Korean Journal of Cosmetic Science
Vol. 1, No. 1, December 2019, 31-43

pISSN 2713-5411
http://dx.doi.org/10.15230/SCSK.2019.1.1.31

Inhibitory Effect of Haplamine on Melanosome Transport and Its Mechanism of Action

Kyung Rhim Lee, Cheol Hwan Myung, and Jae Sung Hwang†

Department of Genetic Engineering & Graduate School of Biotechnology, Kyung Hee University, 1732, Deogyeong-daero, Giheung-gu, Yongin-si, Gyeonggi-do 17104, Republic of Korea

(Received December 22, 2019; Revised December 27, 2019; Accepted December 27, 2019)

Abstract: Melanosomes are specific melanin-containing intracellular organelles of epidermal melanocytes. In epidermal melanocytes, there are three kinds of key player proteins. Rab27a, melanophilin or Slac2-a and Myosin 5a form a tripartite complex connects the melanosome. Mature melanosomes make movements through the tripartite protein complex along actin filaments.In this study, we found that the haplamine (6-Methoxyflindersine) induced melanosome aggregation around the nucleus in epidermal melanocyte. In an attempt to elucidate the inhibitory effect of haplamine on melanosome transport, effect of haplamineon the expression level of Rab27a, melanophilin and Myosin 5a was measured. The results indicated that haplamine up to 5μM effectively suppressed mRNA and protein expression level of melanophilin.To determine the upstream regulator of melanophilin regulated by haplamine, we checked the level of MITF, c-JUN and USF1. Those are possible transcription factor of melanophilin. Among them,treatment of USF1 siRNA decreased mRNA and protein expression level of USF1 as well as melanophilin. Also, treatment of haplamine decreased mRNA and protein expression level of melanophilin as well as USF1 in a dose-dependent manner. Consequently, we found the inhibitory effect of haplamine on melanosome transport in melan-a melanocyte. Treatment of haplamine reduced melanophilin expression level which is a key protein of melanosome transport. We identified that USF1 could be a major transcription factor of melanophilin regulated by haplamine.

Keywords: *Melanosome transport, Haplamine, Melanophilin (Mlph), USF1*

1. Introduction

Melanin plays an important role in determining the skin color. It has been reported that overproduction and accumulation of melanin causes several skin disorders, including freckles, age spots, and others[1].

Melanosomes are specific melanin-containing intracellular organelles of epidermal melanocytes. Melanosomes are lysosome-related organelles specialized in melanin synthesis[2]. Melanins are produced by three melanogenic enzymes: tyrosinase (TYR), tyrosinase-related protein1 (TRP-1) and dopachrome tautomerase (DCT/tyrosinase-related protein2; TRP-2), which converts tyrosine to melanin pigment via a catalytic pathway[3]. Melanosomes are matured four stages with morphological changes[4]. When melanosomes are fully mature, they are transported along microtubules to the peripheral region where they are captured in the cortical actin network. And then, melanosomes are transferred to adjacent keratinocytes[5,6].

In melanosome transport, there are three kinds of key player proteins. Rab27a, melanophilin and Myosin 5a form a tripartite complex and connect the melanosome to the actin cytoskeleton[7,8]. Rab27a is a small GTPase protein present on the membrane of mature melanosomes, which is involved in intracellular vesicular transport and organelle dynamics[5,9]. Rab proteins are regulated by cycle between

† (e-mail: jshwang@khu.ac.kr), 031-201-5638

a guanosine triphosphate (GTP)-bound active form and a guanosine diphosphate (GDP)-bound inactive form[10,11]. Melanophilin is the effector of Rab27a that interacts directly with the GTP-bound form of Rab27a and Myosin 5a[12]. Myosin 5a performs a role of the trafficking of melanosomes in melanocytes[13]. In brief, Rab27a is captured melanosome and Myosin 5a is binding actin filaments. Rab27a and Myosin 5a are linked by melanophilin. Mature melanosomes make movements through the tripartite protein complex along actin filaments. Mutations in any of these pigment transport genes lead to a functional disorder of the tripartite complex, causing aggregation of melanosomes in the perinuclear regions of these mutant melanocytes, because of disruption of their anchoring onto the cell periphery[14,15].

Upstream stimulatory factor 1 (USF1) is basic helix-loop-helix leucine zipper family of transcription factor. This transcription factor binds to characteristic E-box elements via base-specific DNA contacts with its basic region and can homo - and heterodimerize with the related USF2 transcription factor[16-19]. Previous studies implicated the USF1 transcription factors as a key stress-responsive, regulatory element of the pigmentation cascade in response to UV stimulation. Following UV irradiation, USF1 is phosphorylated on threonine 153 and acetylated on lysine 199 by the p38 stress-activated kinase, leading to transcription activation of key upstream components of the pigmentation signaling pathway (POMC and MC1R)[20,21].

Haplamine (6-methoxyflindersine) is an alkaloid of the pyrano-quinolines class (Figure 1A). It was extracted from *Haplophyllumperforatum*[22]. This compound showed cytotoxicity on various solid cancer cell lines and acute leukemia lines. It was noticeably less cytotoxic than other alkaloids against non-cancerous normal skin fibroblasts[23].

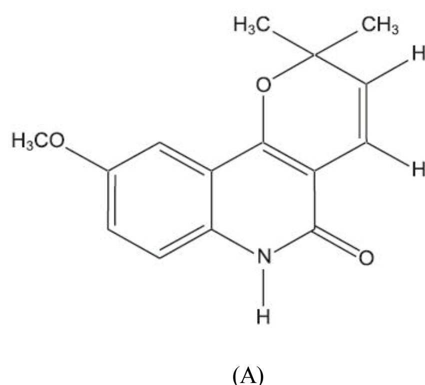
This compound has been shown to have anti-cancer effect, however, the effects of haplamine on skin pigmentation have not been investigated yet.In this study, we examined the effect of haplamine on melanosome transport in melanocyte.

2. Materials and Methods

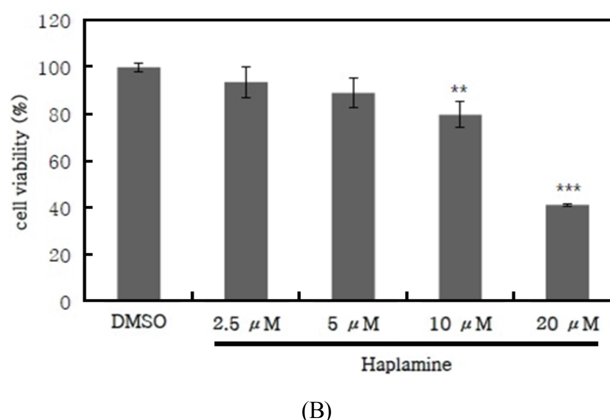
2.1. Materials

Haplamine was purchased from Green Pharma S.A.S (France).

Lipofectamine was purchase from invitrogen (Carlsbad, CA)



(A)



(B)

Figure 1. Effect of haplamine on viability of melan-a melanocyte.
(A) Structure of haplamine. (B) Cell viability was measured in melan-a melanocytes. Melan-a melanocytes were seeded at 1.5 x 104 cells/well in 96 well plates in RPMI-1640 media. After 24 h, melan-a melanocytes treated with diluted sample in RPMI-1640 media with 10% FBS, 1% P/S and 200 nM PMA for 24 h. Cell viability was measured using EZ-Cytox cell viability assay kit based on the cleavage of thetetrazoliumsalt to water-soluble formazan by succinate-tetrazol iumreductase. The experiment was repeated three times. The statistical calculations were performed using SPSS statistics (SPSSversion22.0, Inc., USA). Data were analyzed by one-way analysis of variance (ANOVA) followed by posthoc Tukey's multiple range tests $^{*}p < 0.05$. $^{**}p < 0.01$. $^{***}p < 0.001$.

2.2. Cell Culture

Melan-a melanocytes are immortalized normal murine melanocyte cell line derived from C57BL/6 mice. Melan-a melanocytes were obtained from Dr. Dorothy Bennett (St. George's Hospital, UK). Murine SP-1 keratinocytes were derived from SENCAR mice and were generously provided by Dr. Stuart H. Yuspa (Laboratory of Cellular Carcinogenesis and Tumor Promotion, NIH). Murine cells used included pigmented melan-a melanocytes and SP-1 keratinocytes.

Melan-a melanocytes were maintained in RPMI-1640 (Welgene, Korea) medium supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, 100 μg/mL streptomycin and 200 nM phobol 12-myristate 13-acetate (PMA; Sigma-aldrich, USA). SP-1 keratinocytes were grown in EMEM containing 0.05 mM Ca^{2+}, 8% Chelex treated heat inactivated FCS, 100 U/mL penicillin and 100 μg/mL streptomycin.

2.3. Cell Viability

Cell viability was determined following the treatment of melan-a melanocyte with haplamine using EZ-Cytox cell viability assay kit (Daeil Lab Service CO., LTD, Korea) following the protocol outlined by the manufacturer. Melan-a melanocyte seeded at $1.5 x 10^4$ cells/well in 96 well plates in RPMI-1640 media. After a day, melan-a melanocytes treated with diluted sample in RPMI-1640 media with 10% FBS, 1% P/S and 200 nM PMA for 24 h. After washing in DPBS, melan-a melanocytes media were replaced with RPMI-1640 media, EZ-Cytox assay reagent (10 : 1) was added, and the melan-a melanocytes were incubated for 1 h at 37 ℃ in a 10% CO_2 incubator. The plate was read at 450 nm using a microplate reader (TecanMannedorf, Switzerland). All assays were performed in triplicates. The results are presented as percent of untreated controls.

2.4. Co-Culture

Melan-a melanocytes seeded at $3.75 x 10^4$ cells/well in six-well plates in RPMI-1640 media. Two days later, the plates were rinsed with DPBS. SP-1 cells seeded at $1.5 x 10^5$ cells/well to each well containing the melanocytes. The initial seeding ratio of keratinocytes to melanocytes wasthus 4 : 1 and co-cultures of melanocytes and keratinocytes were maintained thereafter in EMEM media with 0.05 mM Ca^{2+}. 2, 3 and 5 days later, rinsed with DPBS, fresh EMEM with 0.05 mM Ca^{2+} with diluted sample treated. One day after that, the cultures were photographed.

2.5. Measurement of Melanin Contents

Melan-a melanocytes were seeded at $1 x 10^5$ cells in 24-well plates overnight. After washing in DPBS, treated with diluted sample in RPMI-1640 with 10% FBS, 1% P/S and 200 nM PMA for 72 h. After washing in DPBS, the cells were dissolved in 120 μL of 1 N NaOH at 55 ℃ for 30 min. Then, the cell lysates 100 μL transfer 96 well plate. Optical densities (OD) were measured at 490 nm using an ELISA reader. Protein concentration of cell lysate was measured using a BCA Protein Assay Reagent Kit (Pierce Biotechnology, Inc, USA) with bovine serum albumin as the standard.

2.6. Western Blotting

Melan-a melanocytes were lysed by lysis buffer consisted of RIPA solution (Noble Bio, Korea), protease inhibitor cocktail (Sigma, USA) and 1 mM phenylmethanesulfonyl fluoride (PMSF, Sigma, USA) for 30 min at 4℃. The lysates were subjected to centrifugation at 13,000 rpm for 20 min and the supernatant was used. Protein content of supernatant was quantified using the BCA Protein Assay Reagent Kit (Pierce Biotechnology, Inc, USA) with bovin serum albumin as the standard. β-actin antibody (1 : 20000, Sigma, USA), Myosin Va antibody (1 : 800, Cell Signaling Technology, USA), Mlph antibody (1 : 600, ProteinTech Group, Inc, USA), Rab27a antibody (1 : 500, Santa Cruz, USA), MITF antibody (1 : 1000, NeoMarkers, USA), Tyrosinase antibody (1 : 2000, αPEP7, National Institute of Health, Bethesda, USA), TRP-1 antibody (1 : 2000, αPEP1, National Institute of Health, Bethesda, USA), TRP-2 antibody (1 : 2000, αPEP8, National Institute of Health, Bethesda, USA), c-JUN antibody (1 : 1000, Cell Signaling Technology, USA) and USF1 antibody (1 : 10000, UK) were used at 4℃ for 2h or overnight. Horseradish peroxidase conjugated anti-rabbit (1 : 5000,

Bethyl, USA) or anti-mouse (1 : 20000, Bio-Rad, USA) were used at room temperature for 1h. Binding antibodies were detected using an WEST-ZOL® Plus Western Blot Detection System (INtRON Biotechnology, Korea). The bands on membrane were detected with chemiluminescence and visualized with Chemi Doc XRS (Bio-Rad).

2.7. Quantitative Real-Tme PCR

Quantitative real-time PCR was performed using a FastStart Essential DNA Probe Master kit (Roche, Germany) with Universal ProbeLibrary (Roche). The reaction was carried out according to the manufacturer's protocol. The probes for Myosin Va (#63, NM_010864.2), Mlph (#108, NM_053015.3) and Rab27a (#63, NM_023635.5) were designed with the Probe Library Assay Design Center. The cycling condition was 600 s at 95 ℃ and 40 cycles at 95 ℃ for 20 s and 60 ℃ for 40 s on Lightcycler®Nano. Expression level of three genes was normalized to mouse β-actin, a control gene. The obtained cDNA was amplified with the following primers.

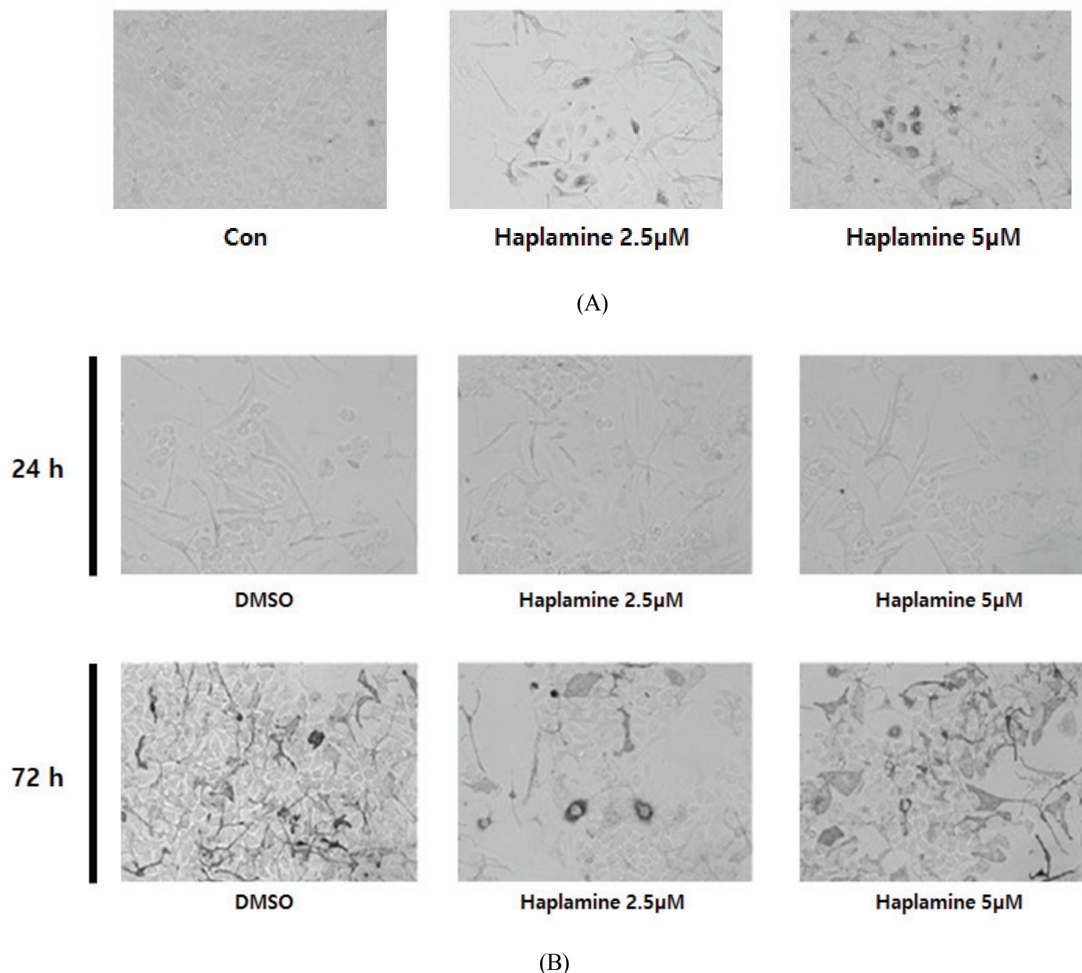


Figure 2. Effect of haplamine on the localization of melanosome.
(A) Bright-field image (x200) of melanocytes treated with DMSO, 2.5 and 5 μM haplamine for 72 h. (B) Effect of haplamine on the localization of melanosome in melan-a / SP-1 co-culture. The initial seeding ratio of kerationcytes to melanocytes was 4:1 and co-cultures of melanocytes and co-culture were maintained in EMEM (with 0.05 mM Ca^{2+}). Every 2 day, the cultured cells were replenished EMEM (with 0.05 mM Ca^{2+}) with DMSO, 2.5 and 5 μM haplamine treated for 24 h and 72 h. Then, photographed in bright field.

Myosin Va	sense	5’-GCGCCATCACCCTAAACA-3’
	antisense	5’-CCAGTTGACTGACAT-TGTACCTG-3’
Mlph	sense	5’-AGCCCCTCAACAGCAAAAA-3’
	antisense	5’-TTCCTCAAAGTCCACATCTCG-3’
Rab27a	sense	5’-GAAGACCAGAGGCAGTGAA-3’
	antisense	5’-ACTGGTTTCAAAA-TAGGGGATTC-3’.

2.8. Small Interfering RNAs (siRNAs)

siRNA oligonucleotides were purchased from Bioneer. Sense and antisense sequences for individual duplexes targeting mouse USF1, Mlph. USF1 was designed by siRNA wizard (www.sirnawizard.com), using the following.

USF1	sense	5’-GAGAGGACCCAACUAGUGUUU-3’
	antisense	5’-ACACUAGUUGGGUCCUCUCUU-3’
Mlph	sense	5'- GGGCAAAAUACAAAAGGAGUUTT–3’
	antisense	5'- CUCCUUUUGU-AUUUUGCCCUUTT-3'.

2.9.Transfection

One day before transfection, melan-a melanocytes were seeded in six-well plate at 60% confluency and 20 nM siRNA mixed with Lipofectamine (Invitrogen) were treated according to the manufacturer’s protocol.

2.10. RT-PCR

cDNA 1 μL mixed with HiPi PCR Mix (ELPIS, Korea), each primer. The cDNA obtained was amplified with the following primers.

Mlph	sense	5’-CCTGCTGGATTACATTAAAGCACTG-3’
	antisense	5’-GTCAAG-GGCATATCCAACAACAAAC-3’;
USF1	sense	5’-AGCTGTTGTTAC-CACCCAGG-3
	antisense	5’-TATGTTGAGCCCTCCGTTTC-3’
β-actin	sense	5’-GTGGGGCTGCCCCAGGCACCA-3’
	antisense	5’-CTCCTTAATGTCACGCACGATTTC-3’.

3. Results

3.1. Effect of Haplamine on the Localization of Melanosome in Melan-a Melanocyte

To identify the effects of haplamine in melanosome transport, we treated haplamine in melan-a melanocytes and observed melanosome aggregation. In cell viability assay, it was found that haplamine under 5 μM showed no cytotoxicity in melan-a melanocytes. Therefore, we treated 2.5 and 5 μM of haplamine for further examination (Figure 1B). Melanosome aggregation was shown in melan-a melanocytes monoculture treated with haplamine for 72 h. Treatment of 2.5 μM and 5 μM haplamine increased melanosome aggregation in a dose-dependent manner compared with control (Figure 2A). The inhibitory effect of haplamine on melanosome transport in sp-1 keratinocyte and melan-a melanocyte co-culture was investigated. Compared with control, significant aggregations of melanosome in the peri-nuclear area by haplamine were shown in a time-dependent manner and a dose-dependent manner (Figure 2B). These results indicated that haplamine induce melanosome aggregation in melan-a melanocytes.

3.2. Effect of Haplamineon the Protein and mRNA Level of Melanophilin

To determine whether haplamine affect the expression of melanosome transport key player protein, we checked expression level of Rab27a, melanophilin and Myosin 5a. Haplamine decreased protein expression level of melanophilin in a dose-dependent manner. But, the expression level of Rab27a, Myosin 5a was not changed by treatment of haplamine (Figure 3A). We also checked the mRNA expression level by quantitative real-time RT-PCR (Figure 3B). Melanophilin mRNA expression level was decreased at 48 h. However, Rab27a and Myosin 5a mRNA expression level was not changed by treatment of haplamine.

3.3. Effect of Haplamine on Melanin Synthesis in Melan-a Melanocyte

To determine whether haplamine affect melanin synthesis, melanin content was measured after treatment with 2.5 μM, 5 μM haplamine. Haplamine treatment did not affect melanin contents (Figure 4A). MITF is known for the transcription factor of Rab27a. We checked the protein expression level of MITF, Tyrosinase, TRP-1 and TRP-2 after treatment of haplamine for 72 h (Figure 4B). The results showed that the protein expression of MITF, Tyrosinase, TRP-1 and TRP-2 were not changed by treatment of haplamine. Thus, haplamine is not involved in melanin synthesis but is associated with melanosome transport.

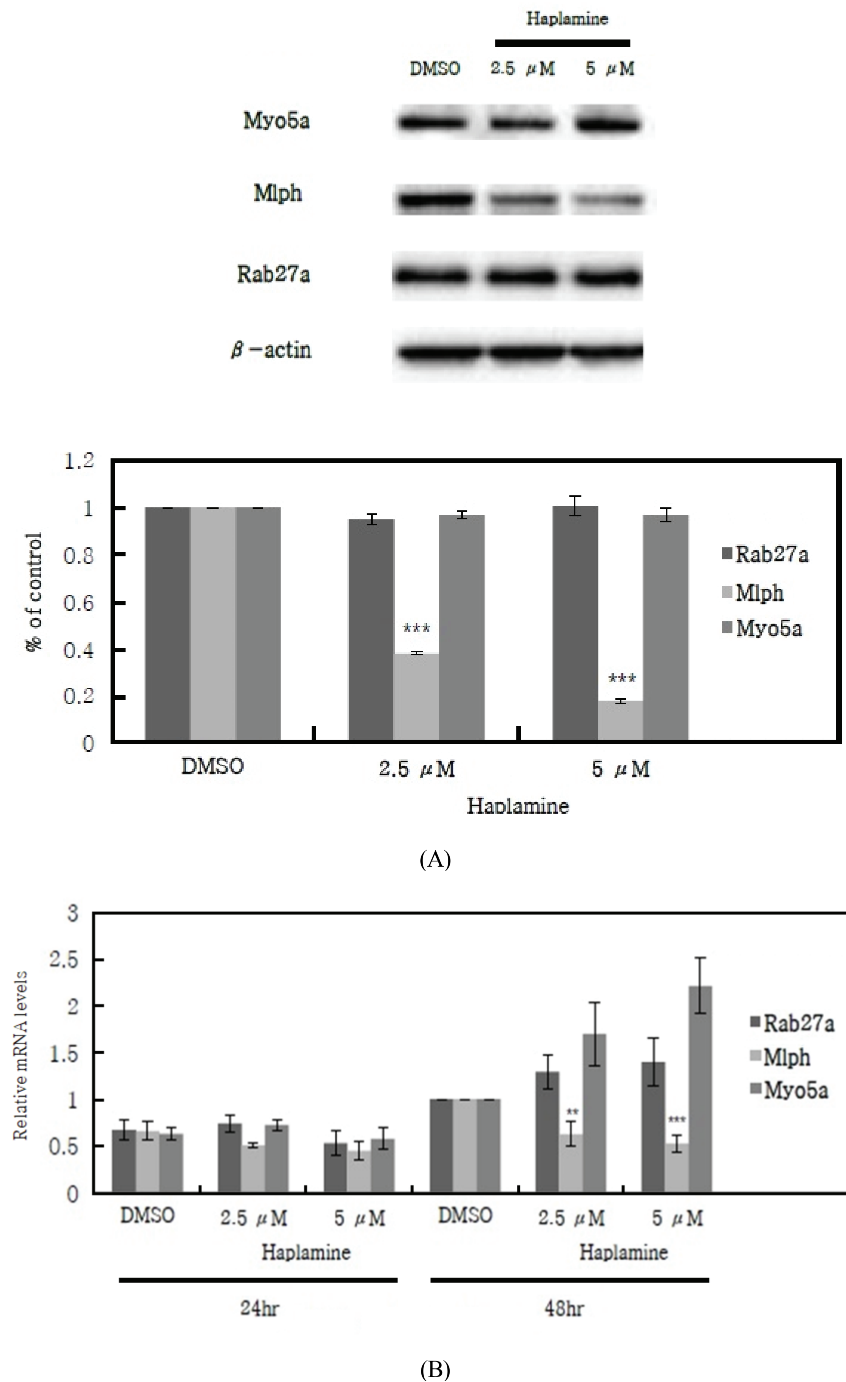


Figure 3. Expression analysis of Rab27a, melanophilin and Myosin 5a on melan-a melanocyte treated with haplamine. (A) Melan-a melanocytes were treated with DMSO, 2.5 and 5 μM haplamine for 72 h. Each transport proteins level of lysates were measured by western blotting. (B) Melan-a melanocytes were with DMSO, 2.5 μM and 5 μM haplamine for 24 and 48 h. Each mRNA level of transport related proteins were determined by quantitative real-time RT-PCR. The experiment was repeated three times. Data were analyzed by Student's unpaired *t* test $^{***}p < 0.001$.

3.4. Effects of Haplamine on Possible Transcription Factor of Melanophilin

The melanophilin mRNA expression level was decreased by haplamine, we are trying to identify which transcription factor is involved on the expression of melanophilin, we checked the expression level of c-JUN and USF1 after treatment with haplamine. c-JUN, which is an early response transcription factor and could be involved in melanophilin expression. USF1, which is known for regulates of cell proliferation and melanogenesis in melanocytes. Treatment of haplamine for 72 h decreased expression level of USF1 and melanophilin, but increased expression level of c-JUN in a dose-independent manner (Figure 5A). These results indicated that USF1 and c-JUN could be a possible transcription factor of melanophilin.

3.5. Effect of si-USF1 on the Localization of Melanosome and Expression Level of Melanophilinin Melan-a Melanocyte

In previous experiments, we identified that USF1 is a possible upstream regulator of melanophilin. To confirm whether USF1 affects melanosome transport, we treated with USF1 siRNA in melan-a melanocytes. Treatment of USF1 siRNA showed melanosome aggregation in melan-a

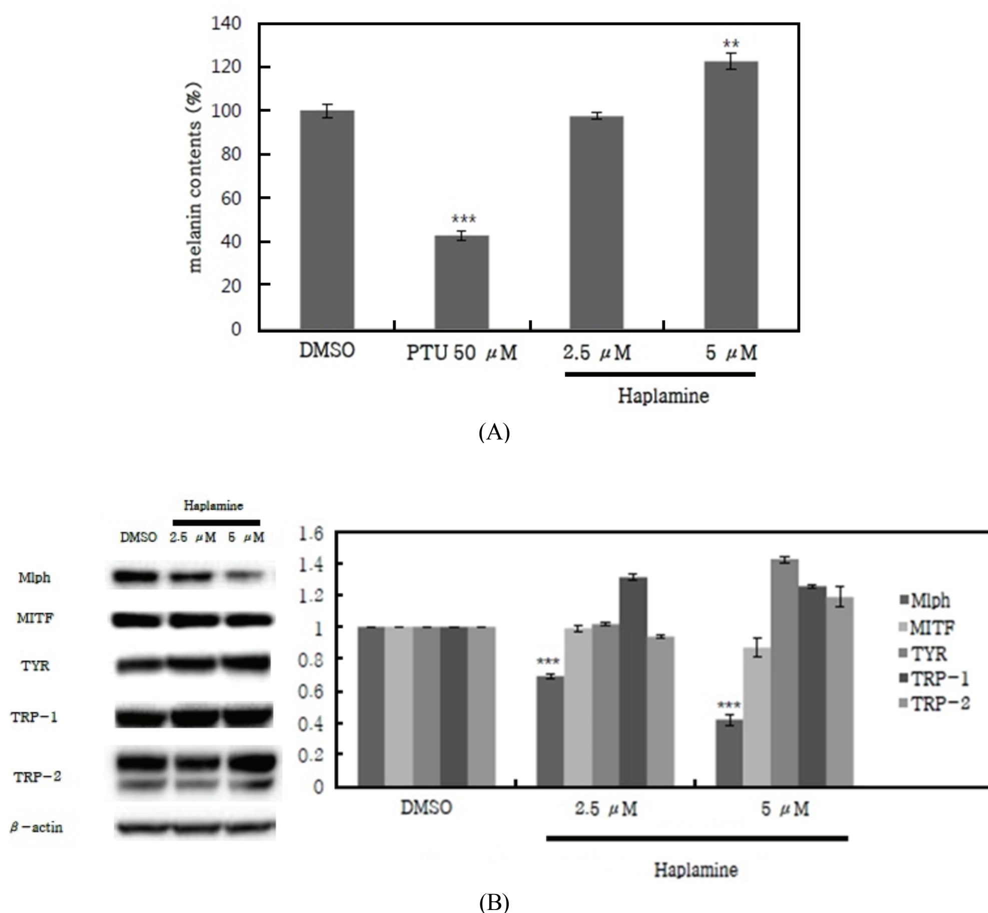
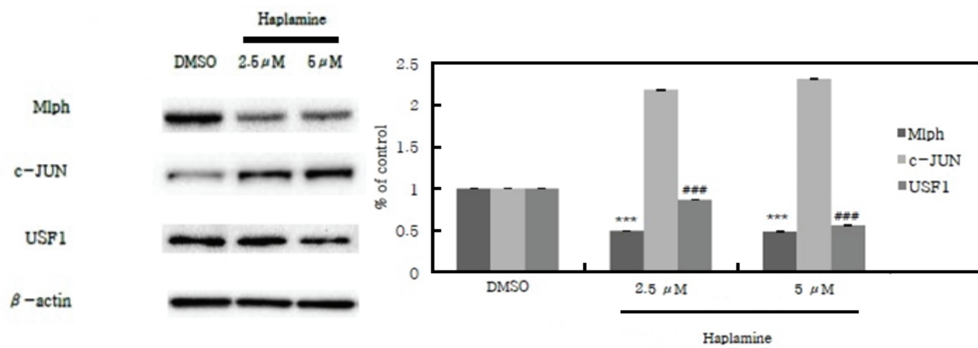


Figure 4. Effect of haplamine on melanin synthesis in melan-a melanocyte.
(A) Melan-a melanocytes were treated with DMSO, 2.5, 5 μM haplamine and 50 μM PTU for 72 h and melanin content disolved by 1 N NaOH was subsequently measured at 490 nm. (B) Effect of haplamine on the melanogenic protein expression. Melan-a melanocytes were treated with DMSO, 2.5 and 5 μM haplamine for 72 h. Protein expression of MITF and melanin synthesis enzymes (tyrosinase, TRP-1 and TRP-2) were measured by western blotting. The experiment was repeated three times. Data were analyzed by Student's unpaired t test $^{**}p < 0.01$, $^{***}p < 0.001$.

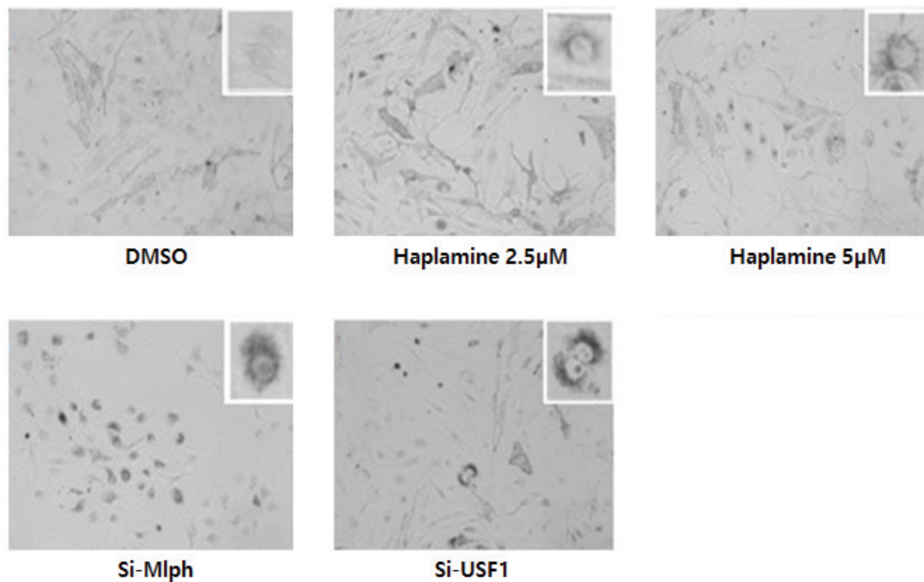
melanocytes as melanophilin siRNA treated group (Figure 5B). These results suggested that USF1 affects melanosome transport in melan-a melanocytes and could be an upstream regulator of melanophilin. We checked expression level of melanophilinwith USF1 siRNA. Treatment of USF1 siRNA for 72 h decreased protein expression level of melanophilin as well as USF1 (Figure6A). Likewise, USF1 siRNA transfection for both 24 and 48 h also decreased mRNA expression level of melanophilin remarkably(Figure 6B).

3.6. Effect of Haplamine on the Expression Level of USF1

To identify the effect of haplamine on the expression of USF1, we checked protein and mRNA expression level



(A)



(B)

Figure 5. Effect of haplamine and USF1 on melanophilin expression in melan-a melanocytes.
(A) Melan-a melanocytes were treated with DMSO, 2.5 and 5 μM haplamine for 72 h. USF1 and melanophilin proteins level were measured by western blotting. The experiment was repeated three times. Data were analyzed by Student's unpaired t test $^{***}p < 0.001$, $^{\#\#\#}p < 0.001$. (B) Effect of si-USF1 on the localization of melanosome. melan-a were transfected with 20 nM siRNA using Lipofectamine. Bright-fieldimage (x200) of melanocytes treated with DMSO, 2.5, 5 μM hapalmine, si-Melanophilin, si-USF1 for 72 h.

of USF1 with haplamine. Treatment with 5 μM haplamine for 72 h decreased protein expression level of USF1 (Figure 7A). Furthermore, USF1 mRNA expression level was decreased by 2.5 and 5 μM haplamine for 48 h as well as melanophilin in a dose-dependent manner (Figure 7B). These results indicated that USF1 affects protein expression level of melanophilin, and the effect of haplamine on the protein expression level of melanophilin was derived from USF1.

4. Conclusion and Discussion

Melanosomes are specific melanin-containing intracellular organelles of epidermal melanocytes. Melanosomes mature four stages with morphological changes[4]. Melanosomes are fully matured during transport from melanocytes nucleus to melanocytes dendrite tips. And then, melanosomes are transferred to adjacent keratinocytes[5,6]. In melanosome transport, there are three kinds of key player proteins. Rab27a, melanophilin and Myosin 5a form a tripartite complex which connects the melanosome to the actin cytoskeleton[7,8]. Mutations in any of these pigment transport genes lead to a functional disorder of the tripartite complex, causing aggregation of melanosomes in the perinuclear regions of these mutant melanocytes, because of disruption of their anchoring onto the cell periphery[14,15].

In this study, we investigated the inhibitory effect of

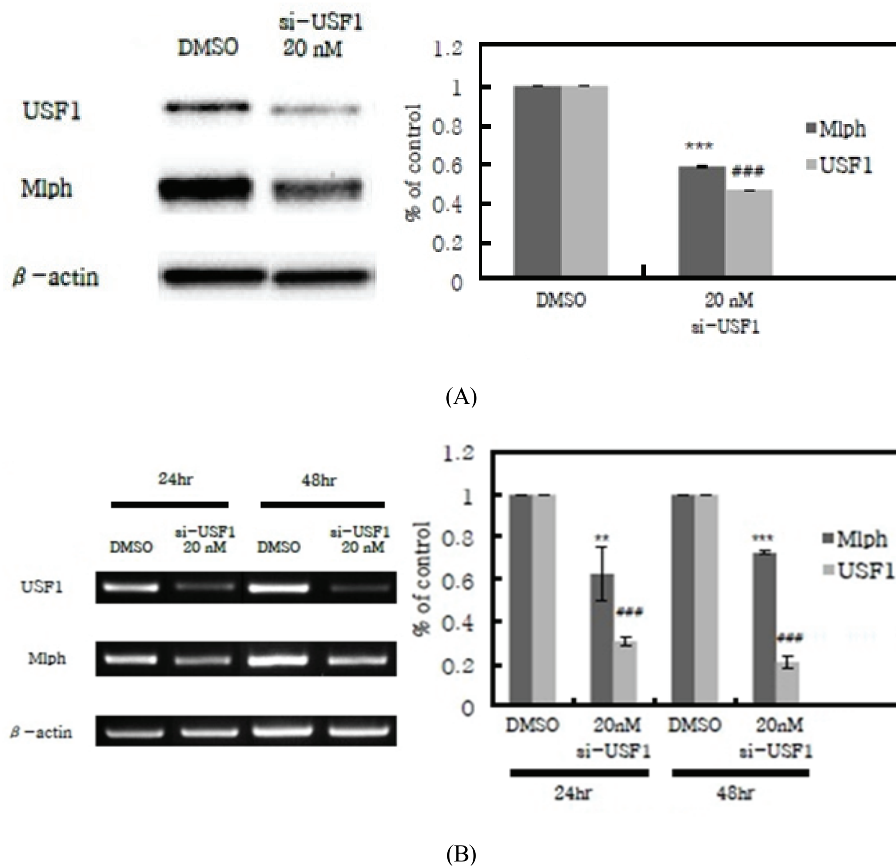


Figure 6. Effect of USF1 on the protein and mRNA expression of melanophilin.
(A) Melan-a cells were treated with DMSO (control) or si-USF1 transfection for 72 h. Protein expression of USF1, melanophilin were examined by western blotting. (B) Melan-a cells were treated with DMSO (control) or si-USF1 transfection for 24, 48 h. mRNA expression level of USF1, melanophilin was measured by RT-PCR. The experiment was repeated three times.Data were analyzed by Student's unpaired t test $^{**}p$ < 0.01. $^{***}p$ < 0.001, $^{###}p$ < 0.001.

haplamine on melanosome transport and trying to elucidate the mechanism of it. In cell viability assay, it was found that haplamine under 5 μM showed no cytotoxicity in melan-a melanocytes (Figure 1B). We observed that haplamine inhibited the melanosome transport in the monoculture of melan-a melanocyte and co-culture of SP-1 keratinocyte and melan-a melanocyte. Significant aggregation of melanosome in perinuclear area was shown by treatment of haplamine in melan-a melanocyte (Figure 2A and B). Haplamineonlydecreased mRNA and protein expression level of melanophilin in a dose-dependent manner (Figure 3A and B). But, haplamine showed no effects on melanin synthesis and three melanogenic enzymes (TYR, TRP-1, and TRP-2) (Figure 4A and B). These results showed that the effect of haplamine on melanosome aggregation was derived from down regulation of melanophilin mRNA and protein expression.

According to a previous study, melanophilin expression is very important for melanosome transports that mutation in melanophilin causes the defect of melanosome transport in melanocytes[24].

MITF is known to be the most important regulator of melanocyte differentiation and pigmentation and is the major transcriptional regulator of the tyrosinase, TRP-1 and TRP-2 genes[25]. Other studies showed that MITF was known for transcription factor of Rab27a [26]. We checked protein expression level of MITF after treatment with haplamine (Figure 4B). MITF protein expression level was not changed by treatment with haplamine. These result indicated that decreased protein expression level of melanophilin was not related with MITF. c-JUN, which is known to form the AP-1. AP-1 is known to be regulator of mela-

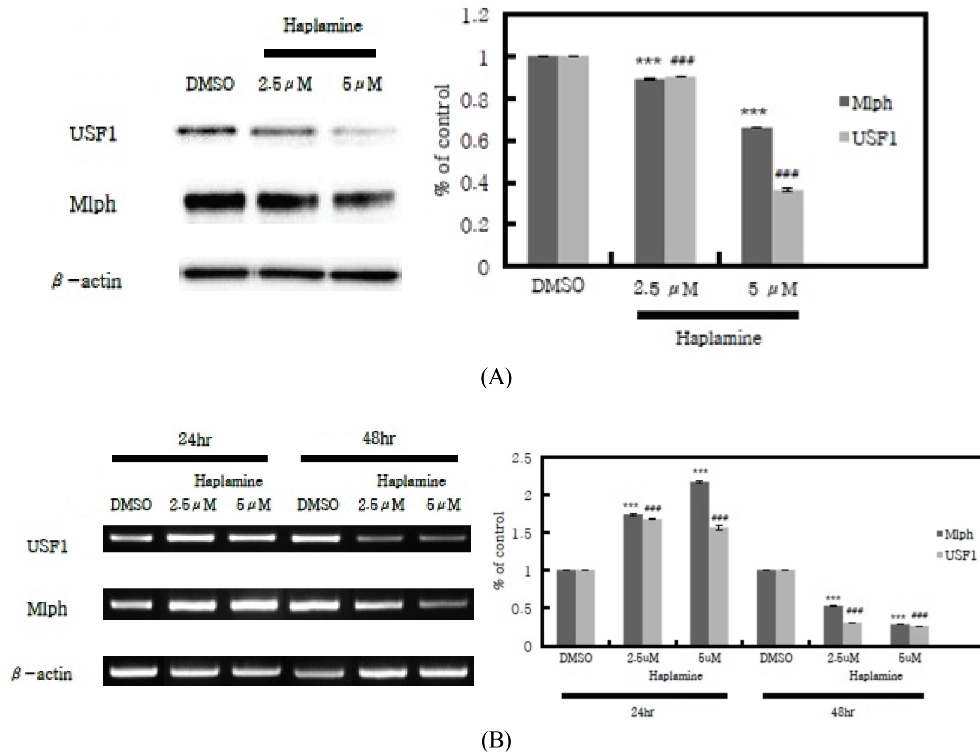


Figure 7. Effect of haplamine on the protein and mRNA expression of USF1.
(A) Melan-a cells were treated with DMSO, 2.5 and 5 μM haplamine for 72 h. Protein expression of USF1, melanophilin were examined by western blotting. (B) Melan-a cells were treated with DMSO, 2.5 and 5 μM haplamine for for 24, 48 h. mRNA expression of USF1, melanophilin were measured by RT-PCR. The experiment was repeated three times.Data were analyzed by Student's unpaired t test $^{***}p < 0.001$, $^{\#\#\#}p < 0.001$.

nogenesis[27]. c-JUN interacts directly with specific target DNA sequences and regulates gene expression. c-JUN was known to be the important regulator of cellular proliferation and stress-induced apoptosis[28]. In melanoma-derived B16-F10 cancer cells, c-JUN suggested a potential anti-tumor strategy through JNK signaling pathway[29]. We checked protein expression level of c-JUN after treatment with haplamine (Figure 5A). The results showed that the expression level of c-JUN was increased in a dose-independent manner. Finally, we checked the expression level of USF1. USF1 is a member of the basic helix-loop-helix leucine zipper family and can function as a cellular transcription factor. Cellular stress stimuli such as DNA damage, oxidative stress and heavy metal exposure increased USF1 transactivation activity[20]. USF1 is also involved in the transcriptional activation of several tumor suppressor genes and cell cycle control[17,30-32]. In previous studies, USF1 was reported to regulate cell proliferation and melanogenesis in melanocytes[20,21]. We checked protein expression level of USF1 by treatment with haplamine (Figure 5A). The expression of USF1 was decreased in a dose-independent manner with haplamine. These results indicated that USF1 is a possible of transcription factor of melanophilin regulated by haplamine.

To confirm whether USF1 is upstream regulator of melanophilin, we treated with siRNA of USF1 in melan-a melanocytes. Treatment of USF1 siRNA decreased mRNA and protein expression level of melanophilin as well as USF1 (Figure 6A,B). These results indicated that USF1 affects expression level of melanophilin, and haplamine decrease melanophilin expression through USF1.

Further studies are needed to identify whether USF1 directly interact with melanophilin promoter region. and to investigate the relationship with USF1 and expression of other transport related proteins or melanogenic proteins.

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