], Chiho Tanaka[1], Momo Okada[1], Yoshikazu Sawaguchi[2], Toshiyuki Yamada[1]

[1] Department of Microbiology and Molecular Cell Biology, Nihon Pharmaceutical University, Saitama, Japan;
[2] Faculty of Biomedical Engineering, Toin University of Yokohama, Yokohama, Japan.

SUMMARY Skin pigmentation is among the defenses against ultraviolet (UV) radiation. During formation of skin pigmentation, melanosomes that are transported to the cell membrane and released are internalized by keratinocytes. We here examined whether vinculin, the origin of actin fibers, is involved in this intracellular transport of melanosomes by using melanocytes with suppressed vinculin expression. Using fluorescence immunostaining, the migration of vinculin to the cell membrane due to exposure to 365-nm LED light was examined. The intracellular distribution of melanosomes after irradiation was weighted toward the pericellular region compared with non-irradiated cells. With the suppression of vinculin expression, the amount of extracellularly released melanin decreased. We conclude that the membrane migration of vinculin after UVA exposure is involved in the intracellular transport of melanosomes.

Keywords vinculin, melanocyte, melanosome trafficking, actin filament, ultraviolet

1. Introduction

Sunlight reaching the Earth's surface includes ultraviolet (UV), visible, and infrared wavelengths. Of these, the UVB and UVA wavelengths are most relevant to skin homeostasis, as both have high energy and trigger exposed skin to produce various responses (*1,2*). UVA and UVB exposure causes erythema, sunburn, and pigmentation (*3*). Such skin responses protect organisms from the harmful effects of UV light, and skin pigmentation is a typical response. After UV exposure, various bioactive factors that enhance melanin formation in melanocytes are produced. Melanin is synthesized within mature melanosomes. Mature melanosomes are transported near the cell membrane and then incorporated into keratinocytes (*4*). Intracellular transport of melanosomes has been reported as a mechanism of microtubule trafficking and transport on actin fibers as in other cell organelles (*5,6*). However, factors determining the destination of melanosomes remain unknown.

While investigating proteins that are preferentially altered after UVB exposure, we found that UVB exposure increased the amounts of vinculin at the cell membrane. Vinculin connects the cell membrane to actin fibers (*7*). Actin fibers are used as rails for the transport of melanosomes after UVB exposure, and inhibition of actin fiber formation inhibits the transport of melanosomes to the cell membrane (*8*).

We here examined whether wavelengths in the UVA region, which have a significant effect on pigmentation, are involved in vinculin translocation to the cell membrane in addition to those in the UVB region. Furthermore, to clarify the function of vinculin transferred to the cell membrane after UVB exposure, we verified our hypothesis that actin fibers, which are formed after vinculin is transferred to the plasma membrane, are involved in melanosome trafficking.

2. Materials and Methods

2.1. Cell lines

The mouse melanoma cell line B16 was supplied by Health Science Research Resources Bank. The B16 cells were cultured in DMEM medium (Nissui Pharmaceutical, Tokyo, Japan) supplemented with 10% fetal bovine serum (Moretate Biotech, Bulimba, Australia). Human melanocytes and cultured media were purchased from Cell Applications (San Diego, CA).

2.2. Sample preparation

A total of 10^6 cells of B16 cells were seeded in a 10-cm petri dish. After culturing the cells for 24 h, they were

exposed to UVA at 20 W m^{-2} by using a 365-nm LED light source. At 24 h after UVA irradiation, the cells were lysed with RIPA buffer and centrifuged at 10,000× *g* and 4°C for 30 min. The supernatants were stored at −80°C for western blot analysis.

2.3. Immunocytochemistry

B16 cells were seeded on culture slides (Corning Inc., Corning, NY). After culturing the cells for 24 h, they were exposed to UV at 20 W m^{-2} by using the 365-nm LED light source. At 24 h after UV irradiation, the cells were fixed with 4% paraformaldehyde for 10 min. After blocking the cells with normal goat serum (1:50) for 30 min at room temperature, they were incubated with rabbit anti-vinculin antibody (1:100; Bethyl Laboratories, Montgomery, TX) overnight at 4°C, followed by secondary Hilyte-488-conjugated anti-rabbit goat antibody (1:500; BioSource International, Camarillo, CA) for 1 h at room temperature. The cells were then stained for actin filaments with rhodamine-conjugated phalloidin for 1 h at room temperature and stained for nucleic acid with Hoechst 33342 (Dojindo, Kumamoto, Japan). Fluorescence was observed under a fluorescence microscope (BZ-X700, Keyence, Osaka, Japan).

2.4. RNAi-induced vinculin knockdown assay

INTERFERin (Polyplus-transfection, Illkirch, France) was used to transfect vinculin siRNA (AGAAUCCAAGUGAACACUA(dTdT), Bioneer Corporation, Daejeon, Republic of Korea) into human melanocytes. At 72 h after transfection, vinculin expression was measured through western blot analysis.

2.5. Western blot analysis

Cell extracts (10 μg) were reduced with mercaptoethanol at 95°C for 10 min and then separated on a 12% polyacrylamide gel. Proteins were blotted on a nitrocellulose membrane (ClearTrans Nitrocellulose Membrane; Fujifilm Wako Pure Chemical, Osaka, Japan) by using a semi-dry blot system (NA-1512S, Nihon eido, Tokyo, Japan). The membranes were blocked with 2% skimmed milk. The blocked membranes were incubated with rabbit anti-vinculin antibody (1:1,000) or mouse anti-glyceraldehyde-3-phosphate dehydrogenase monoclonal antibody (1:5000; Fujifilm Wako Pure Chemical) and then with horseradish peroxidase-conjugated anti-rabbit IgG goat antibody (1:10,000; BioSource International) or HRP-conjugated goat anti-mouse IgG antibody (1:2500; BioSource International). The protein bands were subsequently visualized with a ImmunoStar Zeta by using a luminograph apparatus (Atto, Tokyo, Japan).

2.6. Microscopic observation of melanosome localization in B16 melanoma cells

B16 cells were seeded onto a 6-well plate (TrueLine, San Jose, CA). After incubation for 24 h, the cells were exposed to UVA at 20 W m^{-2} by using the 365-nm LED light source for 15 min. At 24 h after UVA irradiation, the distribution of melanosomes in B16 cells were observed using a microscope (BZ-X700, Keyence).

2.7. Release of melanosomes from human melanocytes

Vinculin siRNA-transfected human melanocytes were treated with 100 nM α-MSH, then the cells were irradiated with UVA at 20 W m^{-2} by using the 365-nm LED light source for 15 min. To evaluate the effect of actin fibers on melanin transport, a similar experiment was conducted using cytochalasin D to inhibit actin fiber formation. After 24 h of UVA irradiation, melanosome release was measured spectrophotometrically at 475 nm.

2.8. Statistical analysis

We analyzed the results *via* Tukey's test in R (version 4.1.2, R Development Core Team), and p values < 0.05 were considered to indicate statistical significance.

3. Results and Discussion

We previously reported that exposure to 312-nm UV light, which is in the UVB region (*7*), changes vinculin localization at the plasma membrane. By using fluorescence immunostaining, we here confirmed that exposure to 365-nm LED light induces the migration of vinculin to the cell membrane (Figures 1A and 1B). Vinculin, stained green, was observed in the cell plasma before irradiation (Figure 1A) but migrated to the cell membrane (Figure 1B) and co-localized with actin after exposure. Vinculin, an adhesion apparatus-lining protein, localizes to both the integrin-mediated cell extracellular

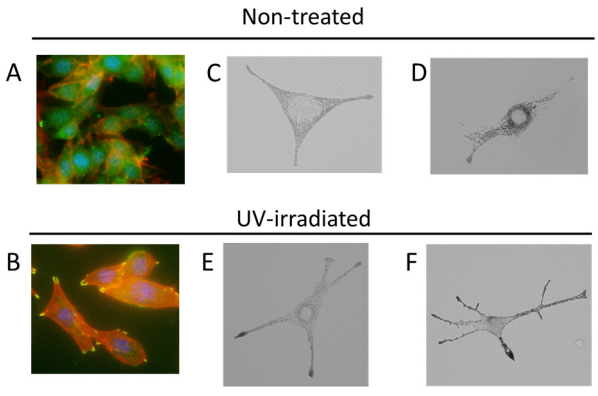

Figure 1. Distribution of vinculin and melanosomes in B16 cells after UVA irradiation. Immunohistochemistry of vinculin in melanocytes treated without (**A**) and with (**B**) UVA radiation. Vinculin is stained with Hylite-488 (green), actin filament is stained with rhodamine-conjugated phalloidin (red), and nuclei are stained with Hoechst (blue). The localization of melanosomes in cells treated with (**C**, **D**) and without UVA radiation (**E**, **F**) is shown.

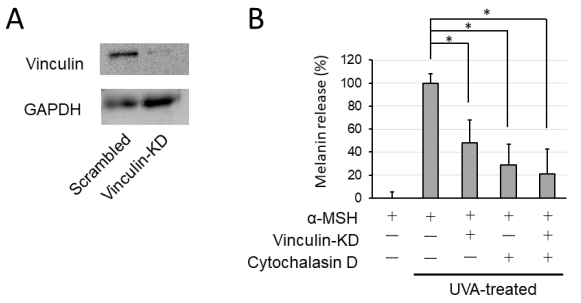

Figure 2. Effect of vinculin expression on melanin release from melanocytes. Western blot analysis of siRNA-mediated suppression of vinculin expression (**A**). Assessment of melanin release from melanocytes (**B**). α-MSH-stimulated melanocytes; melanin release in the absence of UVA irradiation was defined as 0% and that after UVA exposure was defined as 100%. Vinculin-KD indicates cells in which vinculin expression was suppressed with siRNA. Cytochalasin D was also used as an inhibitor of actin fiber formation. Bars indicate means ± SEM ($n = 3$). *: $p < 0.05$.

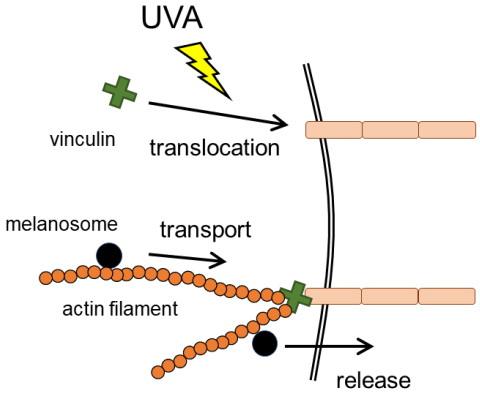

Figure 3. Involvement of vinculin migration and melanosome trafficking after UV exposure. This schematic diagram shows vinculin in membrane migration and melanosome trafficking after UV exposure.

matrix adhesion apparatus and cadherin-mediated cell-cell adhesion apparatus (*9-11*). After binding to actin, vinculin localizes to the force-associated adhesion apparatus to regulate cell adhesion and extension. In this study, exposure to wavelengths in the UVA region caused distribution of vinculin to the cell membrane. Vinculin was uniformly distributed around the cell periphery, with no directionality observed in its localization. This suggests that membrane migration of vinculin causes melanocytes to remain in place rather than enhancing their motility. Moreover, a decrease in cell motility may be consistent with the decrease in melanocyte motility after UV exposure.

Additionally, melanosome localization in the 365-nm LED-exposed cells was observed under a bright-field optical microscope (Figures 1C-1F). In cells cultured without 365-nm LED light, melanosomes were distributed throughout the cells (Figure 1C and 1D), while in some irradiated cells, melanosomes were localized at dendritic sites within the cells (Figures 1E and 1F). Intracellularly formed actin filaments also serve as transport rails for intracellular organelles (*12*). In melanocytes, melanosomes mature at the nuclear periphery because of UV exposure. The motor proteins kinesin and dynein present in the melanosome membrane are involved in the transport of these mature melanosomes; microtubules act as transport rails (*5*). The melanosomes transported to the cell membrane periphery are transferred to actin fibers, which act as new transport rails. They are then transported by myosin-Va, an actin-dependent motor protein, to the cell membrane, from where they are transported to the dendrite terminals (*6*). The pericellular localization of UV-exposed melanosomes was predicted to be due to vinculin migration to the plasma membrane, which promoted actin fiber formation. To determine whether changes in 365-nm LED light-induced intracellular melanosome trafficking involve vinculin translocation to the plasma membrane, we examined changes in the extracellular release of melanin from human melanocytes in which vinculin expression was suppressed with siRNA. This suppressed vinculin expression was evaluated through western blotting. At 48 h after transfection with vinculin siRNA, the density of the vinculin band reduced to approximately 30% (Figure 2A). Next, the human melanocytes were treated with α-MSH to mature intracellular melanosomes. These melanocytes were then exposed to 365-nm LED light, and the amount of melanosomes migrating to the membrane due to LED exposure was evaluated by measuring the amount of melanin released in culture media (Figure 2B). To examine the effect of actin filament formation on melanosome trafficking and membrane migration of melanosomes, we treated cells with cytochalasin D, an inhibitor of actin filament formation. The 365-nm LED light increased the amount of extracellularly released melanin, whereas downregulation of vinculin expression reduced the amount of melanin released to approximately 49%. Similarly, cytochalasin D suppressed melanin release to approximately 28%. Melanin release was suppressed at about the same rate in both vinculin knockdown cells with cytochalasin D treatment and those without. These results indicate that membrane translocation of vinculin after 365-nm LED light exposure increases the number of actin fibers at the cell membrane, thereby increasing melanosome release. Additionally, suppression of vinculin expression in melanocytes decreased the extracellular release of melanosomes, suggesting that vinculin is involved in the intracellular trafficking of melanosomes. In addition, UV exposure regulates the expression of β-catenin, the origin of actin fibers, at the plasma membrane (*13*). This report supports that UV light increased the formation of actin fibers from the cell membrane.

In conclusion, vinculin was translocated to the cell membrane after UVA exposure, indicating its involvement in the intracellular trafficking of melanosomes (Figure 3). In addition, melanosomes

transported to the vicinity of the cell membrane were released extracellularly, thereby facilitating the supply of melanosomes to keratinocytes. Membrane translocation of vinculin increases the number of adhesion points to surrounding cells *via* cadherins. This increase may be involved in the defense of organisms against UVA light through an increase in the number of melanin-supplying keratinocytes or melanin-supplying pathways, which in turn enhances skin pigmentation.

Funding: This work was supported in part by MEXT KAKENHI Grant Number 20K05829.

Conflict of Interest: The authors have no conflicts of interest to disclose.

References

1. Young AR, Chadwick CA, Harrison GI, Nikaido O, Ramsden J, Potten CS. The similarity of action spectra for thymine dimers in human epidermis and erythema suggests that DNA is the chromophore for erythema. J Invest Dermatol. 1998; 111:982-988.
2. Parrish JA, Jaenicke KF, Anderson RR. Erythema and melanogenesis action spectra of normal human skin. Photochem Photobiol. 1982; 36:187-191.
3. Montagna W, Carlisle K. The architecture of black and white facial skin. J Am Acad Dermatol. 1991; 24:929-937.
4. Matsumura S, Mizutani Y, Fukuda M. A new approach to skin brightening based on melanosome transport. Fragrance J. 2011; 139:19-24.
5. Vancoillie G, Lambert J, Mulder A, Koerten HK, Mommaas AM, Van Oostveldt P, Naeyaert JM. Kinesin and kinectin can associate with the melanosomal surface and form a link with microtubules in normal human melanocytes. J Invest Dermatol. 2000; 114:421-429.
6. Wu X, Bowers B, Wei Q, Kocher B, Hammer JA, 3rd. Myosin V associates with melanosomes in mouse melanocytes: evidence that myosin V is an organelle motor. J Cell Sci. 1997; 110 (Pt 7):847-859.
7. Yamamoto H, Yamaguchi H, Yamada T. Vinculin migrates to the cell membrane of melanocytes after UVB irradiation. BPB Rep. 2020; 3:126-129.
8. McGuire J, Moellmann G. Cytochalasin B: effects on microfilaments and movement of melanin granules within melanocytes. Science. 1972; 175:642-644.
9. Geiger B, Tokuyasu KT, Dutton AH, Singer SJ. Vinculin, an intracellular protein localized at specialized sites where microfilament bundles terminate at cell membranes. Proc Natl Acad Sci U S A. 1980; 77:4127-4131.
10. Bakolitsa C, Cohen DM, Bankston LA, Bobkov AA, Cadwell GW, Jennings L, Critchley DR, Craig SW, Liddington RC. Structural basis for vinculin activation at sites of cell adhesion. Nature. 2004; 430:583-586.
11. Bays JL, DeMali KA. Vinculin in cell-cell and cell-matrix adhesions. Cell Mol Life Sci. 2017; 74:2999-3009.
12. Langford GM. Actin- and microtubule-dependent organelle motors: interrelationships between the two motility systems. Curr Opin Cell Biol. 1995; 7:82-88.
13. Bertrand JU, Petit V, Hacker E, Berlin I, Hayward NK, Pouteaux M, Sage E, Whiteman DC, Larue L. UVB represses melanocyte cell migration and acts through β-catenin. Exp Dermatol. 2017; 26:875-882.

Received September 7, 2022; Revised November 21, 2022; Accepted December 11, 2022.

**Address correspondence to:*
Hiroyuki Yamamoto, Department of Microbiology and Molecular Cell Biology, Nihon Pharmaceutical University, 10281 Komuro, Ina-machi, Kitaadachi-gun, Saitama, 362-0806, Japan.
E-mail: yamamoto@nichiyaku.ac.jp

Released online in J-STAGE as advance publication December 16, 2022.