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Inhibition Effects of Mangosenone F from *Garcinia mangostana* on Melanin Formation in B16F10 Cells

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Supporting Information

ABSTRACT: Melanogenesis can be controlled by tyrosinase inhibition or by blocking the maturation processes of tyrosinase and its related proteins. Mangostenone F was isolated from the seedcases of *Garcinia mangostana*. Mangostenone F was shown to be inactive against tyrosinase (IC_{50} > 200 μM) but was a potent α-glucosidase inhibitor in vitro (IC_{50} = 21.0 μM). Mangostenone F was found to inhibit production of melanin in the mouse melanoma cell line B16F10. Importantly, unlike most glycosidase inhibitors, mangostenone F displayed very low cytotoxicity (EC_{50} > 200 μM). The Western blot for expression levels of proteins involved in melanogenesis showed that mangostenone F down-regulated tyrosinase and TRP-2 expression. Treating B16F10 cells with mangostenone F significantly increased the susceptibility of tyrosinase to endoglycosidase H digestion, indicating that tyrosinase was unable to mature fully and pass to the *trans*-golgi apparatus. Consistent with these data, in lysate assays, mangostenone F was shown to be a better inhibitor of α-glucosidases than deoxynojirimycin, a representative glycosidase inhibitor.

KEYWORDS: *mangostenone F, α-glucosidase, endo H, cytotoxicity, melanogenesis*

INTRODUCTION

To protect against UV exposure, and to protect the body from the resulting compounds generated, such as reactive oxygen species, the skin produces melanin.[1] Melanin is a unique pigmented biopolymer produced by melanocytes, which are distributed in the basal layer of the epidermis in animals.[2] Melanin synthesis is typically initiated by ultraviolet (UV) light. However, excessive pigmentation from overproduction of melanin is the cause of numerous skin problems including age spots and melasma.[3] The key enzyme involved in melanin production is tyrosinase.[4] Many studies have focused on direct inhibition of tyrosinase. However, the inhibition of tyrosinase N-glycosylation is an alternative way to affect melanin production.[5] This reduces tyrosinase processing and lowers tyrosinase activity because tyrosinase cannot reach full maturity and remains inactive.

Tyrosinase maturation involves transportation of tyrosinase from the endoplasmic reticulum (ER) via the golgi to the melanosome, where melanin biosynthesis occurs.[6] Procession of tyrosinase to its final destination occurs via several enzymatic steps initiated in the ER. α-Glucosidase I removes the outer α-1,2-linked glucose residue from an asparagine-linked oligosaccharide on tyrosinase. There follows removal of two more glucose residues in the ER by α-glucosidase II.[7] These two steps are required for tyrosinase to leave the ER and enter the golgi via a COPII-mediated process.[8] Inhibition of α-glucosidase I or II, for instance, by the imino sugar deoxynojirimycin or its N-alkylated derivatives, has been extensively characterized in cellular and viral systems.[9] In the presence of these compounds, most *N*-glycans are arrested as glucosylated structures nonspecifically and undergo no further processing.[10] Albinism can also occur due to blockage of tyrosinase glycosylation by mutation of a glycosylation consensus site within tyrosinase. Thus, blocking the maturation processes of tyrosinase N-glycosylation is also good strategy for inhibiting melanogenesis.

We recently isolated a series of α-glucosidase inhibitory xanthones from the seedcases of *Garcinia mangostana*, which is a tropical evergreen tree originating in Southeast Asia.[11] Mangostenone F was found to display significant α-glucosidase inhibition but had very low cytotoxicity and tyrosinase inhibition. This result encouraged us to examine the potential of mangostenone F to reduce melanin production via α-glucosidase inhibition. The aim of the present study is to investigate the potential of mangostenone F for depigmentation in cellular assays on the mouse melanoma line B16F10 and to determine the precise cellular mechanism by which depigmentation occurs.

MATERIALS AND METHODS

General Apparatus and Chemicals. NMR spectra were recorded on a Bruker AM500 instrument (^{1}H NMR at 500 MHz, ^{13}C NMR at 125 MHz, Billerica, MA, USA). Electron

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ionization (EI) and EI−high resolution (HR) mass spectra were obtained on a JEOL JMS-700 instrument (JEOL Ltd., Tokyo, Japan). Optical rotations were measured on a Perkin-Elmer 343 polarimeter (Perkin-Elmer, Shelton, CT, USA). Melting points were measured on a Thomas Scientific Capillary Melting Point Apparatus and are uncorrected. Column chromatography was performed on silica gel (230−400 mesh, Merck, Darmstadt, Germany), RP-18 (ODS-A, 12 nm, S-150 μM, YMC, Kyoto, Japan), and Sephadex LH-20 (GE Healthcare Bio-Science AB, Björkgatan, Sweden). Thin layer chromatography (TLC) was done on precoated TLC plates with silica gel 60 F_{254} (0.25 mm, normal phase, Merck). These were visualized using a UVGL-58 254 nm hand-held UV lamp (UVP, Cambridge, UK) or by spraying with 10% H_2SO_4 in ethanol followed by heating. CD_3OD, acetone-d_6, and $CDCl_3$ were purchased from Cambridge Isotope Laboratory Inc., (Andover, MA, USA). α-Glucosidase (EC 3.2.1.20) type I from *Saccharomyces cerevisiae*, β-glucosidase (EC 3.2.1.21), α-mannosidase (EC 3.2.1.24), β-galactosidase (EC 3.2.1.22), α-L-rhamnosidase (EC 3.2.1.40), *p*-nitrophenyl-α-D-glucopyranoside, *p*-nitrophenyl-β-D-glucopyranoside, *o*-nitrophenyl-α-D-mannopyranoside, *p*-nitrophenyl-β-D-galactopyranoside, *p*-nitrophenyl-α-L-rhamnopyranoside, 4-methylumbelliferyl-α-D-glucopyranoside, mushroom tyrosinase (EC 1.14.18.1), L-DOPA, K_2HPO_4, KH_2PO_4, and DMSO were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Endoglycosidase H (endo H) was purchased from NEB (Ipswich, MA, USA). All solvents were distilled before use.

Plant Materials. The seedcases of *G. mangostana* (Clusiaceae), collected in Vietnam, used for the present study were provided by LanSea Food Co., Ltd. (www.lansea.co.kr, Seoul, Korea) in August 2009. A representative sample has been deposited at the Herbarium of Kyungpook National University (KNU).

Extraction and Isolation of Phenolic Phytochemicals. The dried seedcases of *G. mangostana* (0.5 kg) were extracted three times with ethanol, and the supernatant was evaporated under vacuum at 40 °C. The ethanol extract was dissolved in distilled water and partitioned with chloroform. Evaporation of the solvent under reduced pressure yielded the chloroform extract (65.5 g), which was fractionated on a silica gel column (10 × 30 cm, 230−400 mesh, 700 g) and eluted using hexane/acetone [100:1 (1.5 L), 50:1 (1.5 L), 30:1 (1.5 L), 20:1 (1.5 L), 10:1 (3 L), 6:1 (2.5 L), 4:1 (2 L), 2:1 (2 L), 1:1 (1 L) and then only acetone (2 L)] to give eight pooled fractions, F1−F8, based on comparison of TLC profiles. Subfraction F3 was subjected to flash CC employing a $CHCl_3$/acetone gradient (20:1 → 1:1) to give compound **1** (48 mg) (Figure 1). Finally, the purified compound was identified by comparing its ^1H and ^{13}C NMR data with the literature (Supporting Information).[11,12]

Assay for α-Glucosidase and Tyrosinase Inhibitory Activities. Glycosidase activity was assayed according to standard procedures by following the hydrolysis of the relevant nitrophenyl glycosides (*p*-nitrophenyl-α-D-glucopyranoside, *p*-nitrophenyl-β-D-glucopyranoside, *o*-nitrophenyl-α-D-mannopyranoside, *p*-nitrophenyl-β-D-galactopyranoside, and *p*-nitrophenyl-α-L-rhamnopyranoside, respectively) spectrophotometrically at the optimum pH of each enzyme.[14] The reaction was stopped by adding 2 M NaOH. The inhibitory effects of the tested compounds were expressed as the concentrations that inhibited 50% of the enzyme activity (IC_{50}). The percent inhibition ratio (percent) was calculated according to the following equation: % inhibition = [(rate of control reaction − rate of sample reaction)/rate of control reaction] × 100. The known glycosidase inhibitor deoxynojirimycin (DNJ) (Sigma-Aldrich Co., St. Louis, MO, USA) was used in the assays for comparison. All parameter values were then calculated using SigmaPlot (SPCC Inc., Chicago, IL, USA). Mushroom tyrosinase was used for in vitro assays as described previously with some modifications.[13] In this experiment, L-DOPA was used as a substrate. Enzyme activity was monitored by dopachrome formation at 475 nm with a UV−vis spectrophotometer (Spectro UV−vis double beam; UVD-3500, Labomed, Inc., Culver City, CA, USA) at 30 °C.

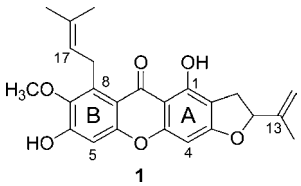

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Figure 1. Chemical structure of compound **1**.

Cell Culture and Chemicals. B16F10 murine melanoma cells were grown in Dulbecco's modified minimum essential medium (DMEM) (Invitrogen, Grand Island, NY, USA) supplemented with 10% heat-inactivated fetal bovine serum (Invitrogen, Carlsbad, CA, USA), L-glutamine (2.5 mM), HEPES [40 mM (Biopure, CA, USA)], and antibiotics [penicillin (50 units/mL) and streptomycin (50 mg/mL) (Gibco, Invitrogen, Carlsbad, CA, USA)] at 37 °C in a 5% CO_2 humidified atmosphere. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolidium bromide (MTT), α-melanocyte-stimulating hormone (α-MSH), and kojic acid (KA) were obtained from Sigma-Aldrich Co. Anti-tyrosinase, anti-TRP-1, and anti-TRP-2 were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Mangostenone F treatment began 24 h after plating, and cells were harvested after 2 days of incubation.

Cell Viability Assay. After treatment with kojic acid or mangostenone F, cells were assayed for growth activity using a MTT-based colorimetric method, as previously described.[15] Briefly, cells were seeded at densities of 5000 cells/well in 96-well culture plates. Cells were treated with mangostenone F for 48 h. After treatment with this compound, the attached cells were incubated with MTT (0.5 mg/mL, 1 h) and subsequently solubilized in DMSO. The absorbance at 550 nm was then measured using a microplate reader (SpectraMax M2Multi-Mode Microplate Reader, Molecular Devices, Sunnyvale, CA, USA). The GI_{20} is the concentration of agent that reduced the cell viability by 20% under the experimental conditions.

Measurement of Melanin Content and Microscopy. The melanin content was measured using a slight modification of the method described by Yang et al.[16] Briefly, the cells were treated with various concentrations of mangostenone F for 48 h. The cell pellets were then dissolved in 1 mL of 1 M NaOH at 100 °C for 30 min and centrifuged for 20 min at 16000*g*, after which time the optical density of the supernatants was measured at 400 nm using a microplate reader (SpectraMax M2 Multi-Mode Microplate Reader). Before the melanin content was measured, the cells were observed under a Leica DM IL microscope from Leica Microsystems (Wetzlar, Germany) supported by Leica Application Suite V3.3.0 software.

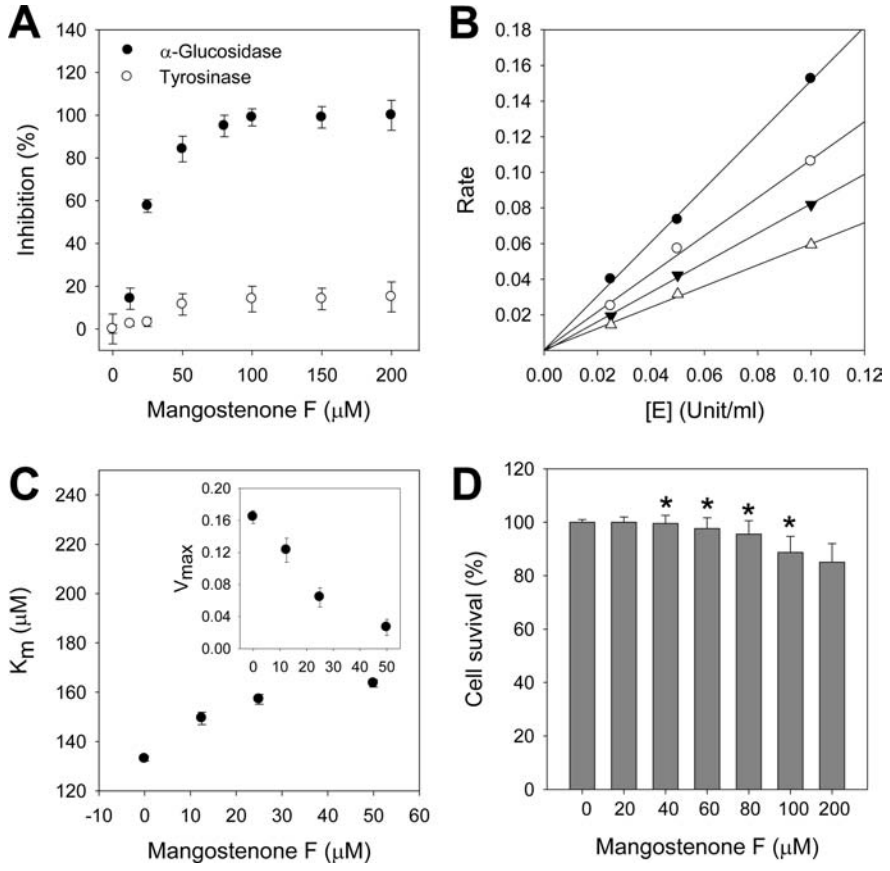

Figure 2. (A) Tyrosinase and α-glucosidase inhibitory activities of mangostenone F from *Garcinia mangostana*. (B) Hydrolytic activity of α-glucosidase as a function of concentration of mangostenone F (0 μM, ●; 12.5 μM, ○; 25 μM, ▼; 50 μM, △). (C) K_m values as a function of the concentrations of mangostenone F. (Inset) Dependence of the values of V_{max} on the concentration of mangostenone F. (D) Effect of mangostenone F on the growth of B16F10 melanoma cells. Cytotoxicity was measured by MTT assay of mangostenone F and control (DMSO) treated B16F10 melanoma cells after 48 h at the indicated concentrations.

Measurement of Cellular Tyrosinase Activity. The tyrosinase activity was measured according to the method of Ohgushi et al. with a slight modification.[17] Briefly, B16F10 cells, which had been pretreated with the indicated concentrations of mangostenone F for 3 h, were then treated for 48 h with α-melanocyte-stimulating hormone (10 ng mL^{-1}). The cells were washed twice with ice-cold phosphate-buffered saline (PBS) (Hyclone Laboratories, Inc., South Logan, UT, USA) and extracted and then centrifuged at 10000*g* for 10 min. Samples of cell extract supernatant were incubated in duplicate for 1 h at 37 °C in sodium phosphate buffer (pH 7.4) containing 0.1% L-DOPA. Dopachrome formation was monitored by measuring the absorbance at 470 nm (SpectraMax M2Multi-Mode Microplate Reader).

Western Blotting. B16F10 cells, pretreated with the indicated concentrations of mangostenone F for 3 h, were treated with α-melanocyte-stimulating hormone (10 ng mL^{-1}) for 48 h. Cells were washed with PBS and scraped in lysis buffer [40 mM Tris-HCl (pH 8.0), 120 mM NaCl, 0.1% Nonidet P-40, and protease inhibitors (Sigma-Aldrich Co.)]. They were then centrifuged at 14000*g* for 15 min at 4 °C, and supernatants (total cell lysates) were collected and aliquoted. These lysates were either used on the day of preparation or immediately stored at −78 °C until required. For Western blot analysis, 30 μg of lysate was resolved on 12.5 and 16% polyacrylamide gels and then transferred to nitrocellulose membranes. Membranes were blocked with 5% fat-free milk in PBS (pH 7.4) for 30 min at room temperature and then incubated with the appropriate mono- or polyclonal primary antibodies in blocking buffer for from 2 h to overnight at 4 °C. After washing, membranes were incubated with anti-mouse or anti-rabbit horseradish peroxidase-conjugated secondary antibodies for 1 h at room temperature and then subjected to ECL solution and visualized using Hyperfilm (GE Healthcare Bi0-Science AB, Björkgatan, Sweden). Densitometric analysis of Western blots was performed using Quantity one software (Bio-Rad XRS, Bio-Rad Laboratories, Hercules, CA, USA).

Endoglycosidase H (Endo H) Digestion. Carbohydrate cleavage was measured using the method reported by Halaban et al. with a slight modification.[18] The lysates [300−500 μg of protein in about 100 μL of lysis buffer (2% CHAPS in 50 mM HEPES and 200 mM NaCl, pH 7.5, 0.1% protease inhibitors)] were centrifuged for 10 min at 12000*g*, and endo H (100 units/mL) was added to the supernatant. After 1 h at 37 °C, the reaction products were subjected to analysis by Western blot (7.5% polyacrylamide gels) and probed with anti-tyrosinase antibodies as described above.

α-Glucosidase Activity in Melanoma Cells. Subconfluent cultures of B16F10 cells were detached with 0.25% trypsin/0.02% EDTA, washed with PBS, and resuspended at 4 × 10^7 cells/mL in Ca^{2+}-/Mg^{2+}-free PBS. The cell suspension was stored at −80 °C and homogenized by an Ultraturrax immediately before use. The protein content of the cell lysate was measured by using a Bio-Rad protein assay kit and bovine

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serum albumin (BSA) as a standard. The glucosidase reaction was initiated by the addition of the B16F10 lysate (10 μL) to the reaction mixture (0.49 mL) containing 25 mM sodium acetate buffer (pH 5.2) and 5 mM 4-methylumbelliferyl glucoside. After incubation at 37 °C, the reaction was stopped by the addition of 0.4 M glycine buffer (pH 10.7; 2.5 mL). 4-Methylumbelliferone was immediately quantified fluorometrically on a SpectraMax M2 Multi-Mode Microplate Reader. The excitation wavelength was 363 nm, and the emission wavelength was 444 nm.[19]

Statistical Analysis. All data are presented as the mean ± SD. The significance of differences between the means of the treated and untreated groups was determined by using Student's t test. A $p < 0.05$ value was considered to be significant.

RESULTS AND DISCUSSION

Mangostenone F was isolated along with a number of other xanthones from seedcases of *G. mangostana* in a previous study. The structure of mangostenone F was confirmed through analysis of spectroscopic data and comparison with previous studies.[12] The principal objective of this study was to investigate the effectiveness of mangostenone F as a depigmentation agent in the mouse melanoma cell line B16F10. We also link the α-glucosidase inhibitory capacity of mangostenone F to depigmentation.

Mangostenone F was screened for in vitro tyrosinase and α-glucosidase inhibitory activities at different concentrations using modified UV assays reported previously by Kubo et al.[13] and Kim et al.[14] Mangostenone F was inactive against mushroom tyrosinase up to 200 μM. However, mangostenone F inhibited α-glucosidase dose-dependently with an IC_{50} of 21.0 μM (Figure 2A). To determine the specificity of mangostenone F against α-glucosidase, its inhibitory activities against various other glycoside hydrolases were determined (Table 1).

Table 1. Effect of Mangostenone F on Sugar-Hydrolyzing Enzyme

enzyme	source	IC_{50}[a] (μM)
α-glucosidase	yeast	21.0 ± 0.8
β-glucosidase	almond	212 ± 18
α-mannosidase	jack been	nd
α-galactosidase	green coffee bean	nd
β-galactosidase	bovine liver	nd
α-rhamnosidase	*Penicillium decumbens*	nd
α-amylase	porcine pancreas	nd

[a]All compounds were examined in a set of experiments repeated three times; IC_{50} values of compounds represent the concentration that caused 50% enzyme activity loss. nd, not detected.

Mangostenone F showed no activity against any of these non-glucoside hydrolases at concentrations up to 200 μM. To determine whether mangostenone F can inhibit α-glucosidase reversibly, we examined the change of the initial velocity as a function of enzyme concentration. This analysis showed the characteristic behavior of a reversible inhibitor because plots of the initial velocity versus enzyme concentration in the presence of different concentrations of mangostenone F gave a family of straight lines, all of which passed through the origin (Figure 2B). As illustrated in Figure 2C, mangostenone F has a mixed inhibition mechanism because increasing concentrations of inhibitor resulted in increased K_m values and diminished V_{max}.

We progressed to investigate the effects of mangostenone F in cultured B16F10 mouse melanoma cells. As shown in Figure 2D, mangostenone F exhibited no cytotoxicity up to 200 μM on B16F10 cells assessed using a calorimetric MTT-based assay as described under Materials and Methods. Kojic acid also showed no cytotoxicity at concentrations ranging from 20 to 200 μM. It is well-known that α-melanocyte-stimulating hormone up-regulates tyrosinase and tyrosinase-related protein production through the activation of a transcription factor, MITF. Therefore, we examined the effect of the target compound on α-melanocyte-stimulating hormone-controlled tyrosinase expression levels. In this assay, B16F10 melanoma cells were incubated with mangostenone F at different concentrations (10, 20, 40, and 60 μM) in the presence of 10 ng mL^{-1} of α-melanocyte-stimulating hormone for 2 days. Importantly, this assay represents a model for hyperpigmentation. As shown in Figure 3A, exposure of cells to α-melanocyte-stimulating hormone more than doubled the melanin content of the cells. However, upon treatment with mangostenone F, the pigmentation and melanin content were both reduced dose-dependently as follows: 19.1% at 10 μM, 31.1% at 20 μM, 38.3% at 40 μM, and 45.5% at 60 μM (Figure 3A). Tyrosinase activity within B16F10 cells was also reduced by mangostenone F dose-dependently (Figure 3B). A 49.1% reduction in tyrosinase activity was observed with 60 μM mangostenone F. These data show that the reduced pigmentation phenotype we observed above in Figure 3A was ultimately due to reduced tyrosinase activity. Because mangostenone F did not inhibit tyrosinase in our in vitro assays, we moved on to investigate depigmentation further. Importantly, cell death was ruled out as a factor, because our experiments were carried out during a time in which cells were completely viable (Figure 3C).

Three enzymes are known to be involved directly in melanin biosynthesis in mammals: tyrosinase, TRP-1, and TRP-2. Therefore, we investigated the dose-dependent effects that mangostenone F exerted on expression levels of these proteins by Western blot. In these experiments we compared expression levels of tyrosinase, TRP-1, and TRP-2 in α-melanocyte-stimulating hormone-treated B16F10 cells versus untreated B16F10 cells. The expression levels of tyrosinase and TRP-2 were found to be reduced dose-dependently by mangostenone F, but the content of TRP-1 was not obviously different from the control (Figure 4A). These results indicated that mangostenone F exerts its potent depigmenting activity at least partially by down-regulating expression levels of the proteins required for the chemical steps involved in tyrosine processing to melanin.

Tyrosinase requires glycolytic processing to mature, and because we had already shown that mangostenone F is glycosidase inhibitor, we reasoned that all of our observations to this point could be explained by the fact that tyrosinase processing was affected by mangostenone F. Digestion of the control B16F10 lysate with endo H had little effect on the Western blot for tyrosinase: a series of bands between 60 and 70 kDa was observed either with or without the deglycosidase, indicating correct processing and passage of tyrosinase to the *trans*-golgi. However, Western blot for tyrosinase of lysates of B16F10 cells treated with mangostenone F showed a shift from a series of bands without endo H digestion to almost a single band at 60 kDa upon endo H treatment (Figure 4B). A shift in molecular weight in tyrosinase upon endo H treatment is diagnostic for incomplete processing.[20−23] An identical result was found for cells treated with deoxynojirimycin, a known

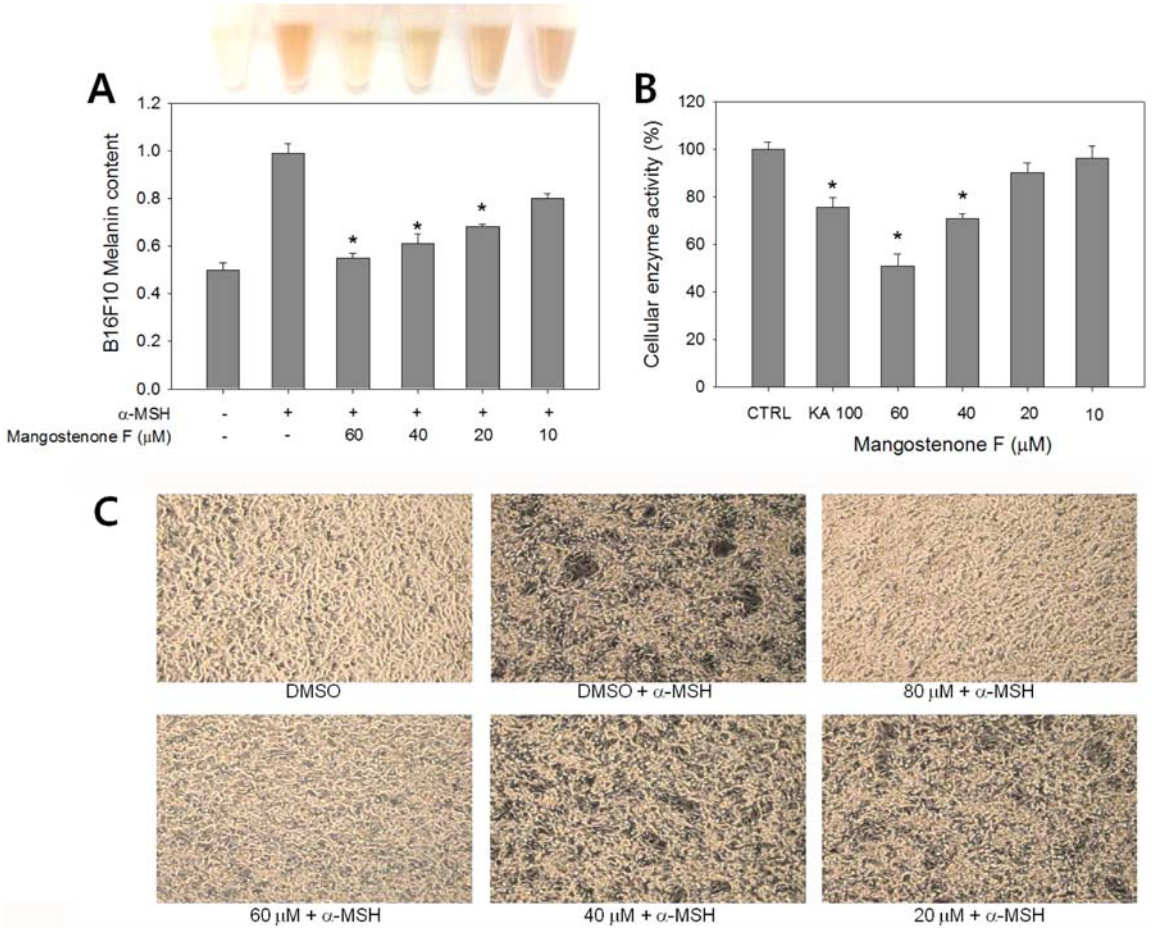

Figure 3. Effect of mangostenone F on melanogenesis in B16F10 cells cultured with 20−80 μM mangostenone F for 2 days: (A) changes of melanin contents that were pretreated with α-melanocyte-stimulating hormone and then cultured with mangostenone F (0−60 μM) for 48 h (∗, $p < 0.01$ when compared to an untreated control); (B) cells (2×10^6 cell per well) were treated with the indicated concentration of mangostenone F in the presence of 10 ng mL^{-1} α-melanocyte-stimulating hormone for 48 h (cellular tyrosinase activity was determined as described under Materials and Methods; ∗, $p < 0.01$ when compared to an untreated control); (C) phase-contrast photomicrographs taken using a digital video camera. The results shown are the average of three replicate experiments ± SD.

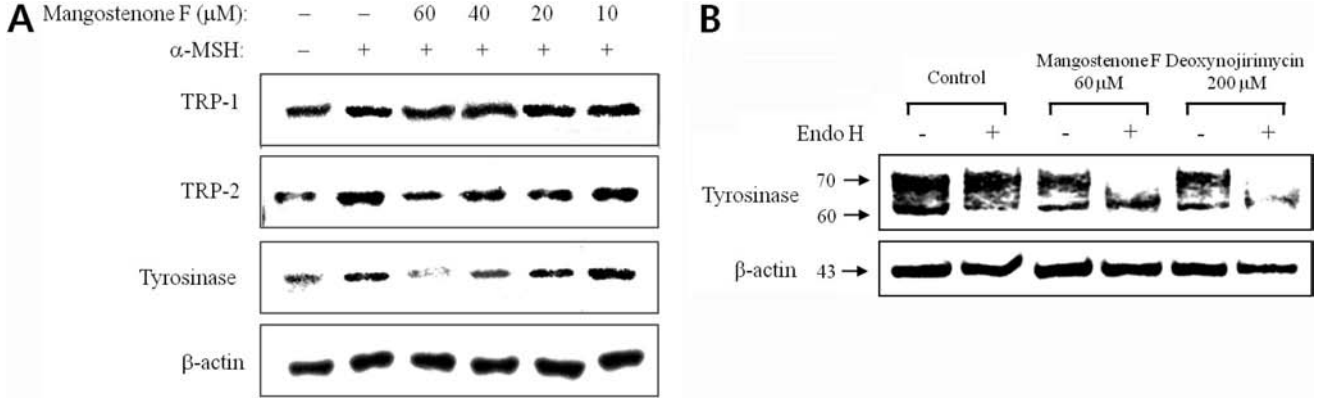

Figure 4. (A) Effect of mangostenone F on α-melanocyte-stimulating hormone-induced increase of tyrosinase, TRP-1, and TRP-2 expression analyzed by Western blot (average of three results). (B) Inhibitory effect of mangostenone F on the glycosylation of tyrosinase in B16F10 cells.

glycosidase inhibitor, which is known to affect tyrosinase processing in vitro. Together, these data firmly indicate that mangostenone F does influence the glycosylation maturation process of tyrosinase, as we postulated.

Because we had linked the apparent tyrosinase inhibition in B16F10 cells by mangostenone F to glycosidase inhibition, we investigated the action of mangostenone F on α-glucosidases in B16F10 cell lysates (Figure 5) using a well-characterized assay involving hydrolysis of 4-methylumbelliferyl-α-D-glucopyroside. Treatment of B16F10 cells with mangostenone F (62.5, 90, 125, and 250 μM) in the culture media for 48 h prior to lysis resulted in a 17.1, 40.8, 59.5, and 79.4% inhibition of cellular α-glucosidase activity, respectively. By means of comparison, deoxynojirimycin caused only a 52% inhibition of cellular α-

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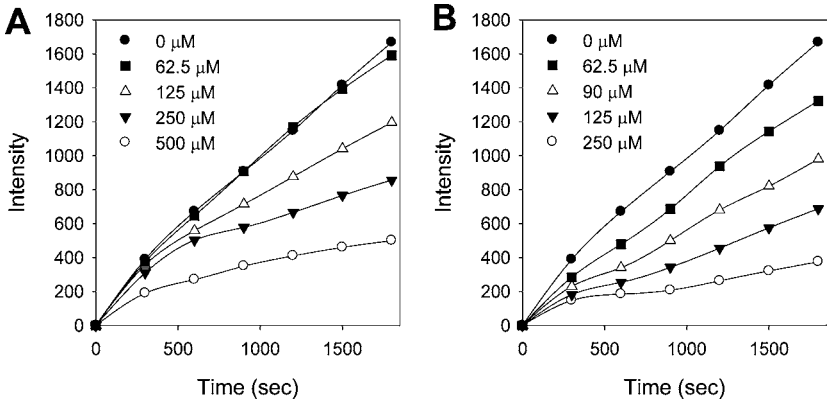

Figure 5. Effect of mangostenone F and deoxynojirimycin on α-glucosidase in B16F10 cells: (A) effects of deoxynojirimycin on the activity of α-glucosidase for hydrolysis of 4-methylumbelliferyl-α-D-*N*-glucopyranoside (0 μM, ●; 62.5 μM, ■; 125 μM, △; 250 μM, ▼; 500 μM, ○); (B) hydrolytic activity of α-glucosidase as a function of the concentration of mangostenone F (0, ●; 62.5 μM, ■; 90 μM, △; 125 μM, ▼; 250 μM, ○).

glucosidase at 250 μM. Thus, mangostenone F is a better inhibitor of mammalian α-glucosidases than deoxynojirimycin in a cell culture assay.

In conclusion, the α-glucosidase inhibitor mangostenone F (IC_{50} = 21.0 μM) from *G. mangostana* showed potent depigmenting ability against B16F10 melanoma cells. This depigmentation was not caused directly by tyrosinase inhibition but by inhibition of tyrosinase processing and maturation in melanoma cells. Our data indicate that mangostenone F down-regulates tyrosinase expression on the basis of its α-glucosidase-inhibitory potential.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

NMR data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS USED

IC_{50}, inhibitor concentration leading to 50% activity loss; endo H, endoglycosidsae H; TRP-1, tyrosinase-related protein-1; TRP-2, tyrosinase-related protein-2; α-MSH, α-melanocyte-stimulating hormone; ER, endoplasmic reticulum.

■ REFERENCES

(1) Kim, Y. J.; Uyama, H. Tyrosinase inhibitors from natural and synthetic sources: structure, inhibition mechanism and perspective for the future. *Cell. Mol. Life Sci.* **2005**, *62*, 1707−1723.

(2) Spritz, R. A.; Hearing, V. J., Jr. Genetic disorders of pigmentation. *Adv. Hum. Genet.* **1994**, *22*, 1−45.

(3) Hu, X.; Wu, J. W.; Wang, M.; Yu, M. H.; Zhao, Q. S.; Wang, H. Y.; Hou, A. J. 2-Arylbenzofuran, flavonoid, and tyrosinase inhibitory constituents of *Morus yunnanensis*. *J. Nat. Prod.* **2012**, *75*, 82−87.

(4) Durairaj, R. B. *Resorcinol: Chemistry, Technology, And Applications*; Springer Verlag: Heidelberg, Germany, 2005; pp 688−690.

(5) Ando, H.; Kondoh, H.; Ichihashi, M.; Hearing, V. J. Approaches to identify inhibitors of melanin biosynthesis via the quality control of tyrosinase. *J. Invest. Dematol.* **2007**, *127*, 751−761.

(6) Petrescu, S. M.; Petrescu, A. J.; Titu, H. N.; Dwek, R. A.; Platt., F. M. Inhibition of N-glycan processing in B16 melanoma cells results in inactivation of tyrosinase but does not prevent its transport to the melanosome. *J. Biol. Chem.* **1997**, *272*, 15796−15803.

(7) Kornfeld, R.; Kornfeld, S. Assembly of asparagines-liked oligosaccharides. *Annu. Rev. Biochem.* **1985**, *54*, 631−664.

(8) Wang, N.; Hebert, D. N. Tyrosinase maturation through the mammalian secretory pathway: bring color to life. *Pigment Cell Res.* **2006**, *19*, 3−18.

(9) Elbein, A. D. Inhibitors of the biosynthesis and processing of N-linked oligosaccharide chains. *Annu. Rev. Biochem.* **1987**, *56*, 497−534.

(10) Lubas, W. A.; Spiro, R. G. Goligi endo-α-D-mannosidase from rat liver, a novel linked carbohydrate unit processing enzyme. *J. Biol. Chem.* **1987**, *262*, 3775−3781.

(11) Ryu, H. W.; Cho, J. K.; Curtis-Long, M. J.; Yuk, H. J.; Kim, Y. S.; Jung, S. I.; Kim, Y. S.; Lee, B. W.; Park, K. H. α-Glucosidase inhibition and antihyperglycemic activity of prenylated xanthones from *Garcinia mangostana*. *Phytochemistry* **2011**, *72*, 2148−2154.

(12) Ryu, H. W.; Curtis-Long, M. J.; Jung, S. I.; Jin, Y. M.; Cho, J. K.; Ryu, Y. B.; Lee, W. S.; Park, K. H. Xanthones with neuraminidase inhibitory activity from the seedcases of *Garcinia mangostana*. *Bioorg. Med. Chem.* **2010**, *18*, 6258−6264.

(13) Kubo, I.; Kinst-Hori, I. Tyrosinase inhibitors from cumin. *J. Agric. Food Chem.* **1998**, *46*, 5338−5341.

(14) Kim, J. Y.; Lee, J. W.; Lee, Y. N.; Ryu, Y. B.; Kim, S. M.; Kim, Y. S.; Ryu, H. W.; Curtis-Long, M. J.; Lee, K. W.; Lee, W. S.; Park, K. H. A novel competitive class of α-glucosidase inhibitors: (*E*)-1-phenyl-3-(4-styrylphenyl)urea derivatives. *ChemBioChem.* **2010**, *11*, 2125−2131.

(15) Lee, Y. J.; Chung, D. Y.; Lee, S. J.; Ja, J. G.; Lee, Y. S. Enhanced radiosensitization of p53 mutant cells by oleamide. *Int. J. Radiat. Oncol. Biol. Phys.* **2006**, *64*, 1466−1474.

(16) Yang, J. Y.; Koo, J. H.; Song, Y. G.; Kwon, K. B.; Lee, J. H.; Sohn, H. S.; Park, B. H.; Jhee, E. C.; Park, J. W. Stimulation of melanogenesis by scoparone in B16 melanoma cells. *Acta. Pharmacol. Sin.* **2006**, *27*, 1467−1473.

(17) Ohguchi, K.; Ito, M.; Yokoyama, K.; Iinuma, M.; Itoh, T.; Nozawa, Y.; Akao, Y. Effects of sesquiterpene lactones on melanogenesis in mous B16 melanoma cells. *Biol. Pharm. Bull.* **2009**, *32*, 308−310.

(18) Halaban, R.; Sevedine, S.; Cheng, E.; Smicun, Y.; Aron, R.; Hebert, D. N. Endoplsmic reticulum retention is a common defect

associated with tyrosinase-negative albinism. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 5889−5894.

(19) Atsumi, S.; Nosaka, C.; Ochi, Y.; Iinuma, H.; Umezawa, K. Inhibition of experimental metastasis by an α-glucosidase inhibitor, 1,6-epi-cyclophellitol. *Cancer Res.* **1993**, *53*, 4896−4899.

(20) Imperiali, B.; Shannon, K. L. Differences between Asn−Xaa−Thr-containing peptides: acomparision of solution conformation and substrate behavior with oligosaccharyl transferase. *Biochemistry* **1991**, *30*, 4374−4380.

(21) Ujvari, A.; Aron, R.; Eisenhaure, T.; Cheng, E.; Parag, H. A.; Smicun, Y.; Halaban, R.; Hebert, D. N. Translation rate of human tyrosinase determines its N-linked glycosylation level. *J. Biol. Chem.* **2001**, *276*, 5924−5931.

(22) Petrescu, S. M.; Nichita, B. N.; Negroiu, G.; Petrescu, A. J.; Dwek, R. A. Tyrosinase and glycoprotein folding: roles of chaperones that recognize glycans. *Biochemistry* **2000**, *39*, 5229−5237.

(23) Halaban, R.; Chang, E.; Zhang, Y.; Moellmann, G.; Hanlon, D.; Michalak, M.; Setaluri, V.; Hebert, D. N. Aberrant retention of tyrosinase in the endoplasmic reticulum mediates accelerated degradation of the enzyme and contributes to the dedifferentiated phenotype of amellanotic melanoma cells. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 6210−6215.