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Effects of 7-methylsulfonylheptyl isothiocyanate on skin whitening and inflammation in cultured mammalian cells

조선대학교 대학원

생명과학과

김 아 주

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배양 포유동물 세포에서 미백 및 염증반응에 미치는
7-methylsulfonylheptyl isothiocyanate의 영향

2020년 2월 25일

조선대학교 대학원

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이 논문을 이학 석사학위 신청 논문으로 제출함.

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ABSTRACT

Effects of 7-methylsulfonylheptyl isothiocyanate on skin whitening and inflammation in cultured mammalian cells

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Skin aging is influenced by intrinsic and extrinsic factors, including genetics, hormonal changes, metabolic processes, dietary habits, smoking, and environmental factors such as ultra-violet (UV) light and air pollution. These factors act synergistically to induce skin aging that is associated with hyperpigmentation, inflammation, wrinkles, and skin cancer. Autophagy is a process that maintains homeostatic balance between the synthesis, degradation, and recycling of cellular organelles and proteins, and plays important regulatory roles in cell differentiation, development, stress, cancer, and aging. The compound 7-methylsulfonylheptyl isothiocyanate (7-MSI; also known as 1-isothiocyanato-7-(methylsulfonyl)-heptane) routinely used in this study is a sulfur-containing phytochemical that is produced by a variety of plants, particularly from cruciferous vegetables such as cauliflower, cabbage, kale, and broccoli *etc*. Although a few studies have showed that the compound seems to have an ability to defense against pathogens and also exhibits an anti-inflammatory effect, to date there is no study on the effects of 7-MSI on

skin whitening and inflammation in detail. Therefore, the present study aimed to examine the effects of 7-MSI on the skin whitening and the inflammation in cultured murine melanoma (B16-F1) and macrophage (Raw 264.7) cell lines. The expression levels of melanogenesis-associated proteins such as microphthalmia-associated transcription factor (MITF), tyrosinase, and tyrosinase-related protein-1 (TRP-1) were also decreased when B16-F1 cells were treated with 7-MSI (1 μg/ml) in the presence of α-melanocyte-stimulating hormone (α-MSH) (10 nM) for 24 h as judged by Western blottings. The rate of melanin synthesis could be decreased to approximately 63% after the treatment with 7-MSI (0.5 μg/ml) for 73 h in B16-F1 cells treated with α-MSH (10 nM) to induce melanin synthesis, compared to that of non-treated control. Taken together, these results suggest that 7-MSI can inhibit the melanin synthesis by suppressing the melanogenesis in B16-F1 cells. The effects of 7-MSI on inflammatory response were examined in Raw 264.7 cells. The expression levels of various inflammatory cytokines and regulators were examined using enzyme-linked immunosorbent assay (ELISA) and reverse transcription-PCR (RT-PCR). The ELISA result showed that the production of tumor necrosis factor-alpha (TNF-α) is decreased to approximately 32% by the treatment with 7-MSI (1 μg/ml) in the presence of lipopolysaccharide (LPS) (1 μg/ml) for 1 h in Raw 264.7 cells. The RT-PCRs also showed that the transcription levels of interleukin (IL)-6, IL-1β, cyclooxygenase-2 (COX-2), and prostaglandin E (PGEs) were decreased by 7-MSI (1 μg/ml) treatments in the presence of LPS (1 μg/ml) for 3 h in Raw 264.7 cells. In addition, the degrees of inhibitory kappa B (IκB) degradation and phospho-IκBα production were significantly reduced by 7-MSI (1 μg/ml) treatments for various time periods. The inhibitory effect of 7-MSI (1 μg/ml) on the nuclear factor kappa B (NF-κB) activation was also confirmed by Confocal microscopic analysis. The confocal analysis showed that the LPS-induced nuclear translocation of NF-κB proteins is inhibited by 7-MSI treatment. Taken together, these results suggest that 7-MSI can inhibit the production of pro-inflammatory cytokines by suppressing the activation of NF-κB signalling pathway. When B16-F1

and Raw 264.7 cells were treated with 7-MSI (1 μg/ml) for 30 min, the mitogen-activated protein kinase (MAPK) signaling pathway could be activated, as judged by Western blottings using antibodies raised against p38, p-p38, c-Jun N-terminal kinase (JNK), p-JNK, extracellular signal-related kinase (ERK), and p-ERK. In addition, the expression levels of autophagy-related proteins, including Beclin-1, autophagy-related protein 12 (Atg12), and microtubule-associated protein light chain 3 (LC3) were increased in both B16-F1 and Raw 264.7 cells treated with 7-MSI (1 μg/ml) for 30 min or 1 h, accompanied with the decreased level of mammalian target of rapamycin (mTOR). Collectively, all the results obtained by this study demonstrate that 1) 7-MSI can inhibit melanin synthesis; 2) it can suppress the expression of pro-inflammatory cytokines and regulators; 3) it can activate MAPK and autophagy signaling pathways. In conclusion, 7-MSI can be an interesting agent to be developed as a cosmetic compound showing skin whitening and anti-inflammatory effects.

Ⅰ. INTRODUCTION

Skin consists of epidermis, dermis, and hypodermis, and plays a variety of physiological functions (Bouwstra *et al.,* 2002). Skin aging is influenced by intrinsic and extrinsic factors, including genetics, hormonal changes, metabolic processes, ultra-violet (UV) light, and air pollution. These factors lead to cumulative alterations of the skin structure, function, and appearance. Consequently, skin aging leads to hyperpigmentation, inflammatory response, wrinkle formation, and skin cancer (Mesa-Arango *et al.,* 2017) (Fig. 1).

Melanin is synthesized by the skin cells to provide protection from UV light exposure (Lim *et al.,* 2018). However, the formation of excessive melanin in the skin causes hyperpigmentation, which can induce skin disorders such as melasma, freckles, and geriatric pigment spots (Ha *et al.,* 2005) (Fig. 2). In mammals, UV exposure stimulates the production of α-melanocyte-stimulating hormone (α-MSH), which binds to melanocortin 1 receptor (MC1R) within the cell membrane. Protein kinase A (PKA) activates the phosphorylation of cAMP response element-binding protein (CREB), which regulates the expression of the microphthalmia-associated transcription factor (MITF) gene. MITF controls the production of tyrosinase and tyrosinase-related protein-1/2 (TRP-1/2) (Lim *et al.,* 2018). Melanin is synthesized by melanocytes, and the melanocytes transport the mature melanosomes to the keratinocytes, resulting in melanogenesis (Mitsunaga & Yamauchi, 2015) (Fig. 3). Melanin is produced as two different forms, eumelanin and pheomelanin, in which tyrosinase and TRP1/2 act as main enzymes (Fig. 4).

Inflammatory response serves as a defense mechanism against injuries and pathogens recognized by macrophages, which produce cytokines, chemokines, and other inflammatory factors (Lim *et al.,* 2017) (Fig. 5). The nuclear factor kappa B (NF-κB) is kept in an inactive state by binding with inhibitory kappa B (IκB), and

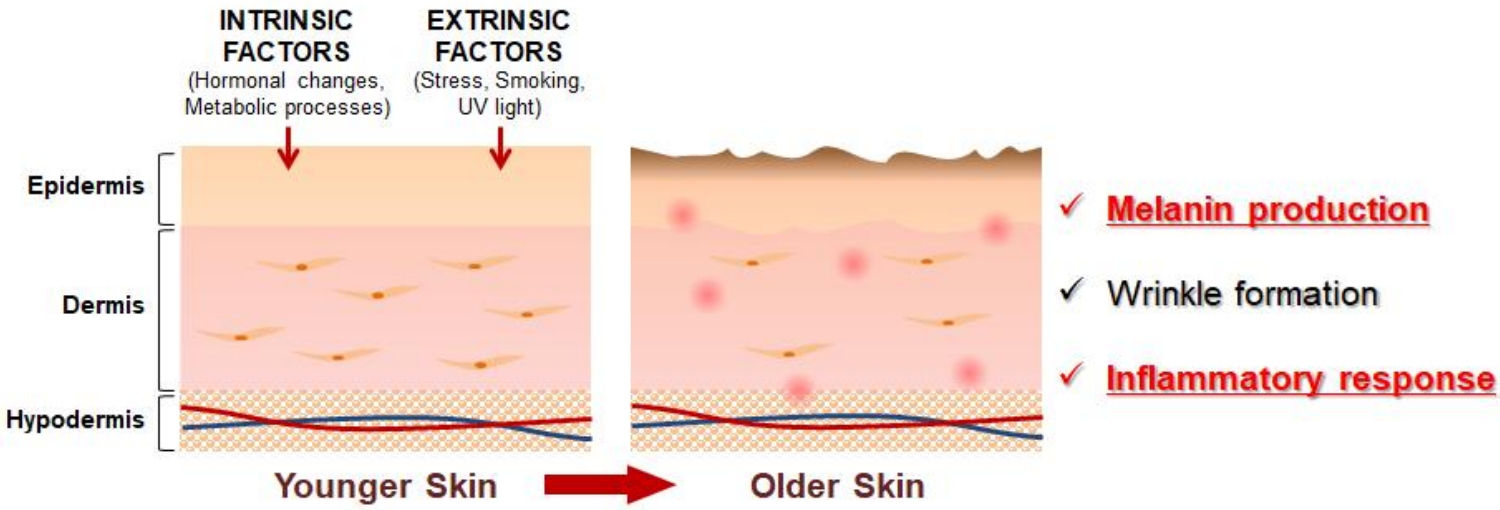

Fig. 1. Schematic diagram of the structure of the skin and the process of skin aging. The skin consists of the epidermis, dermis, and hypodermis (Bouwstra *et al.,* 2002). Skin aging is influenced by intrinsic or extrinsic factors, including genetics, hormonal changes, metabolic processes, UV-light, and air pollution (Mesa-Arango *et al.,* 2017).

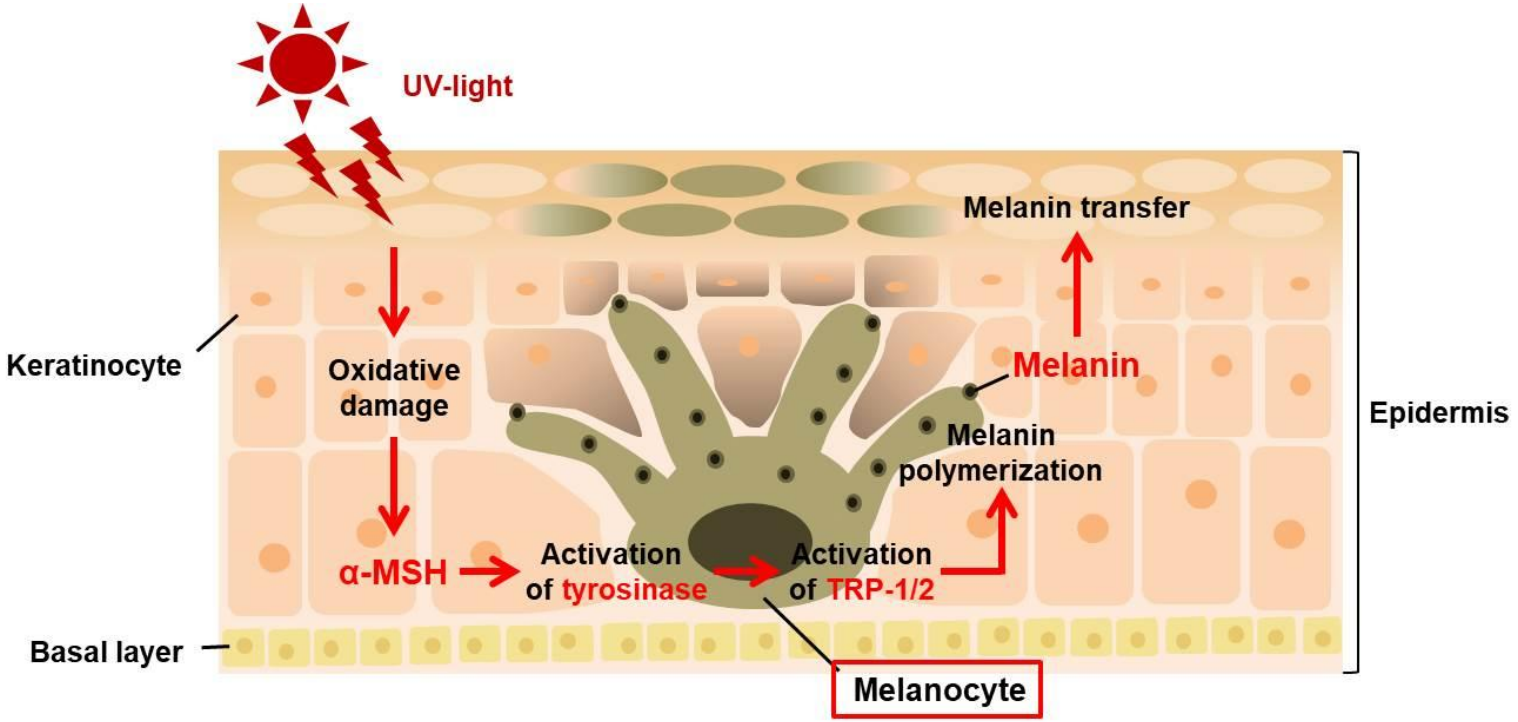

Fig. 2. Melanin formation by UV-light in the skin. Melanin is synthesized for protection of skin from UV-light exposure (Lim *et al.*, 2018). The melanin pigments are first produced in an organelle known as melanosome within melanocytes and then transferred to keratinocytes in the epidermis (Mitsunaga & Yamauchi, 2015).

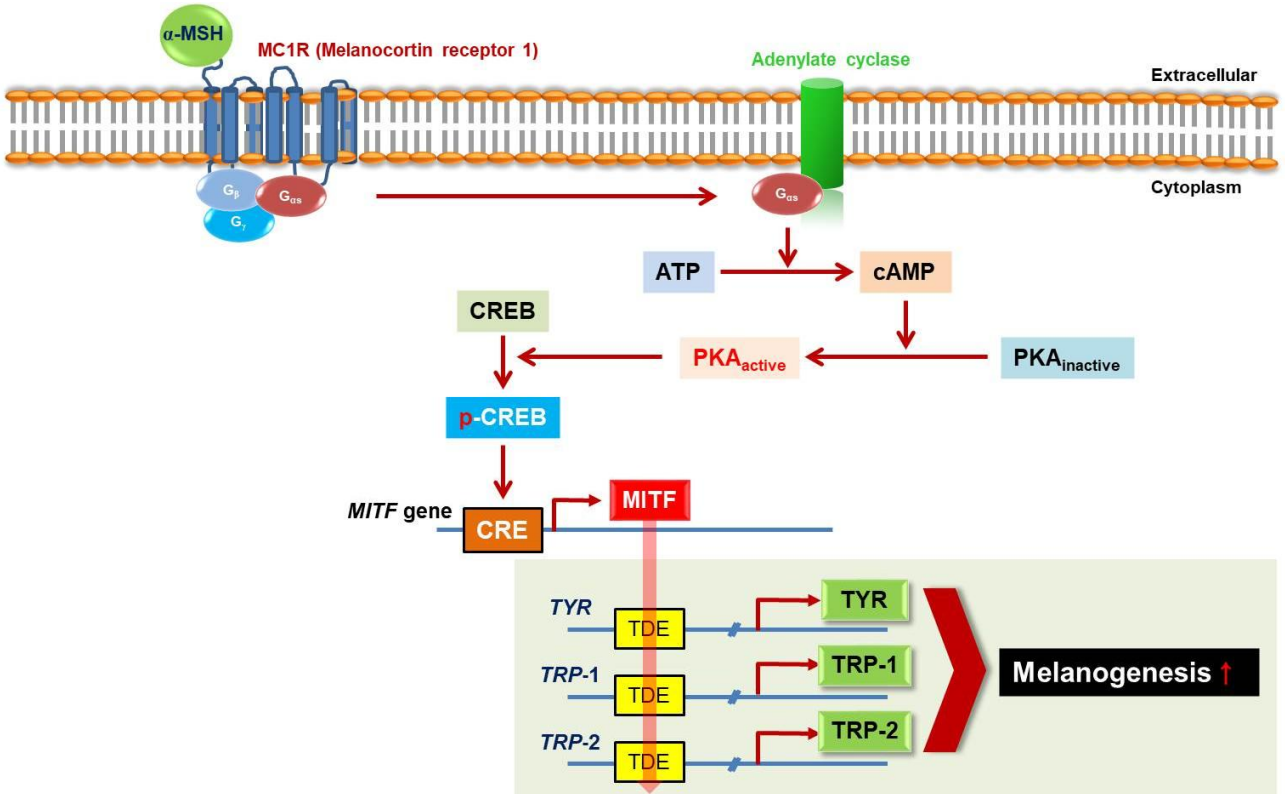

Fig. 3. Melanogenesis in melanocytes. Alpha-MSH stimulates adenylyl cyclase to increase the level of cAMP by binding to MC1R within the cell membrane. PKA subsequently activates the phosphorylation of CREB, which regulates expression of the MITF gene. MITF controls the production of tyrosinase, and TRP-1/2 (Lim *et al.*, 2018).

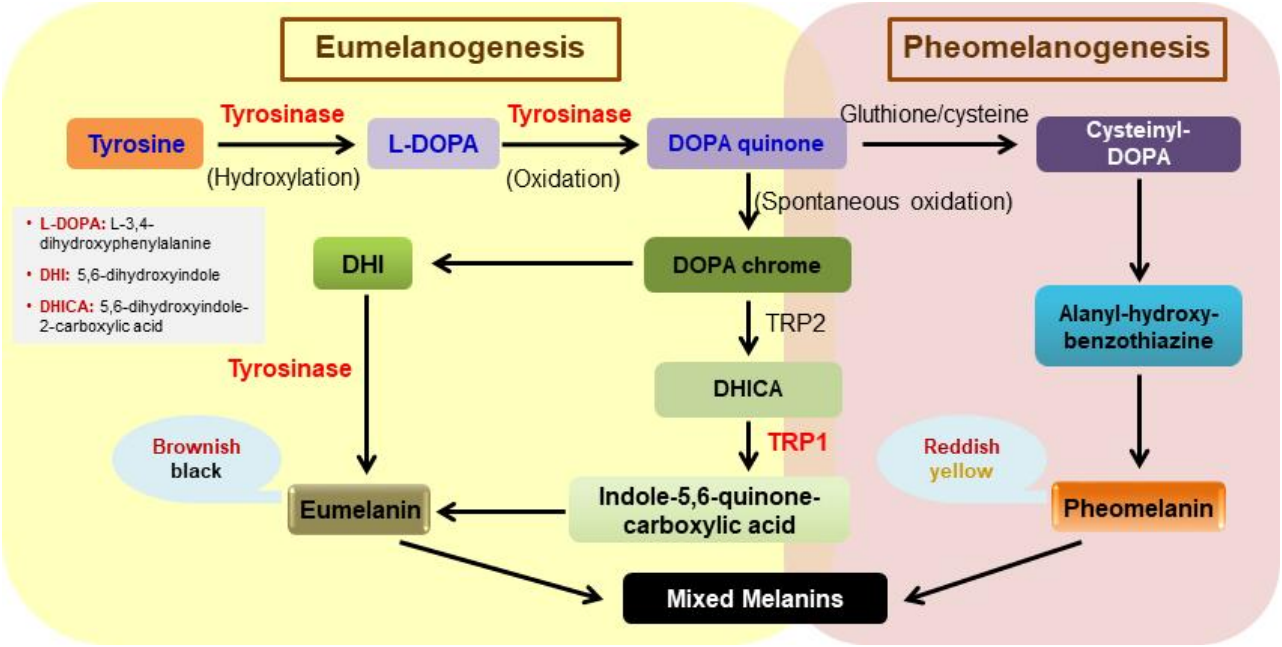

Fig. 4. The synthetic pathway of melanin. Melanogenesis begins with catalysis of the substrate tyrosine to produce L-DOPA by tyrosinase and partly tyrosinase hydroxylase. L-DOPA is then converted to dopaquinone by tyrosinase, and the dopaquinone is catalyzed by TRP and oxidized to eumelanin. Pheomelanin is synthesized through the reaction of dopaquinone and cysteine. DHICA, 5,6-dihydroxyindole-2-carboxylic acid; DOPA, 3,4-dihydroxyphenylalanin; TRP, tyrosinase-related protein. Modified from Yokoyama *et al.* (1994).

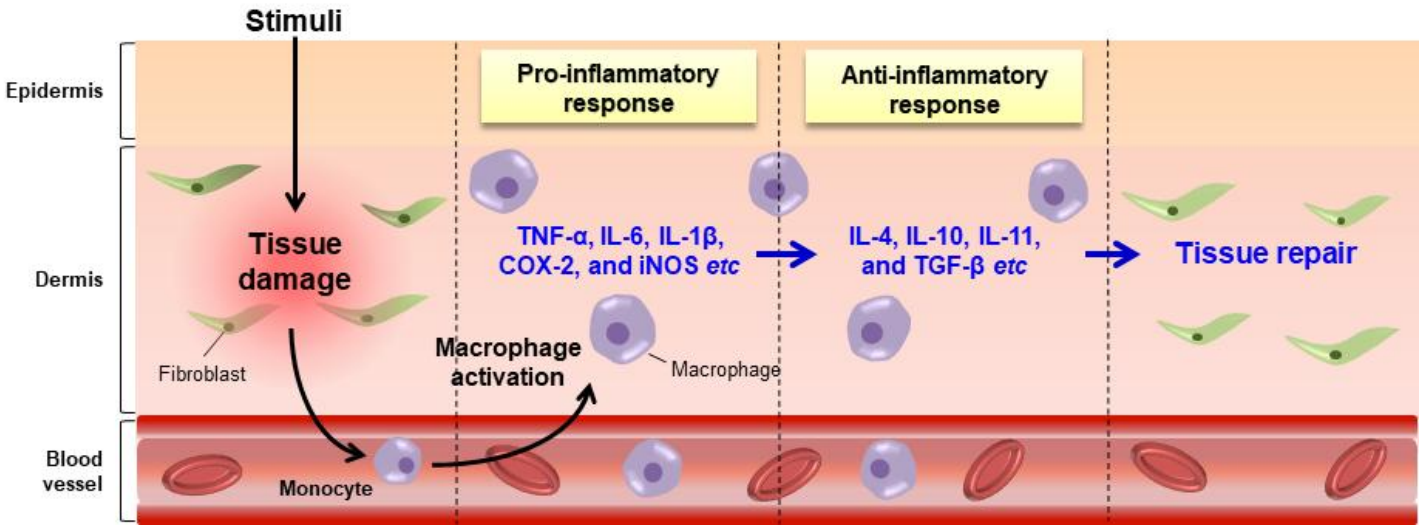

Fig. 5. Inflammatory response in the skin. Inflammatory response as a defense mechanism against injuries and pathogens occurs in macrophages, by producing cytokines, chemokines, and other inflammatory factors (Lim *et al.,* 2017). The pro-inflammatory cytokines are produced predominantly by activated macrophages and are involved in the up-regulation of inflammatory reactions. The anti-inflammatory cytokines are a series of immunoregulatory molecules that control the pro-inflammatory cytokine response (Zhang & An, 2007).

is sequestered in the cytosol. Activation of NF-κB involves the phosphorylation of IκB, thereby releasing NF-κB that is translocated to the nucleus. NF-κB induces the expression of pro-inflammatory cytokines such as interleukin (IL)-1β, IL-6, and tumor necrosis factor-alpha (TNF-α), as well as inducible pro-inflammatory enzymes such as cyclooxygenase-2 (COX-2) (Karunaweera *et al.,* 2015). The pro-inflammatory cytokines induce the up-regulation of inflammatory reactions. The anti-inflammatory cytokines are a series of immunoregulatory molecules that control the pro-inflammatory cytokine response (Zhang & An, 2007) (Fig. 6).

Autophagy is a cellular process for the disposal of damaged organelles, denatured proteins, as well as invaded pathogens through a lysosomal degradation pathway (Qian *et al.,* 2017). The system is generally activated under a state of nutrient deprivation, and its activation is also associated with infection, cancer, and aging (Lemasters *et al.,* 2005). Regulation of the autophagy system involves a very complex process (Faghiri *et al.,* 2010) (Fig. 7). Activated mammalian target of rapamycin (mTOR) suppresses autophagy activation, whereas deactivated mTOR promotes it (Alers *et al.,* 2012). Moreover, Beclin-1 interacts with Bcl-2 to inhibit autophagy induction. Phosphorylation of Bcl-2 by activated mitogen-activated protein kinases (MAPKs), results in the release of Beclin-1 to induce autophagy activation (Zhang *et al.,* 2008). Autophagy-related proteins (Atg proteins) also participate in regulating the autophagy system (Levine *et al.,* 2008). Atg12 combines with Atg5, which then interacts with Atg16 to form a large complex. In addition, microtubule-associated protein light chain 3 (LC3) is a central protein in the autophagy system, and the conversion of LC3-I to LC3-II is an indicator of formation of the autophagosome in the induction of autophagy (Codogno *et al.,* 2012) (Fig. 8).

The compound 7-methylsulfonylheptyl isothiocyanate (7-MSI) is a sulfur-containing phytochemical that is produced by a variety of plants, particularly from cruciferous

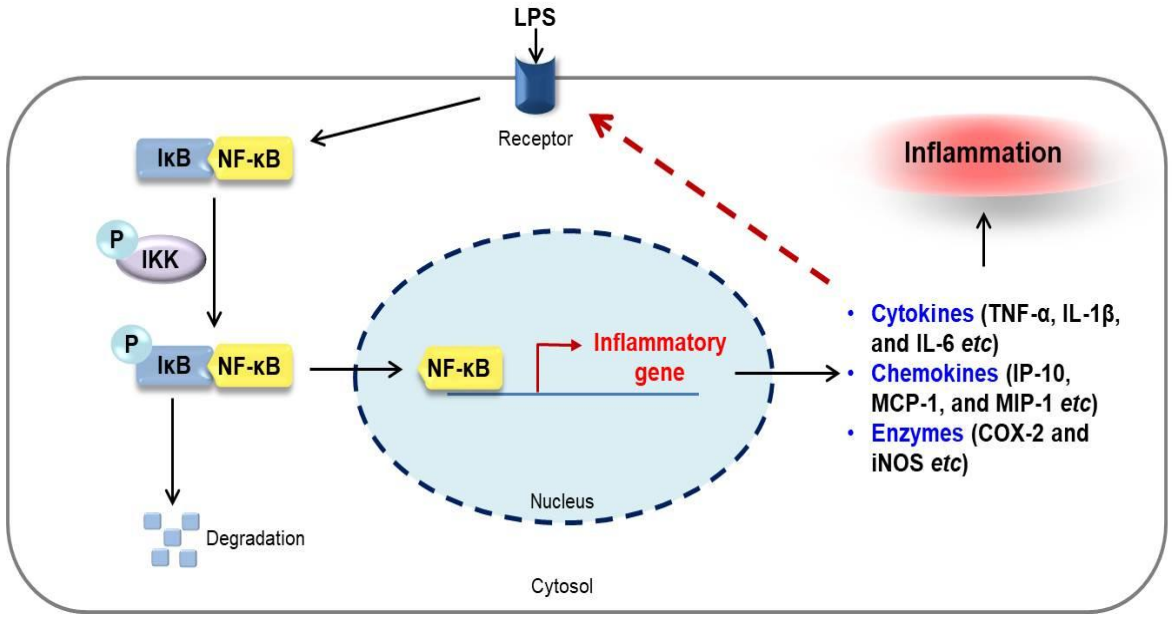

Fig. 6. NF−κB signaling pathway in inflammatory response. NF−κB is kept in an inactive state by binding with IκB in the cytosol. Activation of NF−κB occurs through the phosphorylation of IκB, thereby releasing NF−κB that is translocated to the nucleus where it activates the transcription of numerous genes, including those encoding pro−inflammatory cytokines (Kuo *et al.*, 2015).

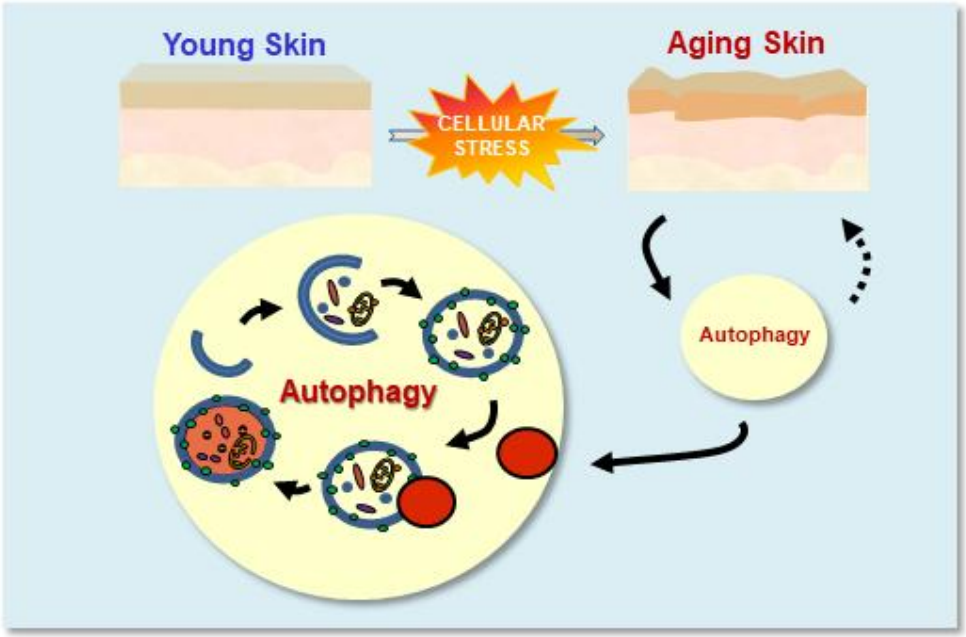

Fig. 7. The autophagy system related to skin aging. Skin aging is related to cellular stresses such as the production of reactive oxygen species (ROS) and various environmental factors, including UV-light, which influences the activation of autophagy (Tashiro *et al.*, 2014).

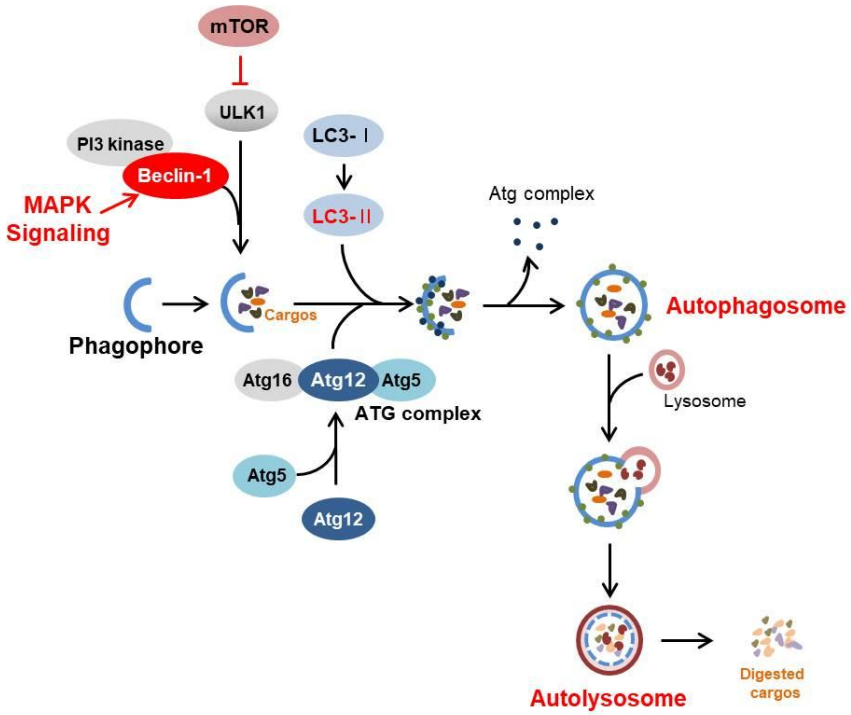

Fig. 8. Autophagy signaling pathway. The mTOR is a major controller for autophagy. Phosphorylation of Bcl-2 by activated MAPKs, results in the release of Beclin-1 to induce autophagy activation (Zhang *et al.*, 2008). Next, the ATG complex and LC3-II are added to the phagophore membrane. The membrane grows to enwrap a protein of the cytosol, forming an autophagosome. In this final step of the process, lysosomes fuse with the autophagosome, resulting in the degradation of the vesicle contents. Modified from Jung *et al.* (2009) and Fujita *et al.* (2008).

vegetables such as cauliflower, cabbage, kale, and broccoli *etc*. Although a few studies have showed that the compound seems to have an ability to defense against pathogens and also exhibits an anti-inflammatory effect (Fig. 9). However, to date there is no study on the effects of 7-MSI on skin whitening and inflammation in detail. Therefore, this study was performed to reveal the followings: 1) the inhibitory effect of 7-MSI on tyrosinase activity, leading the synthesis of melanin in murine melanoma cell lines (B16-F1); 2) the inhibitory effect of 7-MSI on inflammation in murine macrophage cell lines (Raw 264.7); 3) the ability of 7-MSI to induce the activation of autophagy system both in B16-F1 and Raw 264.7 cells.

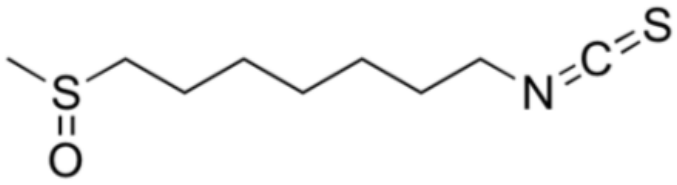

Fig. 9. Chemical structure of 7-MSI. 7-MSI is a sort of isothiocyanates containing sulfor as a form of -N=C=S and its molecular weight is 219.37 M.

Ⅱ. MATERIALS AND METHODS

Ⅱ-1. Materials

7-MSI was obtained from LKT Labs (St. Paul, MN, USA). Dulbecco's modified Eagle medium (DMEM; Lonza, Swiss), fetal bovine serum (FBS; ATLAS, USA), and penicillin-streptomycin (Sigma-Aldrich, St. Louis, MO, USA) were routinely used for cell culture. Mushroom tyrosinase, L-tyrosine, L-DOPA, arbutin, poly-L-lysine, α-MSH, and lipopolysaccharide (LPS) were purchased from Sigma-Aldrich (St. Louis Mo, USA). Transforming growth factor (TGF)-β was purchased from R&D systems (USA). Antibodies raised against NF-κB p65, tyrosinase, and TRP-1 were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Antibodies raised against GAPDH, LC3B, Beclin-1, Atg12, p44/42 MAPK, phospho-p44/42 MAPK (ERK1/2), p38 MAPK, phospho-p38 MAPK, JNK1, phospho-SAPK/JNK, CREB, phospho-CREB, MITF, mTOR, phospho-mTOR, IκB-α, and phospho-IκB-α were purchased from Cell Signaling (Beverly, MA, USA).

Ⅱ-2. Cell culture

Murine melanoma cells (B16-F1) were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). Murine macrophage cell line (Raw 264.7) was purchased from the Korean Cell Line Bank (KCLB, Seoul, Korea). The cells were cultured in DMEM supplemented with 10% FBS and 1% penicillin-streptomycin in a 5% CO_2 incubator at 37°C.

II-3. Cell viability assay

Cell viability was evaluated using the CellTiter 96® AQueous Non-Radioactive cell proliferation assay (Promega, USA). B16-F1 cells were cultured at a density of 0.5 × 10^5 cells/well in 96-well plates overnight, and then treated with various concentrations of 7-MSI (0, 0.1, 0.5, 1, 2, and 4 μg/ml) or TGF-β (0, 0.00002, 0.0002, 0.002, 0.02, and 0.2 ng/ml) as a positive control for 24 h. Raw 264.7 cells were cultured at a density of 0.5 × 10^5 cells/well in 96-well plates overnight, and then treated with various concentrations of 7-MSI (0, 0.1, 0.5, 1, 3, and 5 μg/ml) or TGF-β (0, 0.00002, 0.0002, 0.002, 0.02, and 0.2 ng/ml) for 24 h. At the end of the incubation, 20 μl of MTS dye solution was added to each well. The cells were further incubated at 37°C in 5% CO_2 for 4 h. The absorbance was measured at a wavelength of 490 nm using a 96-well platereader.

II-4. Mushroom tyrosinase activity assay

A mushroom tyrosinase assay was performed with L-tyrosine as a substrate for tyrosinase activity assay in a test tube (Jeong *et al.,* 2013). The reaction mixtures were prepared by adding 240 μl of 7-MSI (0, 0.5, 1, 3, 5, and 10 μg/ml) or arbutin (0.1 mg/ml) diluted in 0.1 M potassium phosphate buffer (pH 6.8), 20 μl of 1,500 U/ml mushroom tyrosinase, and 40 μl of 1.5 mM L-tyrosine. The mixtures were incubated for 15 min at 37°C and the absorbance was measured at a wavelength of 490 nm using a 96-well platereader. Arbutin was used as a positive control. The inhibition rate of tyrosinase activity was calculated using the following formula:

$$\text{Inhibition rate of tyrosinase activity (\%)} = 100 - [(S - B)/(C - B)] \times 100$$

, in which S is the A_{490} value from 7-MSI plus tyrosinase in buffer, B is the A_{490} value from buffer only, and C is the A_{490} value from tyrosinase only in buffer.

II-5. DOPA oxidation assay

The inhibitory effect of 7-MSI on L-DOPA oxidation was determined according to the mushroom tyrosinase activity assay as described above with a minor modification. The reaction mixtures were prepared by adding 450 μl of 7-MSI (0, 0.5, 1, 3, 5, and 10 μg/ml) or arbutin (0.1 mg/ml) diluted in 0.1 M potassium phosphate buffer (pH 6.8) and 25 μl of 500 U/ml mushroom tyrosinase. The mixtures were incubated for 6 min at 37°C, and then 25 μl of 2 mM L-DOPA was added to initiate the reaction. The mixtures were incubated for 1 min at 25°C, and the absorbance was measured at a wavelength of 475 nm using a 96-well platereader. Arbutin was used as a positive control. The inhibition rate of tyrosinase activity was calculated using the following formula:

$$\text{Inhibition rate of tyrosinase activity (\%)} = 100 - [(S - B)/(C - B)] \times 100$$

, in which S is A_{475} value from 7-MSI plus tyrosinase in buffer, B is A_{475} value from buffer only, and C is A_{475} value from tyrosinase only in buffer.

II-6. Melanin content measurement

B16-F1 cells were cultured in 6-well plates at a density of 0.5×10^5 cells/well overnight, and then treated with 7-MSI (0, 0.05, 0.1, 0.5, 1, and 2 μg/ml) in th presence of α-MSH (10 nM) in DMEM without phenol red (WELGENE, Daegu, Korea), and further incubated for 73 h at 37°C in 5% CO_2. The cell-free medium

was collected after centrifugation for 4 min at 13,000 rpm. The extracellular melanin content was measured by analyzing the absorbance at 490 nm.

II-7. Western blot analysis

B16-F1 cells were seeded at a density of 0.5 × 10^5 cells/well in 6-well plates. After culturing overnight, to induce melanogenesis, the cells were treated with α-MSH (10 nM) and then treated with 7-MSI (1 μg/ml) for 24 h at 37°C in 5% CO_2. Raw 264.7 cells were seeded at a density of 2.0 × 10^5 cells/well in 6-well plates. After culturing overnight, to induce inflammatory response, the cells were treated with LPS (1 μg/ml) and then treated with 7-MSI (1 μg/ml) for 0.5, 1, and 3 h at 37°C in 5% CO_2. Protein samples were mixed with an equal volume of 1× SDS-PAGE sample buffer, boiled at 100°C for 3 min and then loaded onto 8%, 10%, or 12% polyacrylamide gel. For Western blot analysis, protein samples were subjected to SDS-PAGE and then transferred onto a PVDF membrane (Bio-Rad, Hercules, CA, USA). The membrane was blocked with 5% skim milk in TBS-T (250 mM Tris-HCl, pH 8.0, 1.5 mM NaCl, and 0.1% Tween 20) at room temperature (RT) for 2 h. The membrane was then incubated with specific primary antibodies (1:1000 in the blocking buffer) overnight at 4°C, and washed six times with TBS-T buffer. The membrane was then incubated with HRP-conjugated secondary antibodies (1:4000 in the blocking buffer) at RT for 2 h. After washing five times with TBS-T buffer, the protein expression levels were determined by analyzing the chemiluminescence captured on the PVDF membrane by EZ-Western Lumi Plus and Lumi Femto (DAEILLAB SERVICE co., Ltd., Seoul, Korea) and exposed on X-ray film (Fuji Film, Japan). Western blot data was quantified using the program ImageJ (National Institutes of Health, Bethesda, Maryland, USA).

II-8. Enzyme-linked immunosorbent assay (ELISA)

Raw 264.7 cells were plated on 24-well plates at a density of 0.25 × 10^5 cells/well and incubated overnight. The cells were treated with 7-MSI (0, 0.1, 1, 2, 3, and 4 μg/ml) in the presence of LPS (1 μg/ml) for 1 h at 37°C in 5% CO_2. The TNF-α protein levels in the culture supernatant were determined using an ELISA kit (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instruction. The absorbance was measured at a wavelength of 450 nm using a 96-well platereader.

II-9. Total RNA purification and cDNA synthesis

Raw 264.7 cells were plated on 12-well plates at a density of 0.1 × 10^5 cells/well and incubated overnight. The cells were treated with 7-MSI (0, 0.5, 1, and 2 μg/ml) in the presence of LPS (1 μg/ml) for 3 h. The cells were lysed and total RNA was isolated by an RNeasy Plus Mini Kit (QIAGEN, Germany) according to the manufacturer's instructions. First-strand cDNA was synthesized from 500 μg of RNA with oligo $(dT)_{18}$ primers using M-MLV reverse transcriptase (Bioneer, Daejeon, Korea). Cycling conditions were for 5 min at 25°C, for 1 h at 42°C, and for 15 min at 70°C.

II-10. Reverse transcription polymerase chain reaction (RT-PCR)

The cDNA products were used as templates for PCR to amplify the target genes. PCR was performed using Top DNA polymerase (Bioneer, Daejeon, Korea). Cycling conditions were as follows; denaturation for 30 sec at 94°C, annealing for

30 sec at 48°C, 55°C, or 60°C, and extension for 30 sec at 72°C for 30 cycles. The sequences of the specific primers used were as follows: IL-1β forward 5′-TGTGAAATGCCACCTTTTGA-3′ and reverse 5′-GTAGCTGCCACAGCTTCTCC-3′, IL-6 forward 5′-AATTTCCTCTGGTCTTCTGG-3′ and reverse 5′-TAGCCACTCCTTCTGTGACTC-3′, COX-2 forward 5′-CAGCAAATCCTTGCTGTTCC-3′ and reverse 5′-CCATCCTTGAAAAGGCGCAG-3′, PGEs forward 5′-ATGCCTTCCCCGGGCCTG-3′ and reverse 5′-TCACAGATGGTGGGCCAC-3′, and GAPDH forward 5′-TCAGCAATGCATCCTGCACCAC-3′ and reverse 5′-TGCCAGTGAGCTTCCCGTTCAG-3′.

II-11. Immunostaining for confocal microscopic analysis

Raw 264.7 cells were cultured (1.0×10^5 cells/well) on poly-L-lysine (0.01% solution)-coated glass coverslip in 12-well plates. After culturing for 24 h, the cells were treated with 7-MSI (1 μg/ml) in the presence of LPS (1 μg/ml) for 1 h at 37°C in 5% CO_2. The cells were washed with PBS and fixed with 3.7% formaldehyde in PBS for 10 min at RT. After washing three times with PBS, the cells were permeabilized with 0.1% Triton X-100 for 10 min at RT and then washed with PBS for three times. The cells were then blocked with 1% BSA in PBS for 20 min at RT. After washing three times with PBS, the cells were incubated with a 1:50 dilution of anti-NF-κB antibody for 1 h at RT. After incubating for 1 h at RT, the cells were washed three times with PBS. The cells were incubated secondary antibody goat anti-rabbit IgG-FITC (diluted 1:200 with PBS; Santa cruz, USA) for 1 h at RT. The cells were washed with PBS for three times, stained with 4'6-diamidino-2-phenylindole (DAPI), and observed using a Zeiss LSM 510 confocal microscope (LePecq, France).

III. RESULTS AND DISCUSSION

III-1. Cytotoxic effects of 7-MSI against B16-F1 cells

To examined that 7-MSI can affect on cell survival, MTS assay was performed with B16-F1 cells after treatments with various concentrations of 7-MSI (0, 0.1, 0.5, 1, 2, and 4 μg/ml) for 24 h. As shown in Fig. 10, 7-MSI did not show any cytotoxic effects up to 4 μg/ml, compared to the non-treated control. These results suggest that 7-MSI has no cytotoxic effect on B16-F1 cells.

III-2. Inhibitory effects of 7-MSI on the hydroxylation of L-tyrosine by tyrosinase

Tyrosinase is a rate limiting enzyme that catalyzes L-tyrosine to L-DOPA and oxidizes it to dopaquinone in the melanogenesis. To observe the inhibitory effect of 7-MSI on the hydroxylation of L-tyrosine by mushroom tyrosinase activity *in vitro*, reaction mixtures composed of L-tyrosine (1.5 mM), mushroom tyrosinase (1,500 U/ml), 7-MSI (0, 0.5, 1, 3, 5, and 10 μg/ml), or arbutin (0.1 mg/ml) that used as a positive control was incubated for 15 min at 37°C. The resulting hydroxylation degree of L-tyrosine was measured by analyzing the absorbance at 490 nm. As shown in Fig. 11, the tyrosinase activity was decreased by approximately 13% with treatment of 5 μg/ml of 7-MSI, and further decreased in a dose-dependent manner compared to that of the non-treated control. These results suggest that 7-MSI can directly inhibit tyrosinase activity on the L-tyrosine.

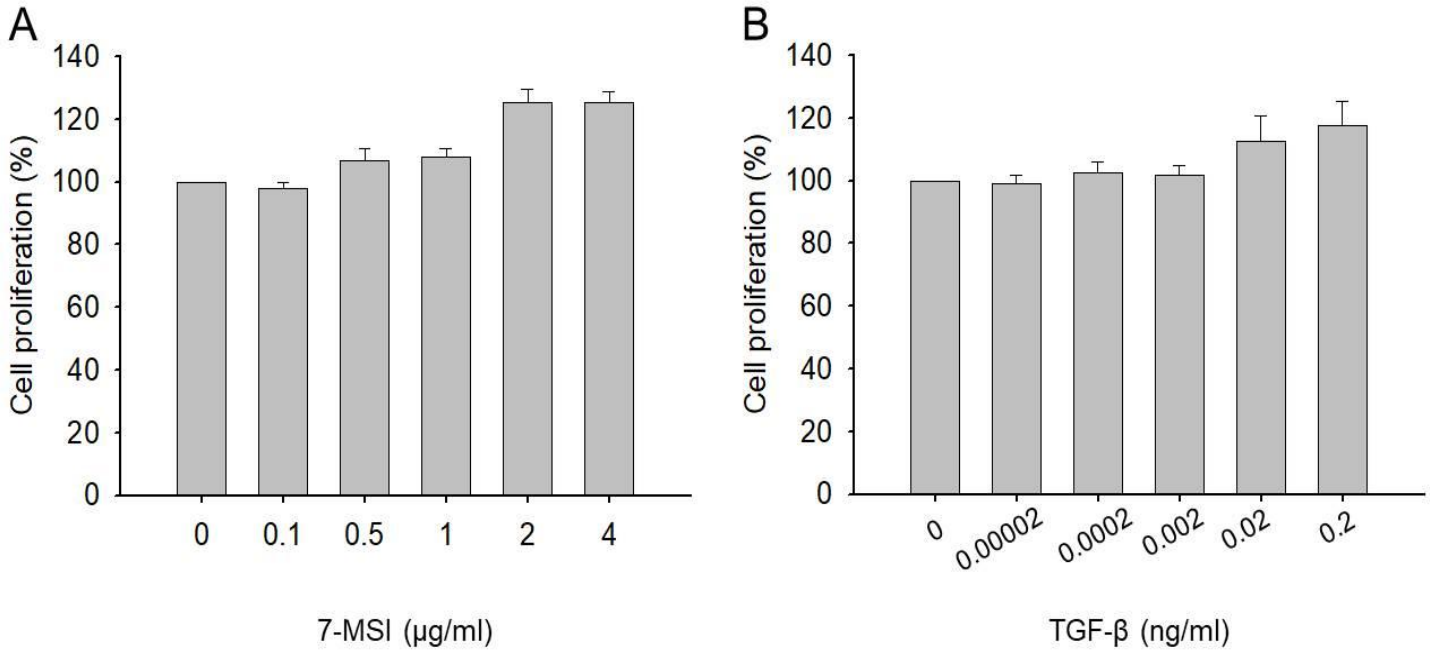

Fig. 10. Effects of 7-MSI on the cell proliferation of B16-F1 cells. B16-F1 cells were treated with various doses of 7-MSI (0, 0.1, 0.5, 1, 2, and 4 μg/ml) (A) and TGF-β (0, 0.00002, 0.0002, 0.002, 0.02, and 0.2 ng/ml) (B) for 24 h and the cell survivals were examined by MTS assay as described in Materials and Methods, in which TGF-β was used as a positive control.

III-3. Inhibitory effects of 7-MSI on the oxidation of L-DOPA by tyrosinase

To observe the inhibitory effect of 7-MSI on the oxidation of L-DOPA by mushroom tyrosinase activity *in vitro*, a reaction mixture composed of mushroom tyrosinase (1,500 U/ml), 7-MSI (0, 0.5, 1, 3, 5, and 10 μg/ml), or arbutin (0.1 mg/ml) was pre-incubated at 37°C for 6 min and the reaction was continued for 1 min at RT after adding 2 mM of L-DOPA. The resulting oxidation degree of L-DOPA was measured by analyzing the absorbance at 475 nm. As shown in Fig. 11, the tyrosinase activity was decreased by approximately 31% with treatment of 5 μg/ml of 7-MSI and then further decreased in a dose-dependent manners compared to that of the non-treated control. These results suggest that 7-MSI can directly inhibit tyrosinase activity on the oxidation of L-DOPA.

III-4. Inhibitory effects of 7-MSI on melanogenesis in B16-F1 cells

It has been known that the phosphorylation of CREB leads the expression of the MITF gene, which controls the production of the tyrosinase and TRP-1 (Lim *et al.,* 2018). In this study, the inhibition ability of 7-MSI to the expression levels of melanogenesis-associated proteins were examined using Western blottings with the corresponding antibodies. As shown in Fig. 12, the production levels of CREB, p-CREB, MITF, tyrosinase, and TRP-1 were decreased by 9%, 27%, 28%, 45%, and 11%, respectively, in the cells treated with 1 μg/ml of 7-MSI in the presence of α-MSH (10 nM) for 24 h, compared to those treated with only α-MSH, in which arbutin (0.1 mg/ml) was used as a positive control. These results suggest that 7-MSI can inhibit the production of melanogenesis-associated proteins.

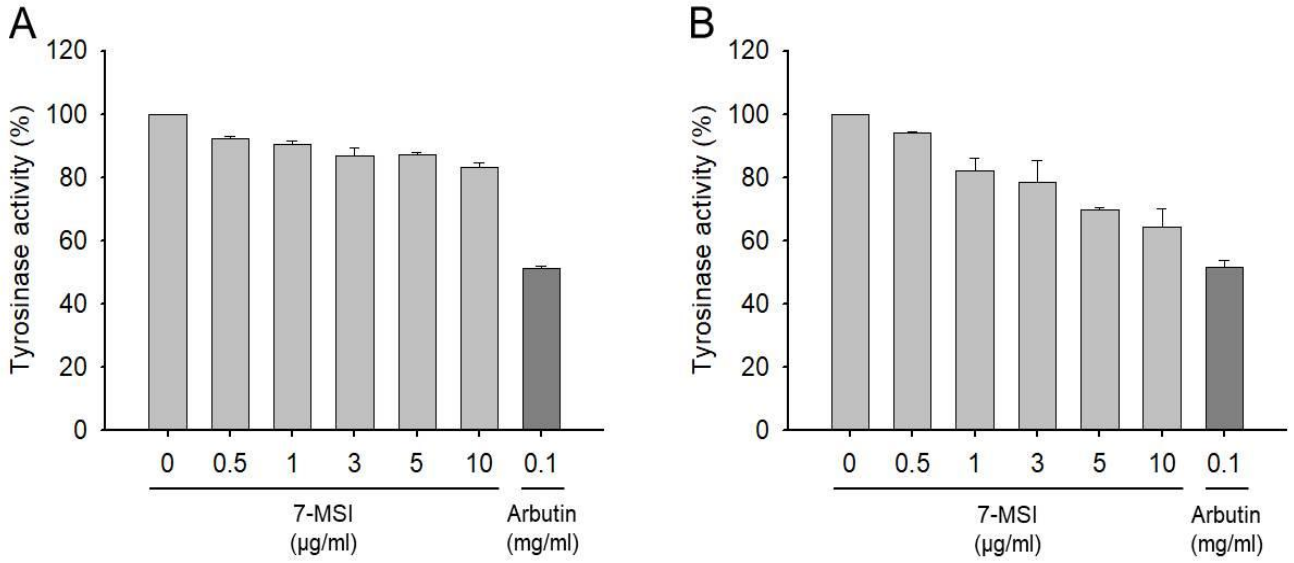

Fig. 11. Effects of 7-MSI on mushroom tyrosinase activity. (A) Various concentrations of 7-MSI (0, 0.5, 1, 3, 5, and 10 μg/ml) were incubated with L-tyrosine (1.5 mM) and mushroom tyrosinase (1,500 U/ml) at 37°C for 15 min *in vitro*. The enzyme activities were measured at A_{490} as described in Materials and Methods. (B) Different concentrations of 7-MSI (0, 0.5, 1, 3, 5, and 10 μg/ml) were pre-incubated with mushroom tyrosinase (500 U/ml) for 6 min at 37°C and the reaction was continued for 1 min at 25°C after adding 2 mM of L-DOPA. The enzyme activities were measured at A_{475}. Inhibitory effect of Arbutin (0.1 mg/ml) on mushroom tyrosinase activity was used as a positive control.

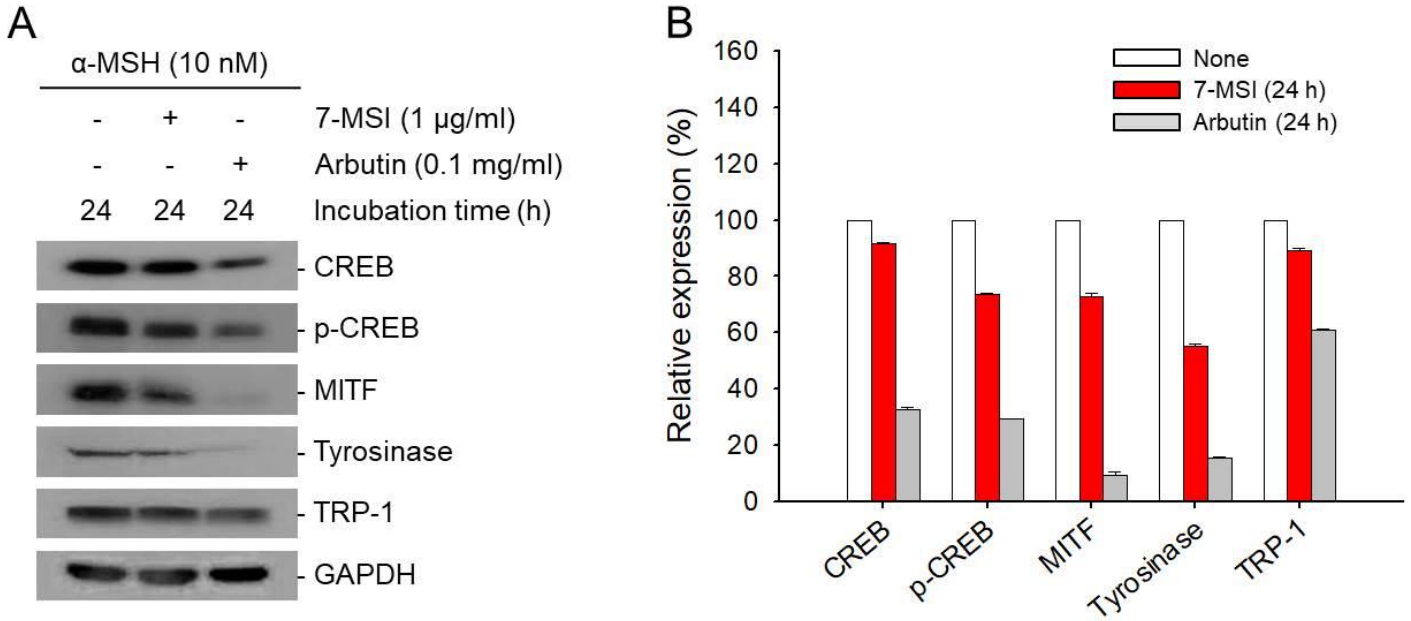

Fig. 12. Effects of 7-MSI on various melanogenesis-associated proteins in B16-F1 cells. (A) B16-F1 cells were treated with 7-MSI (1 μg/ml) in the presence of α-MSH (10 nM) for 24 h. Proteins from each sample were separated by SDS-PAGE and Western blottings were performed with antibodies raised against CREB, p-CREB, MITF, tyrosinase, TRP-1, and GAPDH. Arbutin (0.1 mg/ml) was used as a positive control. (B) Histograms showing the relative rates of expressions. Each value was calculated as the ratio of signal intensity, compared to that of GAPDH.

III-5. Inhibitiory effects of 7-MSI on melanin synthesis in B16-F1 cells

To investigate the inhibitory effect of 7-MSI on melanin production, B16-F1 cells were treated with 7-MSI (0, 0.05, 0.1, 0.5, 1, or 2 μg/ml) in the presence of α-MSH (10 nM) for 73 h. In this experiment, arbutin (0.05 mg/ml) was used as a positive control. As shown in Fig. 13, the rate of melanin synthesis could be decreased dose-dependently by the treatment with 7-MSI, in which 1 μg/ml of 7-MSI decreased to about 62%, compared to that of only α-MSH. These results suggest that 7-MSI can directly inhibit the melanin synthesis in B16-F1 cells.

III-6. Effects of 7-MSI on MAPK signaling pathway in B16-F1 cells

It has been known that MAPK signaling regulates the induction of autophagy and especially the activated ERK can lead the maturation of autophagic vacuoles (Martin *et al.,* 2006). The effects of 7-MSI on activation of MAPK signaling were also examined in B16-F1 cells using Western blottings with antibodies raised against p38, p-p38, JNK, p-JNK, ERK, and p-ERK. When B16-F1 cells were treated with 7-MSI (1 μg/ml) in the presence of α-MSH (10 nM) for 30 min, the expression levels of p-p38, p-JNK, and p-ERK were increased by approximately 181%, 72%, and 158%, respectively, compared to those of control cells (Fig. 14). These results suggest that 7-MSI can induce up-regulation of the MAPK signaling pathway in B16-F1 cells.

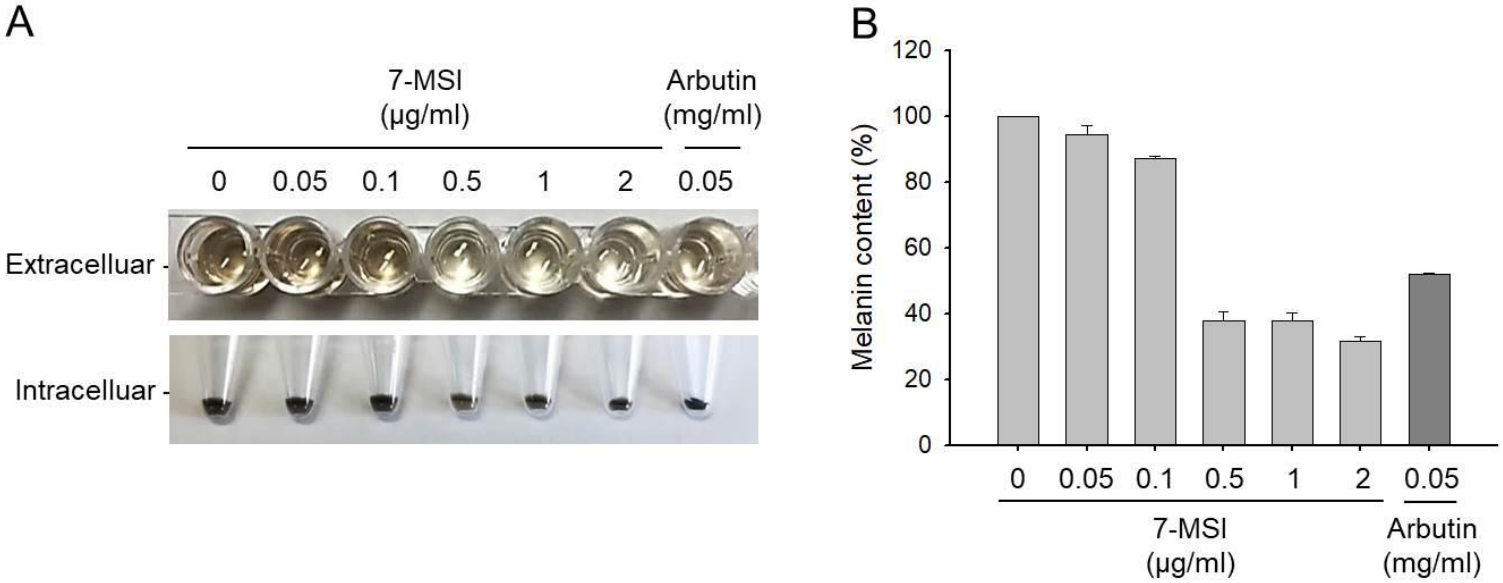

Fig. 13. Inhibition of melanin synthesis by 7-MSI in B16-F1 cells. (A) Photographs showing the inhibitory effects of 7-MSI on melanin production, in which extracellular and intracellular indicate the supernatant and the cell precipitate, respectively. Arbutin (0.05 mg/ml) was used as a positive control. (B) B16-F1 cells were treated with 7-MSI (0, 0.05, 0.1, 0.5, 1, and 2 μg/ml) in the presence of α-MSH (10 nM) for 73 h and the amounts of melanin were examined by measuring the absorbance at 490 nm.

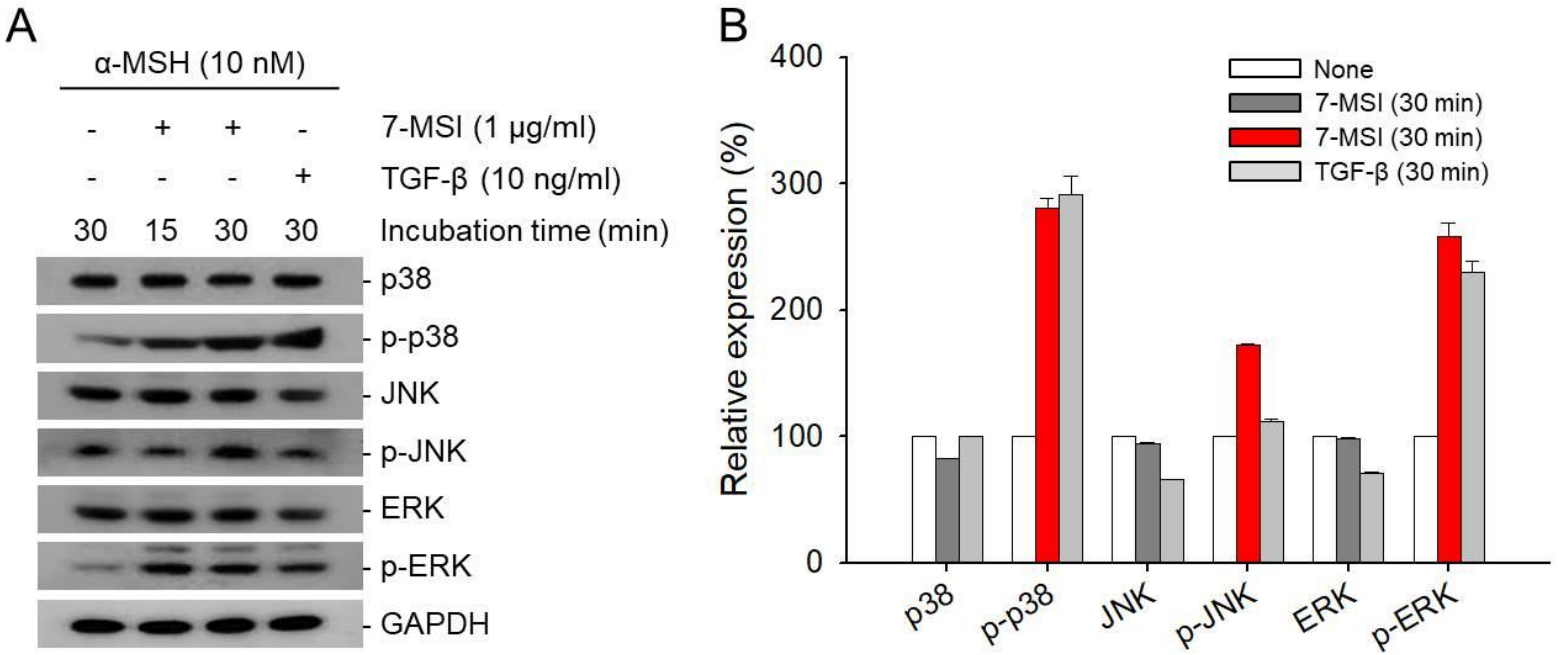

Fig. 14. Activation of MAPK signaling pathway by 7-MSI in B16-F1 cells. (A) B16-F1 cells were treated with 7-MSI (1 μg/ml) in the presence of α-MSH (10 nM) for 15 or 30 min. Proteins from each sample were separated by SDS-PAGE and Western blottings were performed with antibodies raised against p38, p-p38, JNK, p-JNK, ERK, p-ERK, and GAPDH. TGF-β (10 ng/ml) was used as a positive control. (B) Histograms showing the relative expression rates. Each value was calculated as the ratio of signal intensity, compared to that of GAPDH.

III-7. Activation of cellular autophagy by 7-MSI in B16-F1 cells

The activated mTORs suppress autophagy, whereas negative regulation of mTOR promotes it. Beclin-1 is required for the induction of autophagy. The Atg molecules control the formation of autophagosome through Atg12-Atg5 and LC3-II complexes (Codogno *et al.,* 2012). To investigate the activation of autophagy system by 7-MSI, the protein expression levels of autophagy factors were analyzed in B16-F1 cells treated with 1 μg/ml of 7-MSI in the presence of α-MSH (10 nM) for 30 min using Western blottings. As shown in Fig. 15, the expression levels of Beclin-1, Atg12, and LC3 were increased by approximately 34%, 50%, and 46%, respectively, compared to those of the non-treated control. However, the expression level of phospho-mTOR (a negative regulator of the autophagy system) was decreased by about 52% after treatment with 7-MSI. These results showed that 7-MSI can induce the up-regulation of the autophagy-related proteins in B16-F1 cells.

III-8. Cytotoxic effects of 7-MSI against Raw 264.7 cells

To examined that 7-MSI can affect on cell survival, MTS assay was performed with Raw 264.7 cells after treatments with various concentrations of 7-MSI (0, 0.1, 0.5, 1, 3, and 5 μg/ml) for 24 h. As shown in Fig. 16, 7-MSI did not show any cytotoxic effects up to 5 μg/ml, compared to the non-treated control. These results suggest that 7-MSI has no cytotoxic effect on Raw 264.7 cells.

III-9. Effects of 7-MSI on TNF-α production in Raw 264.7 cells

To examine the effects of 7-MSI on the production of TNF-α in LPS-stimulated cells, ELISA was performed. As shown in Fig. 17, when Raw 264.7

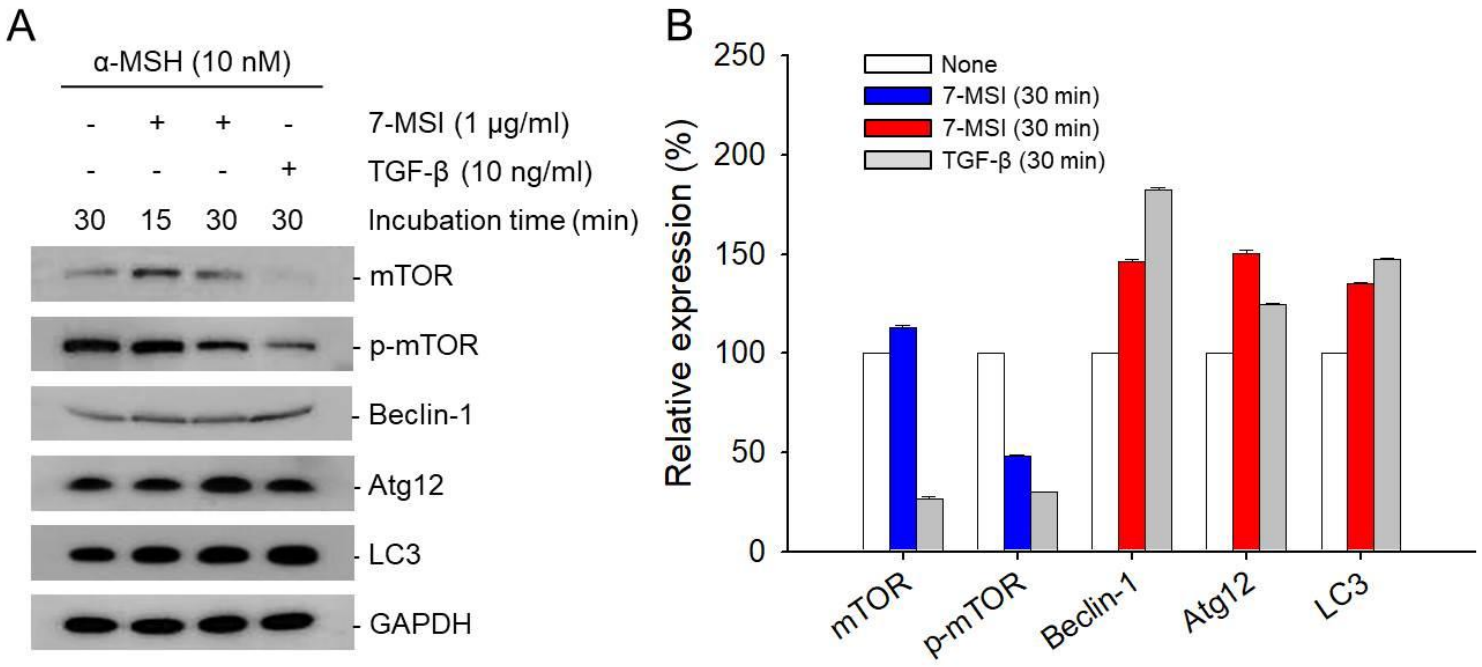

Fig. 15. Activation of cellular autophagy by 7-MSI in B16-F1 cells. (A) B16-F1 cells were treated with 7-MSI (1 μg/ml) in the presence of α-MSH (10 nM) for 15 or 30 min. Proteins from each sample were separated by SDS-PAGE and Western blottings were performed with antibodies raised against mTOR, p-mTOR, Beclin-1, Atg12, LC3, and GAPDH. TGF-β (10 ng/ml) was used as a positive control. (B) Histograms showing the relative expression rates. Each value was calculated as the ratio of signal intensity, compared to that of GAPDH.

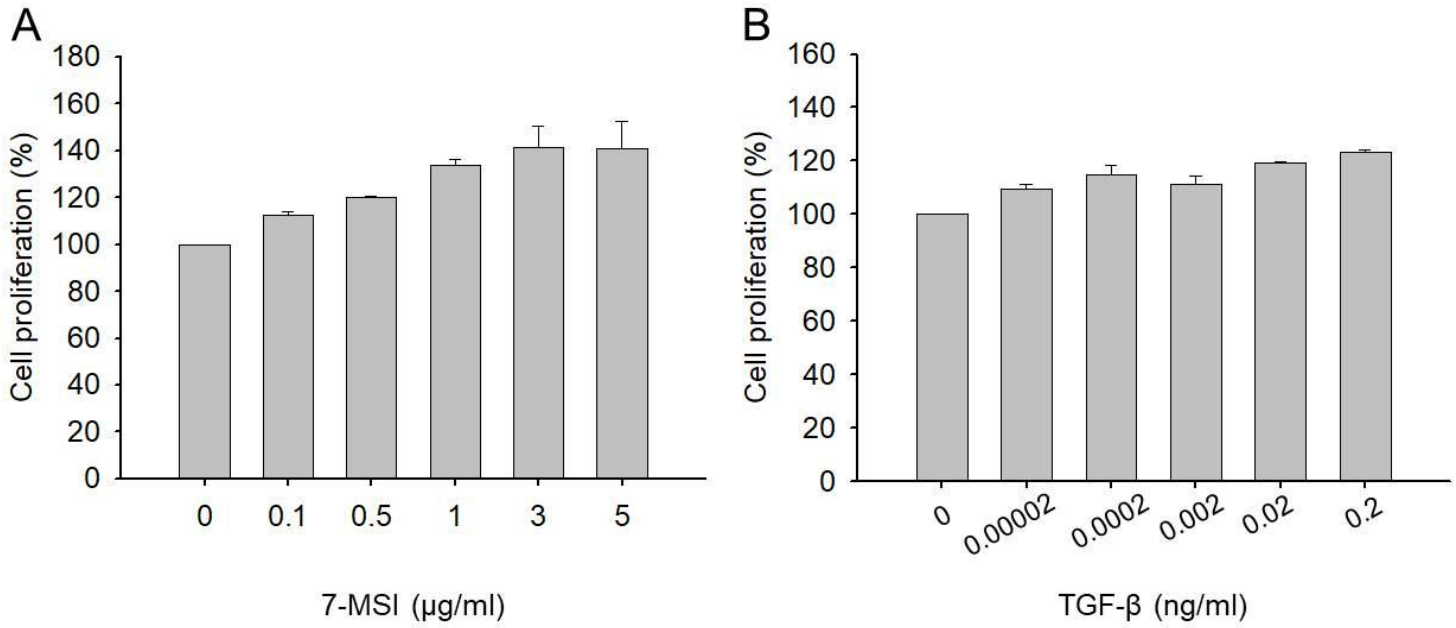

Fig. 16. Effects of 7-MSI on the cell proliferation of Raw 264.7 cells. Raw 264.7 cells were treated with various doses of 7-MSI (0, 0.1, 0.5, 1, 3, and 5 μg/ml) (A) and TGF-β (0, 0.00002, 0.0002, 0.002, 0.02, and 0.2 ng/ml) (B) for 24 h and the cell survivals were examined by MTS assay as described in Materials and Methods, in which TGF-β was used as a positive control.

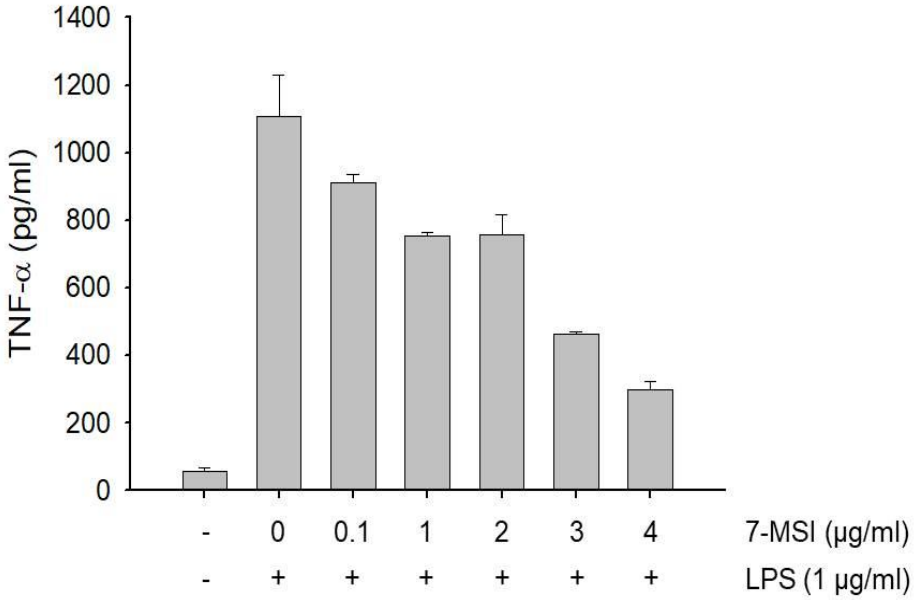

Fig. 17. Effects of 7-MSI on TNF-α production in Raw 264.7 cells. Raw 264.7 cells were treated with 7-MSI (0, 0.5, 1, 2, 3, and 4 μg/ml) in the presence of LPS (1 μg/ml) for 1 h. TNF-α concentrations in the culture supernatants were measured using a TNF-α specific ELISA kit. The concentration of TNF-α was compared to those from a standard curve. Results represent the mean ± S.D. of duplicate determinations from three independent experiments.

cells were treated with 1 μg/ml of LPS for 1 h, the production of TNF-α clearly was increased to 1,106 pg/ml. However, the cells were treated with 7-MSI (1 μg/ml) in the presence of LPS (1 μg/ml) for 1 h, the TNF-α level was decreased to 752 pg/ml. These results suggest that 7-MSI can suppress TNF-α protein production, indicating that it has an anti-inflammatory effect.

III-10. Effects of 7-MSI on the transcription levels of pro-inflammatory cytokines in Raw 264.7 cells

The pro-inflammatory cytokines are produced by the activated macrophages and act an positive regulators for inflammatory reactions. In this study, the expression levels of pro-inflammatory cytokines and regulators were analyzed in Raw 264.7 cells using RT-PCR. When the cells were treated 7-MSI (0, 0.5, 1, and 2 μg/ml) in the presence of LPS (1 μg/ml) for 3 h, the transcription levels of IL-1β, IL-6, COX-2, and PGEs significantly were decreased by approximately 562%, 126%, 209%, and 56%, respectively, compared to those of the LPS-only control group (Fig. 18). These results suggest that 7-MSI can inhibit LPS-induced inflammatory response by suppressing the production of pro-inflammatory cytokines and regulators.

III-11. Effects of 7-MSI on IκB phosphorylation in Raw 264.7 cells

The activation of NF-κB involves the phosphorylation of IκBα, thereby releasing NF-κB that is translocated in to the nucleus where it activates the transcription of numerous pro-inflammatory cytokines genes (Kuo *et al.,* 2015).

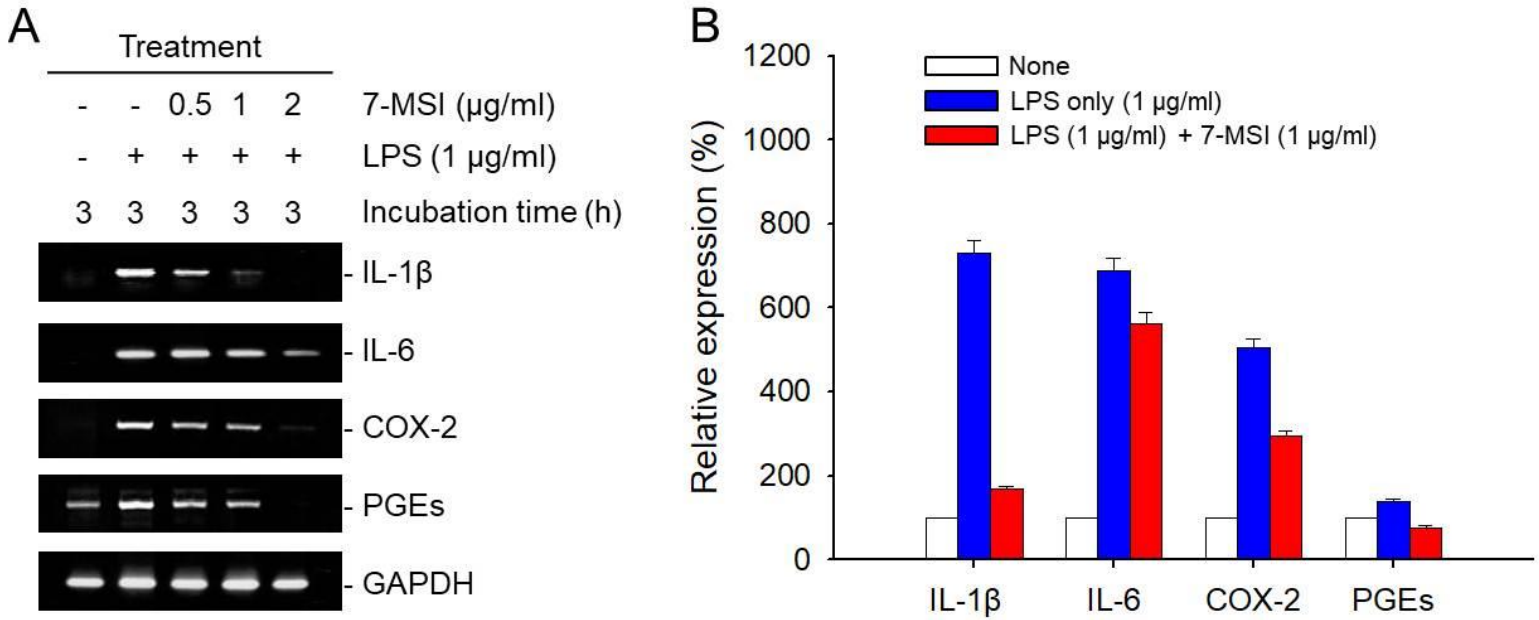

Fig. 18. Effects of 7-MSI on the transcription levels of various pro-inflammatory cytokines in Raw 264.7 cells. (A) Raw 264.7 cells were treated with 7-MSI (0, 0.5, 1, and 2 μg/ml) in the presence of LPS (1 μg/ml) for 3 h, and RT-PCRs were performed with total RNAs isolated from the corresponding experimental groups using primers specific for IL-1β, IL-6, COX-2, and PGEs. The resulting PCR products were analyzed on 1% agarose gel. (B) Histograms showing the relative expression rates. Each value was calculated as the ratio of signal intensity, compared to that of GAPDH.

In this study, the phosphorylation of IκBα was analyzed in Raw 264.7 cells using Western blottings. As shown in Fig. 19, the IκBα was clearly degraded when the cells were treated with LPS (1 μg/ml) for various time periods as shown by Western blottings. However, the degrees of IκBα degradation and phospho-IκBα production were significantly reduced by 7-MSI (1 μg/ml) treatments for various time periods in LPS (1 μg/ml)-treated cells. When Raw 264.7 cells were treated with 7-MSI (1 μg/ml) in the presence of LPS (1 μg/ml) for 1 h, the expression level of phospho-Iκ Bα was decreased by approximately 46% compared to that of the non-treated control, as revealed by Western blottings. These results suggest that 7-MSI can decrease the phosphorylation of IκBα proteins in Raw 264.7 cells.

III-12. Inhibitory effects of NF-κB activation by 7-MSI in Raw 264.7 cells

NF-κB is kept in an inactive state by binding to its inhibitory protein IκBα in the cytosol (Kuo *et al.,* 2015). The Activation of NF-κB induces the expression of pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α, as well as inducible pro-inflammatory enzymes such as COX-2 (Karunaweera *et al.,* 2015). In this study, the inhibitory effect of 7-MSI (1 μg/ml) on the NF-κB activation was confirmed by Confocal microscopic analysis using anti-NF-κB antibody labeled with fluorescein in LPS (1 μg/ml)-treated cells for 1 h. As shown in Fig. 20, the confocal analysis showed that the LPS-induced nuclear translocation of NF-κB proteins is inhibited by 7-MSI treatment by allowing the protein remained in the cytoplasm. Taken together, these results suggest that 7-MSI can inhibit the activation of NF-κB signalling pathway by suppressing the phosphorylation of IκBα proteins.

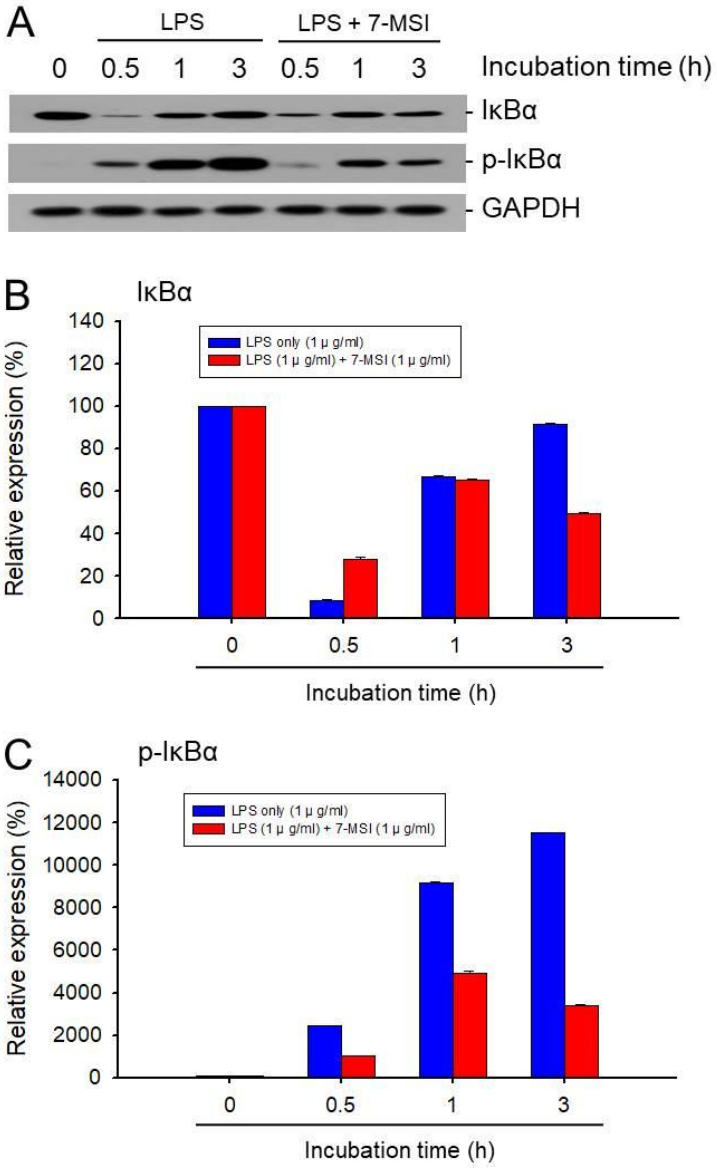

Fig. 19. Inhibition of the phosphorylation of IκB by 7-MSI in Raw 264.7 cells. (A) Raw 264.7 cells were treated with 7-MSI (1 μg/ml) in the presence of LPS (1 μg/ml) for 0.5, 1, and 3 h. Proteins from each sample were separated by SDS-PAGE and Western blottings were performed with antibodies raised against IκBα, p-IκBα, and GAPDH. (B and C) Histograms showing the relative expression rates of IκBα and p-IκBα. Each value was calculated as the ratio of signal intensity, compared to that of GAPDH.

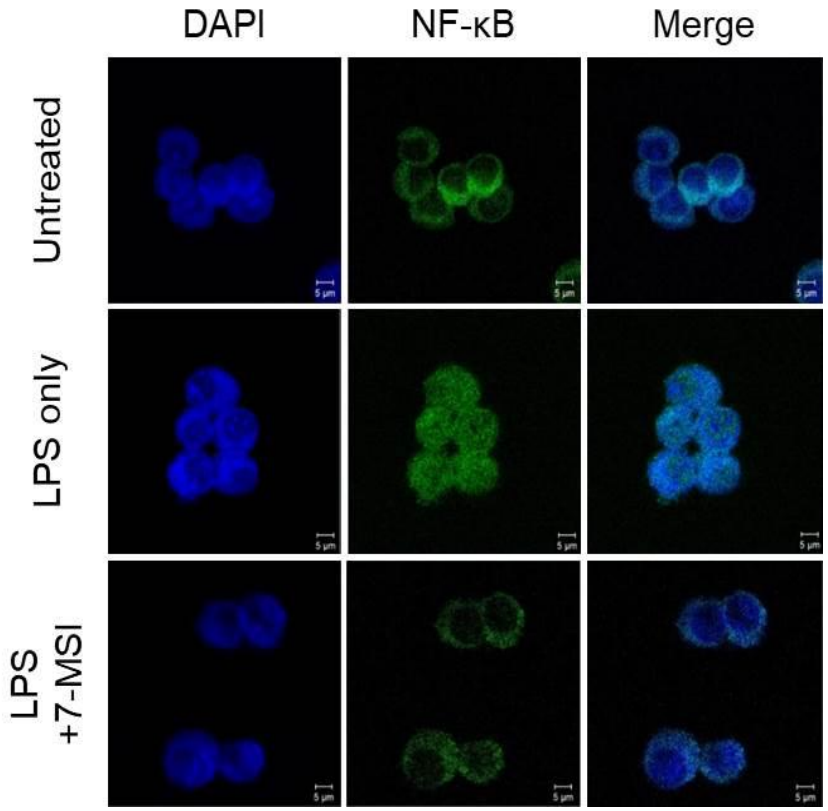

Fig. 20. Confocal microscopic analysis showing the inhibition of NF-κB activation by 7-MSI in Raw 264.7 cells. The cells treated with LPS (1 μg/ml) for 1 h or co-treated with LPS (1 μg/ml) and 7-MSI (1 μg/ml) for 1 h were stained with anti-NF-κB antibody labeled with fluorescein and the green fluorescences emitted were observed with confocal microscope.

III-13. Effects of 7-MSI on MAPK signaling pathway in Raw 264.7 cells

The effects of 7-MSI on activation of MAPK signaling were also examined in Raw 264.7 cells using Western blottings. When Raw 264.7 cells were treated with 7-MSI (1 μg/ml) in the presence of LPS (1 μg/ml) for 15 or 30 min, the expression levels of p-p38, p-JNK, and p-ERK were increased by approximately 89%, 829%, and 1,486%, respectively, compared to those of control cells (Fig. 21). These results further suggest that 7-MSI can also induce up-regulation of the MAPK signaling pathway in Raw 264.7 cells.

III-14. Activation of cellular autophagy by 7-MSI in Raw 264.7 cells

When Raw 264.7 cells were treated with 7-MSI (1 μg/ml) in the presence of LPS (1 μg/ml) for 30 min or 1 h, the expression levels of Beclin-1, Atg12, and LC3 was increased by approximately 377%, 77%, and 27%, respectively, compared to those of the non-treated control, as revealed by Western blottings (Fig. 22). However, the expression level of phospho-mTOR was decreased by about 34% with 7-MSI treatment. These results showed that 7-MSI can induce the up-regulation of the autophagy-related proteins in Raw 264.7 cells.

Collectively, all results obtained by this study suggest that 1) 7-MSI can be a inhibitor of tyrosinase, and inhibit melanogenesis by suppressing the expression of MITF, tyrosinase, and TRP-1 (Fig. 23); 2) it can suppress the production of pro-inflammatory cytokines by inhibiting the activation of NF-κB (Fig. 24); 3) it can

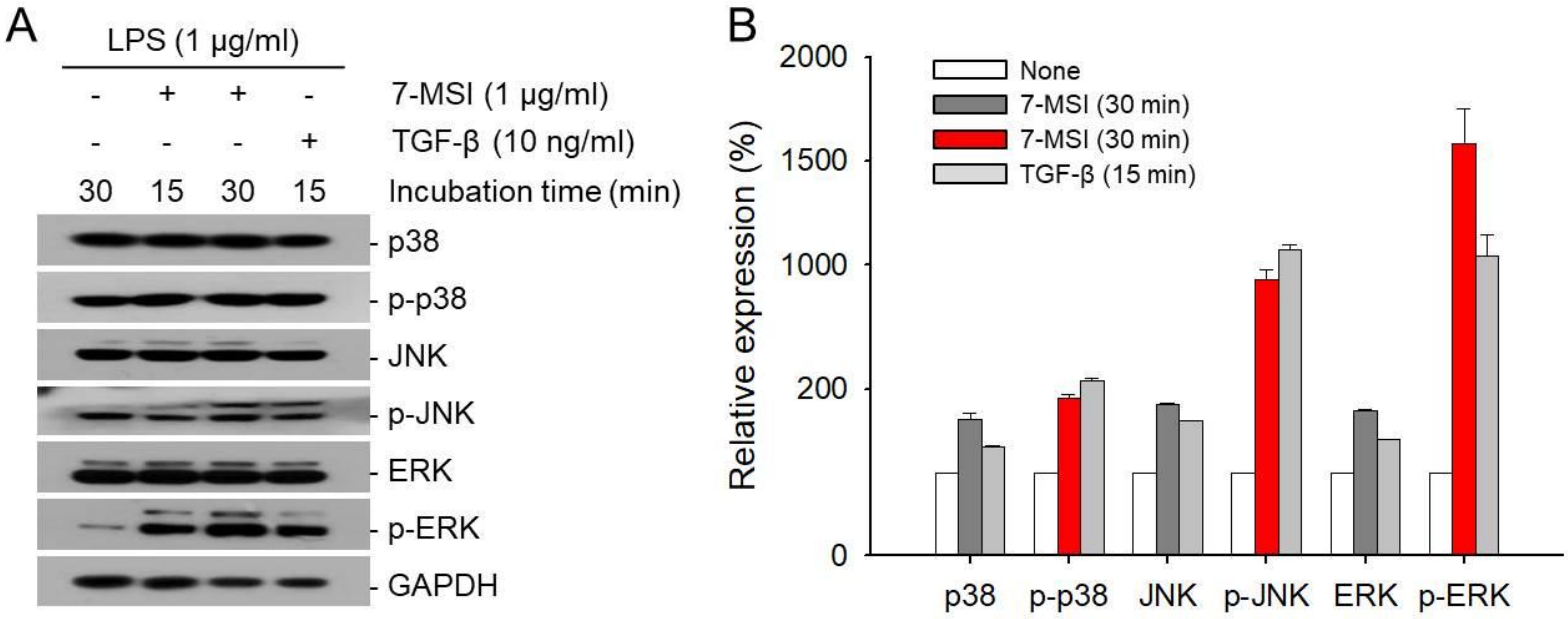

Fig. 21. Activation of the MAPK signaling pathway by 7-MSI in Raw 264.7 cells. (A) Raw 264.7 cells were treated with 7-MSI (1 μg/ml) in the presence of LPS (1 μg/ml) for 15 or 30 min. Proteins from each sample were separated by SDS-PAGE and Western blottings were performed with antibodies raised against p38, p-p38, JNK, p-JNK, ERK, p-ERK, and GAPDH. TGF-β (10 ng/ml) was used as a positive control. (B) Histograms showing the relative expression rates. Each value was calculated as the ratio of signal intensity, compared to that of GAPDH.

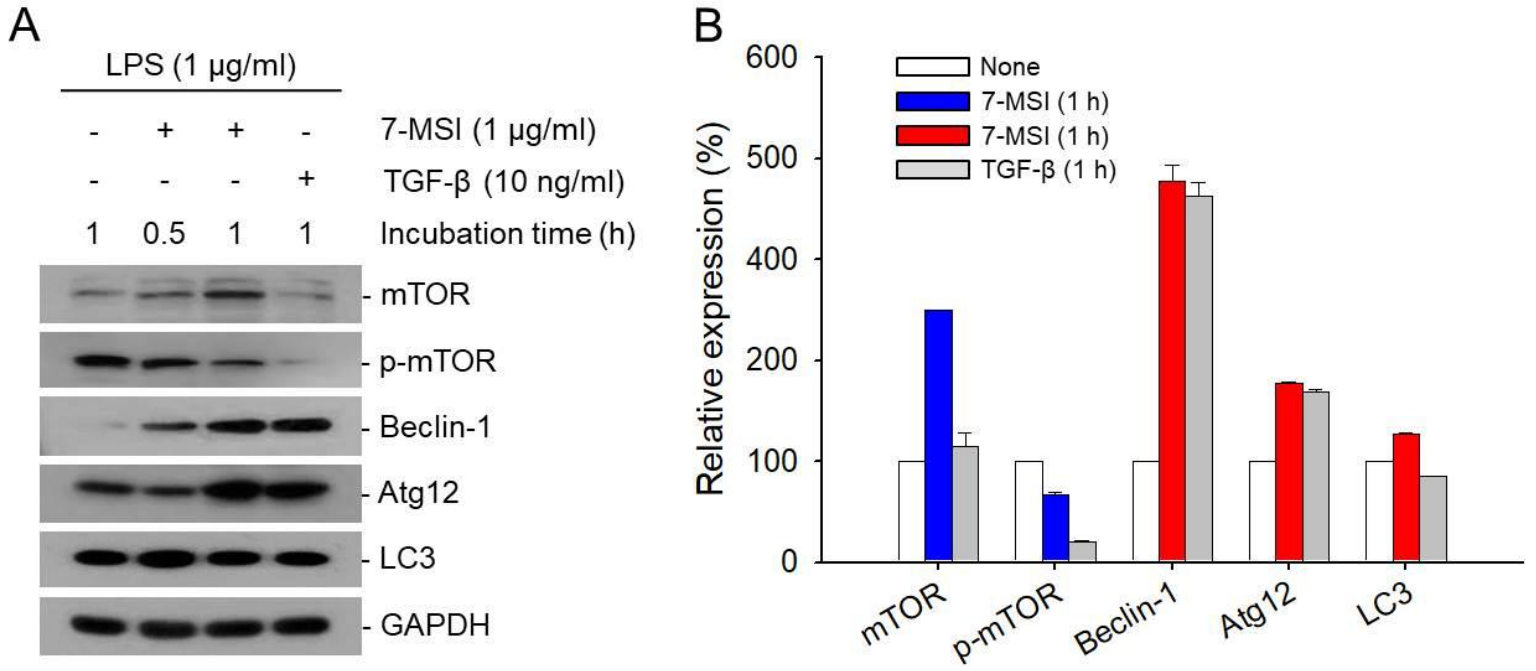

Fig. 22. Activation of cellular autophagy by 7-MSI in Raw 264.7 cells. (A) Raw 264.7 cells were treated with 7-MSI (1 μg/ml) in the presence of LPS (1 μg/ml) for 30 min or 1 h. Proteins from each sample were separated by SDS-PAGE and Western blottings were performed with antibodies raised against mTOR, p-mTOR, Beclin-1, Atg12, LC3, and GAPDH. TGF-β (10 ng/ml) was used as a positive control. (B) Histograms showing the relative expression rates. Each value was calculated as the ratio of signal intensity, compared to that of GAPDH.

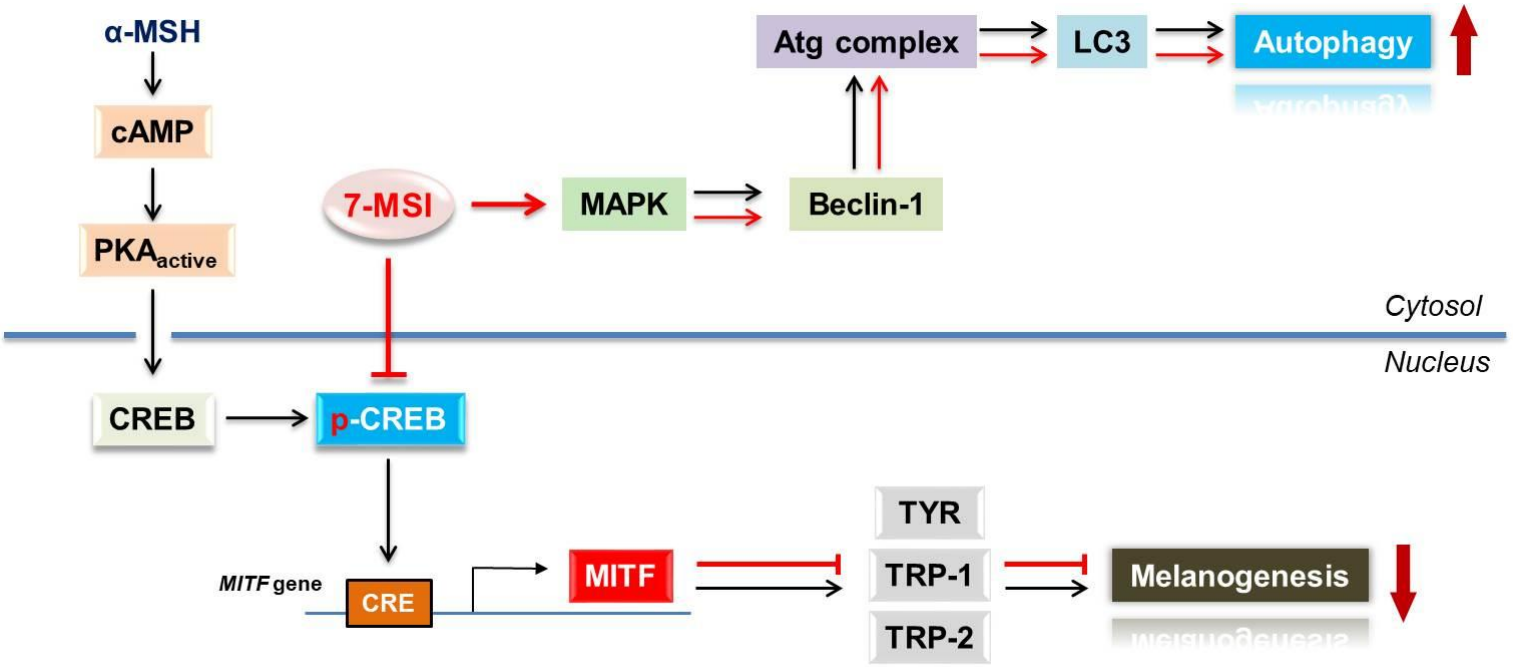

Fig. 23. Possible activation of cellular autophagy and inhibition of melanogenesis by 7-MSI in B16-F1 cells. 7-MSI can induce the activation of autophagy system and leads the degradation of MITF that down-regulates the expression of melanogenesis-associated proteins such as tyrosinase and TRP1/2.

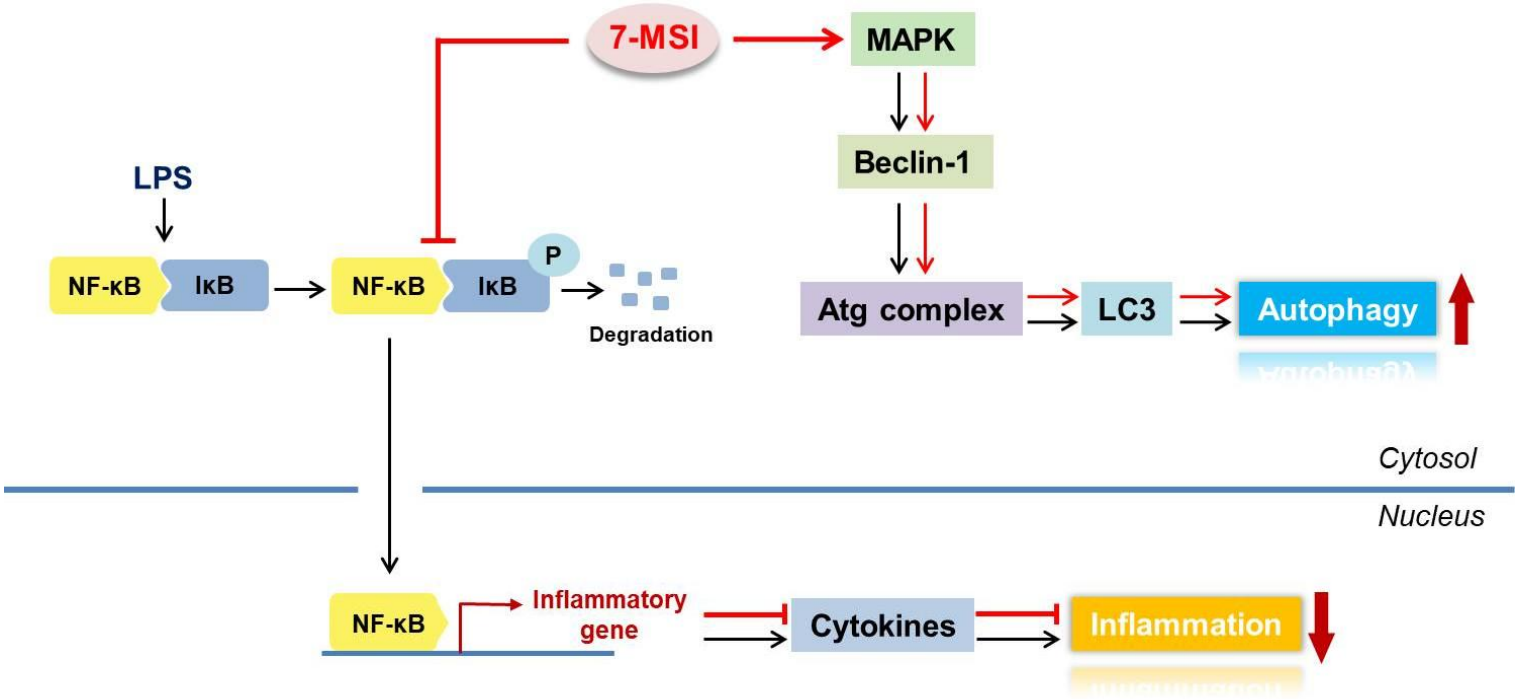

Fig. 24. Possible activation of cellular autophagy and inhibition of inflammatory pathway by 7-MSI in Raw 264.7 cells. 7-MSI can activate MAPK signalling pathway, which induces the activation of autophagy system. In addition, 7-MSI can inhibit the phosphorylation of IκB and in turn down-regulate the expression of pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α.

activate the cellular autophagy by up-regulating autophagy-related proteins, including Beclin-1, Atg12, and LC3, through by inhibiting the expression level of mTOR in both melanoma cells and macrophages. Therefore, the results demonstrate that 7-MSI has a potential property capable of alleviating melasma in skin and also can be an interesting agent to be developed as a cosmetic compound showing skin whitening and anti-inflammatory effects.

Ⅳ. 초록

배양 포유동물 세포에서 미백 및 염증반응에 미치는 7-methylsulfonylheptyl isothiocyanate의 영향

김 아 주
지도교수 : 이 정 섭
생명과학과
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피부노화(skin aging)는 피부의 구조적 및 기능적 특성이 퇴행하는 현상으로 유전적 요인, 호르몬 변화, 대사과정, 식습관, 흡연 및 자외선에 의하여 유발된다. 이러한 요인들에 의한 과도한 자극은 과색소침착, 염증, 주름 및 피부암 등을 유발하기도 한다. Autophagy(자가소화작용 또는 자식작용이라고도 함)는 세포에서 기능을 다한 세포소기관들 또는 단백질들을 제거하는 과정으로 세포의 노화 및 분화과정에서도 중요한 역할을 하는 것으로 알려져 있다. 본 연구에서 사용한 7-methylsulfonylheptyl isothiocyanate(7-MSI; 또는 1-isothiocyanato-7-(methylsulfonyl) heptane이라고 함)는 콜리플라워, 양배추, 케일 및 브로콜리 등과 같은 십자화과 식물에서 풍부하게 존재하는 피토케미컬(phytochemical)로써 박테리아 및 곰팡이 등과 같은 병원성 인자들에 대한 방어 작용을 지녔을 뿐만 아니라 항염증 효과도 지닌 것으로 알려져 있다. 그러나 피부 미백(skin-whitening) 및 항-노화(anti-aging)에 미치는 7-MSI의 영향에 관한 연구결과는 아직 보고된 것이 없다. 본 연구는 7-MSI가 흑색종 세포(B16-F1)에서 멜라닌 생성에 미치는 영향, 대식세포(Raw 264.7)에서 염증성 사이토카인들의 생성에 미치는 영향, 그리고 이 두 가지 세포 모두에서 7-MSI의 autophagy 활성화 효능 등을 분석함으로써 7-MSI의 미백, 항-노화 및 항-염증 효과를

규명하고자 수행되었다. α-melanocyte-stimulating hormone(α-MSH; 10 nM)을 처리하여 멜라닌 생성을 촉진 시킨 B16-F1 세포에 7-MSI (0.5 μg/ml)를 73시간 동안 처리한 결과, 멜라닌 생성이 약 63% 저해됨을 확인하였다. 또한, B16-F1 세포의 멜라닌 생성양상을 항-MITF(microphthalmia-associated transcription factor), 항-tyrosinase 및 항-TRP1(tyrosinase-related protein 1) 항체를 사용한 Western blotting으로 분석하였다. 그 결과, 7-MSI (1 μg/ml)를 처리한 경우, MITF, tyrosinase 및 TRP1의 발현이 모두 감소됨을 확인하였다. 이러한 결과는 7-MSI가 tyrosinase와 TRP1의 발현을 감소시켜 직접적으로 멜라닌 합성을 억제할 수 있음을 시사한다. 또한 본 연구에서는 염증반응에 대한 7-MSI의 영향을 규명하기 위하여 Raw 264.7 세포에 7-MSI (1 μg/ml)를 1시간 동안 처리한 후, ELISA 분석을 통하여 TNF-α의 발현량이 32% 감소됨을 확인하였으며, 7-MSI (1 μg/ml)를 3시간 동안 처리한 후 RT-RCR을 통하여 pro-inflammatory cytokine들의 발현량이 감소됨을 확인하였다. 또한, IκB(inhibitory kappa B)의 인산화에 대한 영향을 분석하기 위하여 Raw 264.7 세포에 7-MSI (1 μg/ml)를 처리하여 항-IκBα 및 항-p-IκBα 항체를 사용한 Western blotting을 통하여 분석한 결과, IκB의 인산화가 억제 된 것을 확인하였다. 또한 NF-κB(nuclear factor kappa B)의 활성화 여부를 분석하기 위하여 Raw 264.7 세포에 7-MSI (1 μg/ml)를 1시간 동안 처리한 후, 면역 형광법을 통해 분석한 결과 NF-κB의 활성화가 감소된 것을 확인하였다. 이러한 결과는 7-MSI가 NF-κB의 활성화의 억제를 통해 Raw 264.7 세포에서 발현되는 염증성 사이토카인들의 발현에 억제 효과를 지닌다는 것을 시사한다. 또한, 본 연구에서는 MAPK(ERK 및 p38 등) 활성화에 미치는 7-MSI의 영향을 규명하기 위하여 B16-F1 세포와 Raw264.7 세포에 7-MSI (1 μg/ml)를 30분 동안 처리한 후, MAPK 유도 단백질들의 발현량을 Western blotting으로 분석하였다. 그 결과, p-p38, p-JNK 그리고 p-ERK의 단백질 발현량이 증가한 것을 확인할 수 있었다. 또한, autophagy system 활성화에 미치는 7-MSI의 영향을 규명하기 위하여 B16-F1과 Raw264.7 세포에 7-MSI (1 μg/ml)를 30분 또는 1시간 동안 처리한 후, autophagy system 유도 단백질들의 발현량을 Western blotting으로 분석하였다. 그 결과, Beclin-1, Atg12 그리고 LC3의 단백질 발현량은 증가되었으나, autophagy의

음성조절자(negative regulator)로 알려진 mTOR의 단백질 발현량은 감소됨을 확인하였다. 이러한 결과는 7-MSI가 MAPK들은 활성화 시키는 반면, mTOR의 발현은 감소시킴으로써 autophagy system을 활성화시킬 수 있음을 시사한다. 이상의 결과들은 7-MSI가 멜라닌 생성 유도 단백질들의 발현을 억제하여 멜라닌 합성을 억제 시킬 수 있을 뿐만 아니라, NF-κB의 활성화를 감소시켜 pro-inflammatory cytokine의 발현량을 감소시킴으로써 염증반응을 억제 할 수 있음을 시사하는 것이다. 또한, 피부세포의 항상성에 중요한 역할을 하는 autophagy의 활성화를 제어할 수 있음을 시사한다. 결론적으로 7-MSI는 항-염증 및 미백 치료제로 개발될 가능성을 보여주고 있다.

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