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A derivative of imidazobenzimidazole, ML106, inhibits melanin synthesis via p38 MAPK activation

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We investigated the effects of ML106 on melanogenesis in B16F10 melanoma cells. Our results showed that ML106 decreased melanin content and tyrosinase activity in a dose-dependent manner. Interestingly, ML106 did not inhibit microphthalmia-associated transcription factor (MITF) expression, but did decrease tyrosinase expression. Thus, we further investigated the expression and degradation of tyrosinase and related signal transduction pathways. Although ML106 increased glycogen synthase kinase 3β (GSK3β) activation, the level of β-catenin level was not affected. Thus, we excluded the involvement of GSK3β and β-catenin in ML106-induced hypopigmentation. However, ML106 induced the phosphorylation of p38 mitogen-activated protein kinase (MAPK), causing down-regulation of tyrosinase. Thus, we next investigated whether tyrosinase down-regulation was due to proteasomal degradation by p38 MAPK activation. We found that ML106-induced tyrosinase down-regulation was restored by MG132, a proteasome inhibitor. Thus, we propose that ML106 has hypopigmentary activity through tyrosinase degradation *via* p38 MAPK phosphorylation.

1. Introduction

Melanocytes produce melanin in the basal layer of the epidermis, and surrounding keratinocytes receive and distribute melanosomes, thereby regulating human skin color (Yamaguchi et al. 2007; Yamaguchi and Hearing 2009). Melanin synthesis occurs within melanosomes that contain three major melanogenic enzymes: tyrosinase, tyrosinase-relate protein 1 (TRP1), and TRP2 (Uyen et al. 2008). Tyrosinase is a key enzyme involved in melanin synthesis, and microphthalmia-associated transcription factor (MITF) is an important transcription factor that regulates tyrosinase (Takeda et al. 2000). Tyrosinase has been shown to undergo post-translational degradation. Tyrosinase down-regulation by degradation can result in decreased melanin synthesis in melanocytes (Francis et al. 2003; Park et al. 2009). Degradation of abnormal proteins misfolded in the endoplasmic reticulum (ER) and short-lived proteins occurs selectively *via* the ubiquitin-proteasome pathway. Proteasomes are multicatalytic proteinase complexes located within cells that selectively degrade ubiquitinated proteins (Hiller et al. 1996; Hochstrasser 1996).

Abbreviations: GSK3β, glycogen synthase kinase 3β; MAPK, mitogen-activated protein kinase; MC1R, melanocortin 1 receptor; MITF, microphthalmia-associated transcription factor; α-MSH, α-melanocyte stimulating hormone; TRP, tyrosinase-relate protein; USF-1, upstream stimulating factor-1.

There are discrepancies in the reported effects of glycogen synthase kinase 3β (GSK3β) on melanogenesis. First, activation of the Wnt pathway results in activation of Frizzled receptors, inhibition of GSK3β, and stimulation of β-catenin accumulation (Bellei et al. 2008; Wu et al. 2003). Accumulated *β*-catenin forms a complex the lymphoid-enhancing factor/T-cell factor (LEF/TCF), and formation of this complex leads to upregulation of MITF (Widlund et al. 2002; Wu et al. 2003). Thus, the GSK3β pathway can control melanogenesis *via* β-catenin regulation. Second, activation of Akt signaling plays a role in the regulation of melanogenesis through phosphorylation (inactivation) of GSK3β on serine 9 (Khaled et al. 2002). Therefore, GSK3β inactivation inhibits MITF phosphorylation at serine 298 and decreases MITF biding affinity to the tyrosinase promoter, leading to (down-regulation) of tyrosinase expression and inhibition of melanogenesis (Khaled et al. 2002). Thus, the GSK3β pathway can control melanogenesis *via* regulation of MITF phosphorylation.

Mitogen-activated protein kinases (MAPKs), which include extracellular signal-regulated protein kinase (ERK), p38 MAPK, and c-jun N-terminal kinase (JNK), are known to be involved in cell proliferation, differentiation, stress response, and apoptosis (Harper and LoGrasso 2001; Kim et al. 2007). It was reported that the p38 MAPK signaling pathway is also involved in stress-induced melanogenesis (Corre et al. 2004). Moreover, p38 MAPK activated upstream stimulating factor-1 (USF-1) is necessary for UV-induced tyrosinase expression

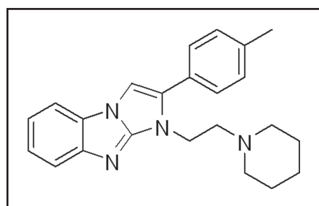

Fig. 1: Chemical structure of ML106.

(Corre and Galibert 2005; Park et al. 2010). However, it has also been reported that tyrosinase degradation is related to p38 MAPK activation (Bellei et al. 2010). Thus, the role of p38 MAPK in melanogenesis needs to be investigated in greater detail.

To developed a new skin-whitening agent, we screened 35 compounds from our in-house chemical library. Among these, we selected ML106 (Fig. 1) for further study because it had the best hypopigmentary effects. In the present study, we investigated the effects of ML106 on melanin synthesis and tyrosinase activity using B16 mouse melanoma cells. In addition, we examined the molecular mechanisms by which ML106 regulates melanogenesis.

2. Investigations and results

2.1. *Effects of ML106 on cell viability and melanin synthesis*

B16F10 cells were treated with ML106 at various concentrations ranging from 0.1 to 10 μM for 24 h. Cell viability was determined using a crystal violet assay. As shown in Fig. 2A, ML106 was not cytotoxic at concentrations of 0.1–10 μM. Therefore, we used concentrations ranging from 0.1 to 10 μM of ML106 for further experiments. First, we measured melanin content after ML106 treatment for 3 days in the presence of α-MSH (1 μM). Our results showed that ML106 treatment decreased melanin synthesis in a dose-dependent manner (Fig. 2B). Next, we examined tyrosinase activity in cells treated with ML106 and the direct effect of ML106 on tyrosinase activity using mushroom tyrosinase. We found that ML106 treatment decreased α-MSH-stimulated tyrosinase activity significantly (Fig. 2C).

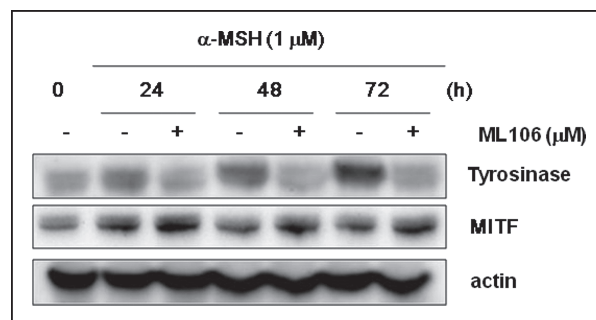

Fig. 3: Effects of ML106 on MITF and tyrosinase protein expression. B16F10 cells were treated with ML106 (5 μM) in the presence of α-MSH (1 μM) for 24–72 h. Whole cell lysates were then subjected to western blot analysis using antibodies against MITF and tyrosinase. Equal protein loading was confirmed using anti-actin antibody.

However, ML106 did not inhibit tyrosinase activity directly (Fig. 2D). These results suggest that the hypopigmentary activity of ML106 may result from inhibition of the melanogenic pathway involving tyrosinase expression, but not from the direct inhibition of tyrosinase.

2.2. *Effects of ML106 on MITF and tyrosinase protein level*

Next, we performed western blot analysis to examine MITF and tyrosinase protein levels in α-MSH-stimulated B16F10 cells treated with ML106 (5 μM). Interestingly, tyrosinase protein expression decreased clearly from 24 h to 72 h, while MITF levels did not change (Fig. 3). These data suggest that ML106 may induce tyrosinase protein degradation.

2.3. *Effects of ML106 on signaling pathways involved in melanogenesis*

Several reports have indicated that Akt and GSK3β phosphorylation regulate melanogenesis. Accordingly, we examined whether ML106 (5 μM) induced Akt and GSK3β phosphorylation in a time course experiment. As shown in Fig. 4A, ML106 inhibited Akt and GSK3β phosphorylation. The level

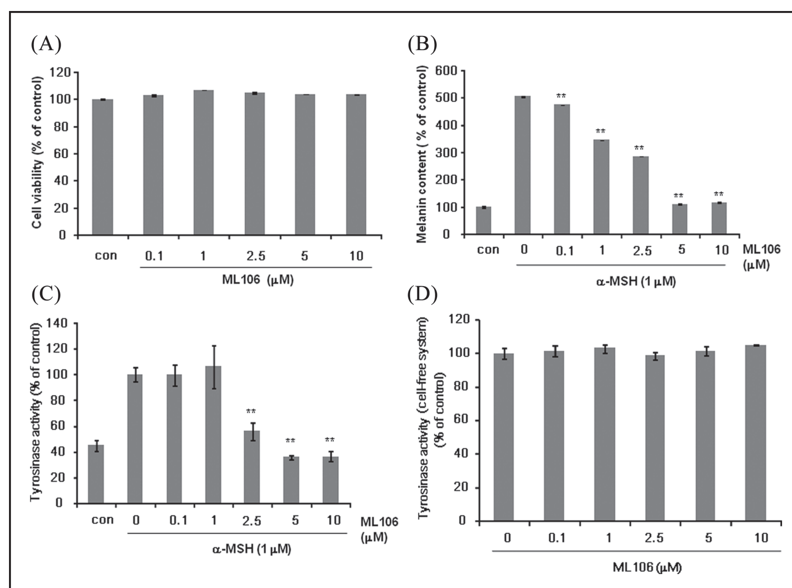

Fig. 2: Effects of ML106 on melanogenesis in B16F10 cells. (A) After serum starvation for 24 h, cells were treated with 0.1–10 μM ML106 for 24 h in serum-free media. Cell viability was determined by the crystal violet assay. Cells were treated with ML106 (0.1–10 μM) in the presence of α-MSH (1 μM) for 3 days. Melanin content (B), tyrosinase activity (C), and tyrosinase activity in a cell-free system (D) were measured, as described in the Experimental section. Results shown are the averages of triplicate experiments ± SD. ** $P < 0.01$ versus α-MSH-treated controls.

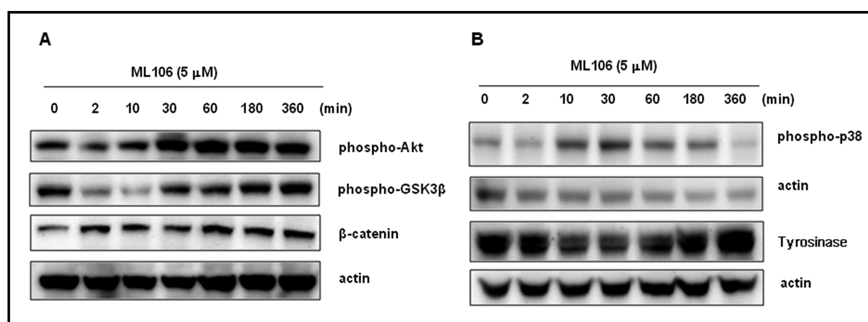

Fig. 4: Effects of ML106 on signal transduction pathways in B16F10 cells. After 24 h of serum starvation, cells were treated with 5 μM ML106 for the times indicated. (A) Whole cell lysates were then subjected to western blot analysis using antibodies against phospho- specific Akt, phospho-specific GSK3β, and β-catenin. (B) Western blot analysis was performed using antibodies against phospho-specific p38 MAPK and tyrosinase. Equal protein loading was confirmed using anti-actin antibody.

of β-catenin level did not change. In contrast, we found that ML106 induced phosphorylation of p38 MAPK. The level of p38 MAPK peaked after 30 min (Fig. 4B). In accordance with the increase in p38 MAPK phosphorylation, the tyrosinase level decreased (Fig. 4B).

2.4. ML106 decreases tyrosinase protein expression via proteasomal degradation

Next, we evaluated whether the ML106-induced down-regulation of tyrosinase was due to post-translational degradation of tyrosinase. To accomplish this, we used a proteasome inhibitor, MG132. To inhibit protein synthesis, cycloheximide was added to B16F10 cells. Cells were pretreated with 150 nM of MG132 for 3 h followed by 30 min of ML106 treatment. Tyrosinase levels were then analyzed using western blot analysis. The ML106-induced decrease in tyrosinase level was restored by MG132 pretreatment, but p38 phosphorylation was not affected (Fig. 5). These data indicate that tyrosinase down-regulation is due to proteasomal degradation.

3. Discussion

Human skin pigmentation is regulated by complex and intricate interactions between melanocytes and keratinocytes in the epidermis (Choi et al. 2010). Many studies have focused on developing new therapeutic agents to prevent hyperpigmentation. Therefore, we investigated whether ML106 inhibited hyperpigmentation induced by α-MSH in B16F10 melanoma cells. Moreover, we focused the mechanistic basis of ML106 regulation of melanogenesis.

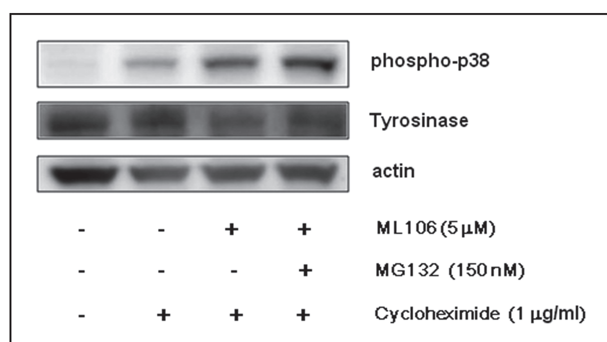

Fig. 5: Effects of inhibition of proteasomal degradation on melanogenesis. Following serum starvation for 24 h, cells were pretreated with 1 μg/ml of cycloheximide for 1 h and with 150 nM MG132 for 3 h as indicated, after which they were treated with 5 μM ML106 for 30 min. Whole cell lysates were then subjected to western blot analysis with antibodies against phospho-specific p38 MAPK or tyrosinase. Equal protein loading was confirmed using anti-actin antibody.

MITF is a major transcriptional regulator of melanogenic enzymes such as tyrosinase and TRP1 (Bentley et al. 1994; Busca and Ballotti 2000; Kim et al. 2003). Several reports have suggested that Akt inhibition and subsequent GSK3β activation stimulate MITF biding to the M-box of the tyrosinase promoter *via* MITF phosphorylation at serine 298, thereby up-regulating melanogenic protein expression and inducing melanogenesis (Kang et al. 2011; Lin et al. 2012; Takeda et al. 2000). For example, we reported that GSK3β inactivation by KHG25855 reduced MITF binding affinity and subsequently decreased tyrosinase expression (Kim et al. 2011). However, we showed in the present study that ML106 did not stimulate melanin synthesis, even though it inhibited Akt and activated GSK3β. It has also been reported that activation of GSK3β can cause β-catenin degradation, leading to decreased melanin synthesis (Bellei et al. 2008; Wu et al. 2003). However, the level of β-catenin was not altered by ML106 treatment. Instead, ML106 reduced tyrosinase protein expression without decreasing MITF protein expression (Fig. 3). These results indicate that GSK3β activation is not related to ML106-induced hypopigmentation.

As mentioned in the introduction, the ERK and p38 MAPK pathways are involved in the regulation of melanin synthesis (Hirata et al. 2007; Smalley and Eisen 2000; Tsang et al. 2012). In our experiment, however, ERK levels did not change after ML106 treatment (data not shown). Rather, ML106 induced p38 MAPK phosphorylation. On the one hand, p38 MAPK has been shown to increase tyrosinase expression with the concomitant accumulation of melanin (Jiang et al. 2009; Lee et al. 2011). On the other hand, the phosphorylation of p38 MAPK is related to tyrosinase degradation (Bellei et al. 2010). The major pathways of protein degradation in eukaryotic cells include proteolysis in proteasomes and lysosomes. Tyrosinase could be degraded in proteasomes *via* ER-associated protein degradation after the post-Golgi stage, as reported in linoleic acid-treated melanoma cells (Ando et al. 2007, 2006), or it could be degraded *via* the endosomal/lysosomal degradation system, as reported in phenylthiourea-treated melanoma cells (Hall and Orlow 2005). To investigate tyrosinase degradation, we treated cells with the proteasome inhibitor MG132 and evaluated changes in the levels of tyrosinase expression by western blot analysis. As shown in Fig. 5, the ML106-induced decrease in tyrosinase level was restored by treatment of cells with MG132. These results indicate that ML106-induced tyrosinase down-regulation is due to tyrosinase degradation by the proteasomal pathway.

In summary, our results suggest that ML106 functions as an inhibitor of melanin synthesis in B16F10 cells. Furthermore, ML106-induced tyrosinase down-regulation is associated with p38 MAPK phosphorylation and subsequent tyrosinase degradation. These results suggest that ML106 is a new potential skin whitening agent that functions by decreasing tyrosinase.

4. Experimental

4.1. Materials

ML106 was synthesized at the Medicinal Chemistry Laboratory (Chung-Aug University, Seoul, Korea). The chemical name of ML106 is 2-(4-methylphenyl)-1-[2-(1-piperidinyl)ethyl]-1*H*-Imidazo[1,2-*a*]benzimidazole. The structure of ML106 is shown in Fig. 1. ML106 was dissolved in DMSO and stored at -20°C as a stock solution (10 mM). Synthetic melanin, 3,4-dihydroxy-L-phenylalanine (L-DOPA), and mushroom tyrosinase were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). SB203580, MG132, and cycloheximide were purchased from Calbiochem (Darmstadt, Germany). Antibodies specific for phospho-p38 MAPK (#9211), phospho-Akt (ser473, #9271), phospho-GSK3β (#9336), and β-catenin (#9581) were purchased from Cell Signaling Technology (Beverly, MA, USA). Antibodies specific for tyrosinase (C-19) and actin (I-19) were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA), and microphthalmia Ab-1 (C5, MS-771-P0) was obtained from NeoMarkers (Fremont, CA, USA).

4.2. Cell culture

B16F10 murine melanoma cells were obtained from the Korean Cell Line Bank (Seoul, Korea). Cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) fetal bovine serum (FBS; Hyclone, Logan, UT, USA) and 1% (v/v) penicillin-streptomycin in 5% CO_2 at 37 °C.

4.3. Cell viability assay

Cell viabilities were determined using a crystal violet assay. After incubating cells with ML106 for 24 h, media was removed, and cells were stained with 0.1% crystal violet in 10% ethanol for 5 min at room temperature. Cells were then rinsed four times with distilled water, and the crystal violet retained by adherent cells was extracted with 95% ethanol. Absorbance was determined at 590 nm using an ELISA reader (VERSAMax; Molecular Devices, Sunnyvale, CA, USA).

4.4. Measurement of melanin content

B16F10 cells were incubated at a density of $1x10^5$ cells in 6-well plates overnight. α-MSH (1 μM) was then added and cells were treated with increasing concentrations of ML106 in phenol red-free DMEM for 3 days. Aliquots of media (200 μL) were then placed in 96-well plates and the optical density (OD) of each culture well was measured using an ELISA reader at 400 nm. Cell numbers were then counted using a hemocytometer. Melanin production was expressed as a percentage of the untreated control.

4.5. Tyrosinase activity

Tyrosinase activity was assayed as DOPA oxidase activity. B16F10 cells were incubated at a density of $1x10^5$ cells in 6-well plates, and incubated with ML106 in DMEM for 3 days. Cells were washed with PBS and lysed with lysis buffer (0.1 M phosphate buffer [pH 6.8] containing 1% Triton X-100). They were then disrupted by freeze-thawing, and lysates were clarified by centrifugation at 13,000 rpm for 30 min. After quantifying protein content using a protein assay kit (Bio-Rad, Hercules, CA, USA), cell lysates were adjusted to the same amount of protein with a lysis buffer, 90 μl of each lysate was placed in a well of a 96-well plate, and 10 μl of 10 mM L-DOPA was added. Control wells contained 90 μl lysis buffer and 10 μl 10 mM L-DOPA. After incubation at 37 °C for 20 min, dopachrome formation was monitored by measuring absorbance at 475 nm using an ELISA reader.
A cell-free assay system was used to investigate the direct effect of ML106 on tyrosinase activity. Seventy microliters of phosphate buffer containing ML106 was mixed with 20 μl of 10 μg/ml mushroom tyrosinase, and 10 μl of 10 mM L-DOPA was then added. Following incubation at 37 °C, absorbance was measured at 475 nm.

4.6. Western blot analysis

Cells were lysed in cell lysis buffer (62.5 mM Tris-HCl [pH 6.8], 2% SDS, 5% β-mercaptoethanol, 2 mM phenylmethylsulfonyl fluoride, and protease inhibitors [Complete^{TM}; Roche, Mannheim, Germany], 1 mM Na_3VO_4, 50 mM NaF, and 10 mM EDTA). Twenty microgram of protein per lane was separated by SDS-polyacrylamide gel electrophoresis and blotted onto PVDF membranes, which were then saturated with 5% dried milk in Tris-buffered saline containing 0.5% Tween 20. Blots were then incubated with the appropriate primary antibodies at a dilution of 1:1000, and further incubated with horseradish peroxidase-conjugated secondary antibodies. Bound antibodies were detected using an enhanced chemiluminescence plus kit (Amersham International, Little Chalfont, UK).

4.7. Statistics

Significance of differences between treatment groups was assessed by Student's t-test. *P* values < 0.01 were considered significant.

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References

Ando H, Kondoh H, Ichihashi M, Hearing VJ (2007) Approaches to identify inhibitors of melanin biosynthesis via the quality control of tyrosinase. J Invest Dermatol 127: 751–761.

Ando H, Wen ZM, Kim HY, Valencia JC, Costin GE, Watabe H, et al. (2006) Intracellular composition of fatty acid affects the processing and function of tyrosinase through the ubiquitin-proteasome pathway. Biochem J 394: 43–50.

Bellei B, Flori E, Izzo E, Maresca V, Picardo M (2008) GSK3beta inhibition promotes melanogenesis in mouse B16 melanoma cells and normal human melanocytes. Cell Signal 20: 1750–1761.

Bellei B, Maresca V, Flori E, Pitisci A, Larue L, Picardo M (2010) p38 Regulates pigmentation via proteasomal degradation of tyrosinase. J Biol Chem 285: 7288–7299.

Bentley NJ, Eisen T, Goding CR (1994) Melanocyte-specific expression of the human tyrosinase promoter: activation by the microphthalmia gene product and role of the initiator. Mol Cell Biol 14: 7996–8006.

Busca R, Ballotti R (2000) Cyclic AMP a key messenger in the regulation of skin pigmentation. Pigment Cell Res 13: 60–69.

Choi W, Wolber R, Gerwat W, Mann T, Batzer J, Smuda C, et al. (2010) The fibroblast-derived paracrine factor neuregulin-1 has a novel role in regulating the constitutive color and melanocyte function in human skin. J Cell Sci 123: 3102–3111.

Corre S, Galibert MD (2005) Upstream stimulating factors: highly versatile stress-responsive transcription factors. Pigment Cell Res 18: 337–348.

Corre S, Primot A, Sviderskaya E, Bennett DC, Vaulont S, Goding CR, et al. (2004) UV-induced expression of key component of the tanning process, the POMC and MC1R genes, is dependent on the p-38-activated upstream stimulating factor-1 (USF-1). J Biol Chem 279: 51226–51233.

Francis E, Wang N, Parag H, Halaban R, Hebert DN (2003) Tyrosinase maturation and oligomerization in the endoplasmic reticulum require a melanocyte-specific factor. J Biol Chem 278: 25607–25617.

Hall AM, Orlow SJ (2005) Degradation of tyrosinase induced by phenylthiourea occurs following Golgi maturation. Pigment Cell Res 18: 122–129.

Harper SJ, LoGrasso P (2001) Signalling for survival and death in neurones - The role of stress-activated kinases, JNK and p38. Cellular Signal 13: 299–310.

Hiller MM, Finger A, Schweiger M, Wolf DH (1996) ER degradation of a misfolded luminal protein by the cytosolic ubiquitin-proteasome pathway. Science 273: 1725–1728.

Hirata N, Naruto S, Ohguchi K, Akao Y, Nozawa Y, Iinuma M, Matsuda H (2007) Mechanism of the melanogenesis stimulation activity of (-)-cubebin in murine B16 melanoma cells. Bioorg Med Chem 15: 4897–4902.

Hochstrasser M (1996) Ubiquitin-dependent protein degradation. Ann Rev Genet 30: 405–439.

Jiang Z, Xu J, Long M, Tu Z, Yang G, He G (2009) 2,3,5,4'-Tetrahydroxystilbene-2-O-beta-D-glucoside (THSG) induces melanogenesis in B16 cells by MAP kinase activation and tyrosinase upregulation. Life Sci 85: 345–350.

Kang YG, Choi EJ, Choi Y, Hwang JK (2011) 5,7-dimethoxyflavone induces melanogenesis in B16F10 melanoma cells through cAMP-dependent signalling. Exp Dermatol 20: 445–447.

Khaled M, Larribere L, Bille K, Aberdam E, Ortonne JP, Ballotti R, Bertolotto C (2002) Glycogen synthase kinase 3beta is activated by cAMP and plays an active role in the regulation of melanogenesis. J Biol Chem 277: 33690–33697.

Kim DS, Hwang ES, Lee JE, Kim SY, Kwon SB, Park KC (2003) Sphingosine-1-phosphate decreases melanin synthesis via sustained ERK activation and subsequent MITF degradation. J Cell Sci 116: 1699–1706.

Kim DS, Park SH, Kwon SB, Na JI, Huh CH, Park KC (2007) Additive effects of heat and p38 MAPK inhibitor treatment on melanin synthesis. Arch Pharm Res 30: 581–586.

Kim SY, Hahn HG, Nam KD, Park KC, Yun HY, Baek KJ, Kwon NS, Kim DS (2011) A derivative of 2-aminothiazole inhibits melanogene-

sis in B16 mouse melanoma cells via glycogen synthase kinase 3beta phosphorylation. J Pharm Pharmacol 63: 1031–1036.

Lee SA, Son YO, Kook SH, Choi KC, Lee JC (2011) Ascorbic acid increases the activity and synthesis of tyrosinase in B16F10 cells through activation of p38 mitogen-activated protein kinase. Arch Dermatol Res 303: 669–678.

Lin YS, Chuang MT, Chen CH, Chien MY, Hou WC (2012) Nicotinic acid hydroxamate downregulated the melanin synthesis and tyrosinase activity through activating the MEK/ERK and AKT/GSK3beta signaling pathways. J Agric Food Chem 60: 4859–4864.

Park SH, Kim DS, Lee HK, Kwon SB, Lee S, Ryoo IJ, Kim WG, Yoo ID, Pove KC (2009) Long-term suppression of tyrosinase by terrein via tyrosinase degradation and its decreased expression. Exp Dermatol 18: 562–566.

Park SY, Kim YH, Park G, Lee SJ (2010) Beta-carboline alkaloids harmaline and harmalol induce melanogenesis through p38 mitogen-activated protein kinase in B16F10 mouse melanoma cells. BMB Rep 43: 824–829.

Smalley K, Eisen T (2000) The involvement of p38 mitogen-activated protein kinase in the alpha-melanocyte stimulating hormone (alpha-MSH)-induced melanogenic and anti-proliferative effects in B16 murine melanoma cells. FEBS Lett 476: 198–202.

Takeda K, Takemoto C, Kobayashi I, Watanabe A, Nobukuni Y, Fisher DE, Tachibana M (2000) Ser298 of MITF, a mutation site in Waardenburg syndrome type 2, is a phosphorylation site with functional significance. Human Mol Genet 9: 125–132.

Tsang TF, Ye Y, Tai WCS, Chou GX, Leung AKM, Yu ZL, Hsiao WL (2012) Inhibition of the p38 and PKA signaling pathways is associated with the anti-melanogenic activity of Qian-wang-hong-bai-san, a Chinese herbal formula, in B16 cells. J Ethnopharmacol 141: 622–628.

Uyen LDP, Nguyen DH, Kim EK (2008) Mechanism of skin pigmentation. Biotechnol Bioproc Engin 13: 383–395.

Widlund HR, Horstmann MA, Price ER, Cui J, Lessnick SL, Wu M, He X, Fisher DE (2002) Beta-catenin-induced melanoma growth requires the downstream target Microphthalmia-associated transcription factor. J Cell Biol 158: 1079–1087.

Wu J, Saint-Jeannet JP, Klein PS (2003) Wnt-frizzled signaling in neural crest formation. Trends Neurosci 26: 40–45.

Yamaguchi Y, Brenner M, Hearing VJ (2007) The regulation of skin pigmentation. J Biol Chem 282: 27557–27561.

Yamaguchi Y, Hearing VJ (2009) Physiological factors that regulate skin pigmentation. Biofactors 35: 193–199.