Arch Dermatol Res (2011) 303:737–744
DOI 10.1007/s00403-011-1155-7

ORIGINAL PAPER

Aromatic-turmerone inhibits α-MSH and IBMX-induced melanogenesis by inactivating CREB and MITF signaling pathways

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Received: 6 March 2011 / Revised: 16 May 2011 / Accepted: 24 May 2011 / Published online: 10 June 2011

Abstract This study investigated the anti-melanogenic effect of aromatic (ar)-turmerone on alpha-melanocyte stimulating hormone (α-MSH) and 3-isobuty-1-methxlzanthine (IBMX)-induced tyrosinase (Tyr), tyrosinase-related protein 1 (TRP-1), and tyrosinase-related protein 2 (TRP-2) expression in B16F10 melanoma cells. We demonstrated that ar-turmerone inhibits α-MSH and IBMX-induced melanin synthesis and tyrosinase activity. Data also showed that ar-turmerone inhibits the expression of tyrosinase, TRP-1, and TRP-2 in α-MSH- and IBMX-stimulated B16F10 cells. In addition, ar-turmerone exhibits stronger anti-melanogenic effects than curcumin. Furthermore, ar-turmerone strongly inhibited α-MSH- and IBMX-induced microphthalmia-associated transcription factor by suppressing the activity of cyclic adenosine monophosphate (cAMP)-responsive element binding protein in α-MSH-stimulated B16F10 cells. Our data revealed that ar-turmerone is a novel, effective, anti-melanogenic agent that functions by down-regulating tyrosinase, Trp-1, and Trp-2 gene expression. Therefore, ar-turmerone may be a useful therapeutic agent for treating hyperpigmentation disorders, such as freckles and melasma, and as a beneficial additive in whitening cosmetics.

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Keywords Ar-turmerone · Melanin · Tyrosinase · cAMP-responsive element binding protein (CREB) · Microphthalmia-associated transcription factor (MITF)

Introduction

Melanogenesis is a biosynthetic pathway that occurs in differentiated cells known as melanocytes, located in the lowest layer of epidermis in human skin [10, 34]. Melanogenesis has many important physiological functions, including photo-protection of human skin from ultraviolet (UV) irradiation [9]. Melanin synthesis is stimulated by various molecules and conditions, such as α-melanocyte-stimulating hormone (α-MSH), cyclic AMP (cAMP) elevating agents, including forskolin, glycyrrhizin, and isobutylmethylxanthine, UV-B radiation, and the placental total lipid fraction [14, 16, 31]. Excessive melanin production in the skin has negative hyperpigmentation effects, inducing melasma, freckles, age or liver spots, and actinic damage [2, 29]. Various dermatologic disorders result from the accumulation of excessive levels of epidermal pigmentation. Epidermal and dermal hyperpigmentation may depend on either increased numbers of melanocytes or melanogenic enzyme activities [20]. Melanin synthesis occurs in melanocytes and melanoma cells in an enzymatic process catalyzed by tyrosinase, tyrosinase-related protein 1 (TRP-1), and tyrosinase-related protein 2 (TRP-2), which converts tyrosine to 3,4-dihydroxyphenylalanine (DOPA) and catalyzes oxidation of DOPA into DOPAquinone [5, 6, 8]. Dopaquinone is converted to dopachrome, which is in turn converted to dihyroxyindole or dihydroxyindole-2-carboxylic acid (DHICA) to form eumelanin. The cascade of enzymatic reactions in melanin synthesis involves

tyrosinase, TRP-1, and dopachrome tautomerase (DCT), also known as TRP-2 [7, 13, 22].

cAMP-dependent protein kinase A (PKA) signaling pathways are the predominant cascades participating in melanin synthesis. Furthermore, stimulators, such as α-MSH, forskolin and IBMX, control the expression of tyrosinase, TRP-1 and TRP-2 by modulating the activation of transcription factors such as MITF and CREB and through protein kinase A signaling pathways [18]. MITF is the most important transcriptional regulator of tyrosinase activity and is involved in melanocyte pigmentation, proliferation, survival, and differentiation [3, 33]. Alpha-melanocyte stimulating hormone (α-MSH) induces MITF expression by increasing cAMP levels after binding to the melanocortin 1 receptor [22]. The *MITF* gene contains two promoters, one of which is a cAMP-responsive element (CRE). Phosphorylation of a CRE binding protein (CREB) promotes MITF expression [12, 19].

Aromatic (ar)-turmerone is a naturally occurring turmeric oil, which was initially isolated from *Curcuma longa*, that has been used for centuries in Southeast Asia as both a remedy and a food. Curcumin, demethoxycurcumin, bisdemethoxycurcumin, ar-turmerone, α-turmerone, and *β*-turmerone are the major bioactive compounds found in *C. longa.* In modern pharmacological studies, *C. longa* constituents, particularly curcumin, have been shown to have anti-inflammatory, anti-cancer [11], anti-oxidative, chemopreventive, immunomodulatory, and potentially chemotherapeutic properties [1, 15]. Recently, the anti-melanogenic effects of partially purified *C. longa* and curcumin have been reported. However, the effects of other components of this plant on the melanogenesis signaling pathway have not been investigated. Ar-turmerone is a component of *C. longa.* Therefore, in this study we examined the effect of ar-turmerone on α-MSH and IBMX-induced melanogenesis and signaling pathways in B16F10 melanoma cells.

Materials and methods

Materials

Ar-turmerone, α-MSH, IBMX, L-3,4-dihydroxyphenylalanine (L-DOPA), curcumin, 3-(4,5-dimetylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), H89 and all other reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA). Antibodies recognizing phospho-CREB and CREB were obtained from Cell Signaling Technology (Danvers, MA, USA). Tyrosinase, TRP-1, TRP-2, and MITF antibodies were supplied by Santa Cruz Biotechnology (Santa Cruz, CA, USA).

Cell culture

The B16-F10 murine melanoma cell line obtained from the American Type Culture Collection (ATCC; Rockville, MD, USA) was grown as monolayers in Dulbecco's modified Eagle's medium (DMEM; GIBCO BRL, Carlsbad, CA, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS; GIBCO BRL). The cells were incubated at 37°C in a humidified atmosphere containing 5% CO_2 and 95% air. To avoid changes in cell characteristics produced by extended cell culture periods, cells were used for experiments between passages 15 and 25. Each cell suspension was split every 2 days to maintain exponential growth.

Cell viability assay

Cells were incubated in wells of a 24-well plate at a density of 4×10^4 cells/well. MTT solution (50 μg/ml) was added to each well. The plates were then incubated for an additional 3 h at 37°C in a 5% CO_2 atmosphere, after which the supernatant was removed. Formazan crystals that had formed in viable cells were solubilized using dimethylsulfoxide (DMSO). The absorbance of each well was measured at 570 nm using an enzyme-linked immunosorbent assay (ELISA) reader (Wallace, Boston, MA, USA).

Determination of intracellular melanin content

The melanin content was measured using slight modification of a previously described method [32]. Briefly, cells were treated with 0.5 μM α-MSH in the absence or presence of ar-turmerone for 48 h. Then the media was aspirated and the cells were washed twice with PBS. Cells were harvested from each well in a phosphate buffered saline (PBS). Cells were pelleted by centrifugation (2,000 rpm) and the melanin was dissolved by treatment with 500 μl of 1 N NaOH in 10% DMSO at 80°C for 1 h. Relative melanin content was determined by measuring absorbance at 475 nm in a microplate reader. Melanin production was calculated by normalizing melanin values with protein content (absorbance/μg protein). Protein content was determined using the Bradford dye reagent (Bio-Rad, Hercules, CA, USA).

B16F10 cell tyrosinase activity assay

Tyrosinase activity was determined by measuring the rate of dopachrome formation of L-DOPA and was measured by the method of Akao et al. [24] with a slight modification. Briefly, cells grown in 6-well dishes were treated with 0.5 μM α-MSH in the absence or presence of ar-turmerone

for 48 h. Cells were then washed in ice-cold PBS and lysed in PBS containing 1% (w/v) Triton X-100 and 0.1 mM phenylmethanesulfonylfluoride (PMSF). The lysates were then centrifuged at 13,000 rpm for 30 min to obtain the supernatant as the crude tyrosinase extract for the activity assay. The tyrosinase substrate, L-DOPA (2 mg/ml), was prepared in phosphate lysis buffer. Each extract was placed in a 96-well plate and the enzymatic reaction was initiated by adding L-DOPA solution. After incubation, dopachrome formation was assayed by measuring absorbance at 475 nm using a microplate reader. The value of each measurement was expressed as a percentage of the control. Tyrosinase activity was calculated by normalizing tyrosinase activity values with protein content (absorbance/μg protein). Protein content was determined using the Bradford dye reagent (Bio-Rad, Hercules, CA, USA).

Determination of intracellular cAMP level

cAMP concentration was measured using a cyclic AMP EIA kit (Cayman Chemical Company, Ann Arbor, MI, USA). Briefly, cells were lysed in 0.1 M HCl to inhibit phosphodiesterase activity. After neutralization and dilution, a fixed amount of cAMP conjugate was added to compete with cAMP in the cell lysate for sites on rabbit polyclonal antibody immobilized on a 96-well plate. Protein content in the cell lysate was determined using the Bradford dye reagent. For cAMP measurements, 50 μg of protein was used for sample analysis, which was performed according to the manufacturer's instructions.

Quantitative real-time polymerase chain reaction (PCR)

Total RNA was isolated from cells using RNA spin mini RNA isolation kit (GE Healthcare, Uppsala, Sweden) according to the manufacturer's instructions. cDNA was synthesized from 1 μg of total RNA using *Maxime* RT PreMix (Intron Biotechnology, Gyeonggi-do, Korea) and anchored oligo-dT_{15}-primers. Real-time PCR was performed using a Chromo4^{TM} instrument (Bio-Rad) and SYBR Green Master Mix (Applied Biosystems, Foster City, CA, USA). Relative amounts of target mRNA were determined using the comparative threshold (C_t) method by normalizing target mRNA C_t values to those for glyceraldehyde 3-phosphate dehydrogenase (ΔC_t).

Western blot analysis

Cells were harvested in ice-cold lysis buffer consisting of 1% Triton X-100, 1% deoxycholate, and 0.1% sodium dodecyl sulfate (SDS). The protein content of cell lysates was determined using the Bradford reagent. Protein amounts in each sample (50 μg total protein) were resolved by 7.5% SDS-polyacrylamide gel electrophoresis (SDS-PAGE), transferred to a polyvinylidene difluoride (PVDF) membrane, and exposed to the appropriate antibodies. Proteins were visualized using an enhanced chemiluminescence detection system (Amersham Biosciences, Piscataway, NJ, USA) and horseradish peroxidase-conjugated anti-rabbit or anti-mouse secondary antibodies. Images were acquired using an ImageQuant 350 analyzer (Amersham Biosciences, Uppsala, Sweden) and densitometry was performed using the ImageQuant TL software (Amersham Biosciences).

Statistical analysis

Data is expressed as mean [standard error (SE)]. Each experiment was repeated at least three times. Statistical analysis was performed using SPSS, version 16.0 software (SPSS, Inc., Chicago, IL, USA) to determine significant differences. We used either one- or two-way analysis of variance (ANOVA) followed by Dunn's post hoc tests for analyses. Values of $*p < 0.05$ were considered statistically significant.

Results

Ar-turmerone inhibits α-MSH- and IBMX-induced melanin synthesis and tyrosinase activity in B16F10 cells

The effect of ar-turmerone on melanogenesis, activated by α-MSH and IBMX with its intracellular signaling pathway, was examined. We determined whether ar-turmerone inhibited cellular melanin synthesis in α-MSH- and IBMX-stimulated cells. B16F10 mouse melanoma cells were exposed to 0.5 μM α-MSH and 0.1 mM IBMX in the presence of ar-turmerone or curcumin, and melanin synthesis was examined. As shown in Fig. 1a, ar-turmerone reduced the cellular melanin content in a dose-dependent manner. Since tyrosinase plays a key role in melanogenesis [21, 25] and tyrosinase activity is directly involved in abnormal accumulation of melanin pigments [30], the effect of ar-turmerone on tyrosinase activity was determined in α-MSH- and IBMX-induced cells (Fig. 1b). Tyrosinase activity in α-MSH and IBMX-stimulated cells was decreased by ar-turmerone in a dose-dependent manner, consistent with the suppression of melanin synthesis by ar-turmerone. We assayed the cell viability in the presence of ar-turmerone by treating B16F10 cells with ar-turmerone (0–40 μM) for 24, 48, and 72 h determined by MTT assay. Cell viability was not significantly affected by the presence of ar-turmerone (0–20 μM) for 48 h, as compared with the untreated control (Fig. 1c). To avoid inhibition of cell viability in the subsequent experiment, we

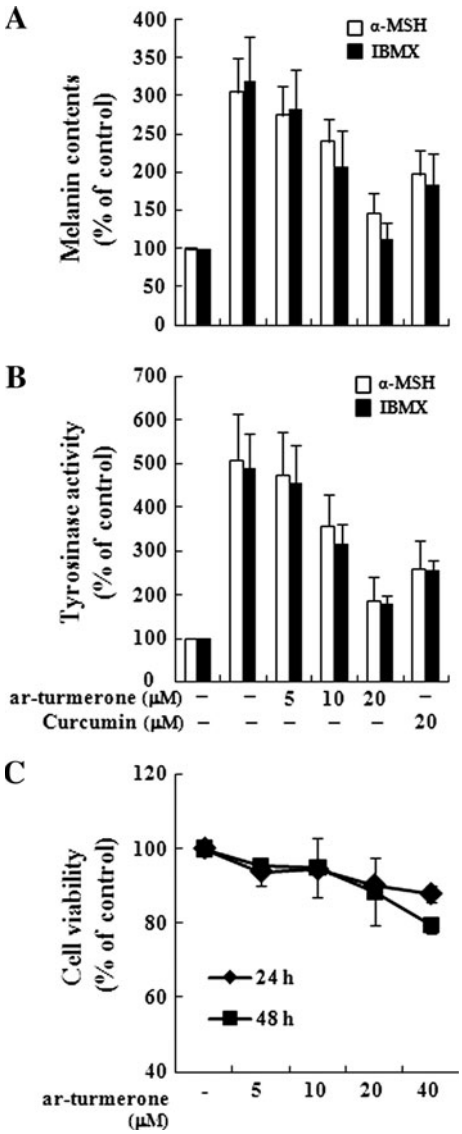

Fig. 1 Effect of ar-turmerone on α-MSH- and IBMX-induced cellular melanin synthesis, tyrosinase activity, and cell viability in B16F10 cells. Cells were incubated in the presence of various concentrations of ar-turmerone or curcumin (20 μM) for 2 h, and then stimulated with 0.5 μM of α-MSH and 0.1 mM of IBMX for 48 h. **a** Melanin content assay and **b** tyrosinase activity assay were conducted as described in "Materials and methods". **c** Cell viability was determined using an MTT assay. Values are given as the mean (SE) of at least three independent experiments. *$p < 0.05$ compared with α-MSH and IBMX alone groups

chose to use ar-turmerone concentrations between 5 and 20 μM and an incubation time of 48 h. These results suggest that ar-turmerone reduces melanin synthesis by inhibiting tyrosinase activity. Curcumin, a well-studied compound present in *C. longa*, was used for comparison. By treating cells with curcumin and ar-turmerone at equivalent concentrations of 20 μM, the inhibitory effect of ar-turmerone was shown to be more effective than that of curcumin. Collectively, these results demonstrate that ar-turmerone have an inhibitory effect on α-MSH- and IBMX-induced melanin synthesis and tyrosinase activity.

Inhibition of α-MSH and IBMX-induced expression of tyrosinase, TRP-1, and TRP-2 by ar-turmerone

Next, we examined the effect of ar-turmerone on expression of melanogenesis-related proteins, including tyrosinase, TRP-1, and TRP-2. TRP-1 and TRP-2 are involved in stabilizing tyrosinase and modulating its catalytic activity as well as in maintenance of melanosome structure. B16F10 cells were exposed to α-MSH and IBMX in the presence of ar-turmerone or curcumin and mRNA and protein extracts were subjected to western blot and real-time PCR analysis. Protein and mRNA levels of tyrosinase, TRP-1, and TRP-2 in α-MSH- and IBMX-stimulated cells were reduced by ar-turmerone treatment in a dose-dependent manner (Fig. 2). These results suggest that ar-turmerone inhibits α-MSH- and IBMX-induced melanogenesis by suppressing the transcription of tyrosinase, TRP-1, and TRP-2.

Inhibition of α-MSH- and IBMX-induced expression of MITF by ar-turmerone

MITF is an important transcription factor in the regulation of tyrosinase activity and expression of *Trp1* and *Trp2* genes. Tyrosinase, TRP-1, and TRP-2 harbor an MITF binding site, allowing for activation regulation of melanocyte differentiation [33]. MITF is a transcription factor and a member of the basic-helix-loop-helix-leucine-zipper family (b-HLH-LZ). This protein plays an important role in melanogenesis as the major transcriptional regulator of tyrosinase, TRP-1, and TRP-2 [7, 8]. To elucidate details of the melanogenesis signaling pathway, the effect of ar-turmerone on MITF expression was examined. B16F10 cells were exposed to α-MSH and IBMX in the presence of ar-turmerone or curcumin, and MITF expression was examined by western blot and real-time PCR analysis. Consistent with the effects on tyrosinase and TRP1 expression, MITF protein and mRNA levels in α-MSH- and IBMX-stimulated cells were decreased by ar-turmerone in a dose-dependent manner (Fig. 3). These results indicated that the inhibitory activity of ar-turmerone on melanogenesis is associated with the inhibition of tyrosinase, TRP-1, and TRP-2 expression resulting from decreased MITF expression.

Ar-turmerone inhibits tyrosinase expression through suppression of α-MSH- and IBMX-induced cAMP production and CREB phosphorylation

α-MSH binds to the melanocortin-1 receptor (MC1R) in melanocyte membranes [23, 26]. Stimulating MC1R with α-MSH increases the level of intracellular cAMP by

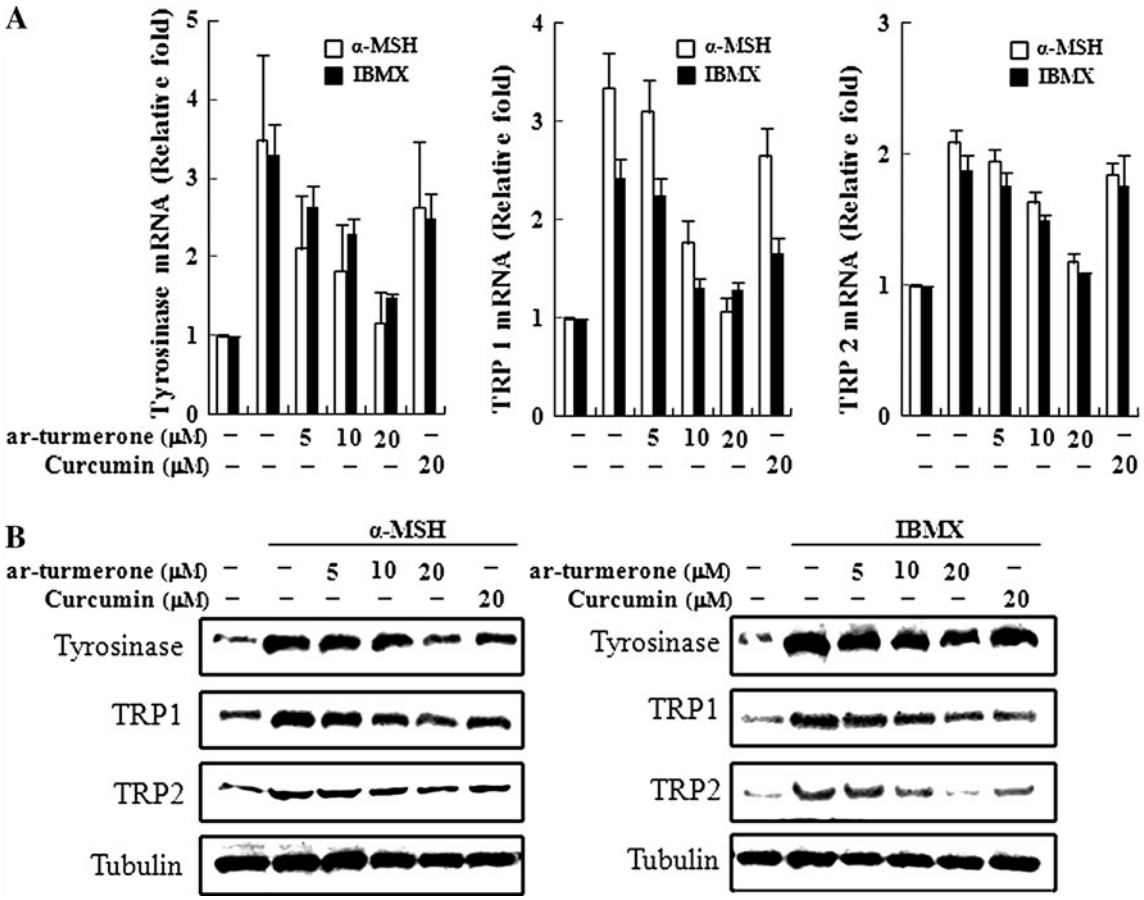

Fig. 2 Effect of ar-turmerone on expression of tyrosinase, TRP-1, and TRP-2 in α-MSH- and IBMX-induced B16F10 cells. **a** Cells were pre-treated with ar-turmerone or curcumin (20 μM) for 2 h, and then exposed to 0.5 μM of α-MSH and 0.1 mM of IBMX for 24 h. mRNA levels of tyrosinase, TRP-1, and TRP-2 were examined using real-time PCR. Relative tyrosinase, TRP-1, and TRP-2 mRNA expression ($2^{-\Delta C_t}$) was determined using real-time PCR and calculated by subtracting the C_t value for GAPDH from the C_t value for tyrosinase, TRP-1, and TRP-2 as determined using real-time RT-PCR relative to GAPDH mRNA ($\Delta C_t = C_t$ of tyrosinase, TRP-1, and TRP-2 C_t of GAPDH). The mRNA relative content is represented as the fold change over control values. Values are given as the mean (SE) of at least 3independent experiments. $^*p < 0.05$ compared with α-MSH and IBMX alone groups. **b** Cells were incubated with ar-turmerone or curcumin (20 μM) for 2 h, and then exposed to 0.5 μM of α-MSH and 0.1 mM of IBMX for 48 h. Tyrosinase, TRP-1, and TRP-2 protein expression decreased after exposure to ar-turmerone and curcumin as shown by western blot analysis. Western blot detection of α-tubulin was used as a protein loading control for each lane

activating adenylate cyclase [22]. cAMP activates protein kinase A, which is then able to phosphorylate and activate CREB [28]. Activated CREB binds to the *MITF* gene promoter and upregulates MITF expression [4]. As shown in Fig. 4 a and b, CREB was phosphorylated after α-MSH and IBMX treatment for 30 min, and ar-turmerone decreased α-MSH- and IBMX -induced CREB phosphorylation and cAMP levels. However, curcumin had no effect on α-MSH- and IBMX-induced CREB phosphorylation or cAMP levels. Next we further examined whether activation of CREB was involved in α-MSH induced tyrosinase, TRP-1 and TRP-2 expression in B16F10 cells, using a selective protein kinase A inhibitor (H89). B16F10 cells were pretreated with H89 for 1 h and then stimulated with α-MSH in the presence or absence of ar-turmerone for 48 h. As shown in Fig. 4c; H89 inhibited α-MSH induced tyrosinase, TRP-1 and TRP-2 expression; and combination treatment with H89 and ar-turmerone synergistically reduced α-MSH induced tyrosinase, TRP-1 and TRP-2 expression. Furthermore, H89 inhibited the α-MSH induced CREB phosphorylation and combination treatment with H89 and ar-turmerone synergistically reduced α-MSH induced CREB phosphorylation. This suggests that ar-turmerone suppresses MITF expression by inhibiting CREB activity, which in turn decreases the levels of tyrosinase, TRP-1, TRP-2, and melanogenesis.

Discussion

Numerous chemicals and cosmetic agents inhibit melanin biosynthesis by inhibiting tyrosinase catalytic activity.

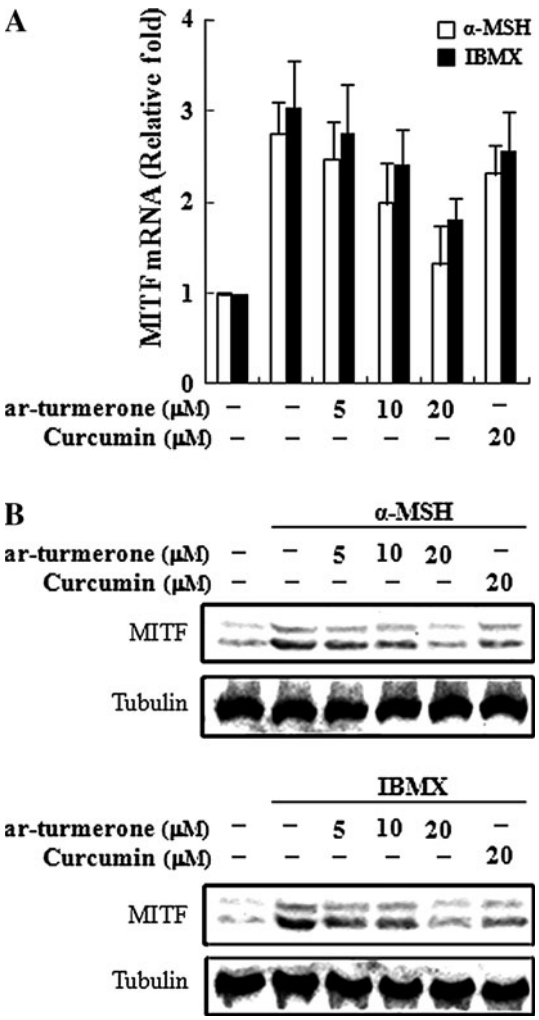

Fig. 3 Effect of ar-turmerone on MITF expression in α-MSH- and IBMX-induced B16F10 cells. **a** Cells were incubated with various concentrations of ar-turmerone or curcumin (20 μM) for 2 h, and then stimulated with 0.5 μM of α-MSH and 0.1 mM of IBMX for 24 h. mRNA levels of MITF were examined using real-time PCR. Relative MITF mRNA expression ($2^{-\Delta C_t}$) was determined using real-time PCR and calculated by subtracting the C_t value for GAPDH from the C_t value for MITF as determined using real-time RT-PCR relative to GAPDH mRNA ($\Delta C_t = C_t$ of MITF $- C_t$ of GAPDH). The mRNA relative content is represented as the fold change over control. Values are given as the mean (SE) of at least three independent experiments. $^*p < 0.05$ compared with α-MSH and IBMX alone groups. **b** Cells were incubated with ar-turmerone or curcumin (C, 20 μM) for 2 h, and then exposed to 0.5 μM of α-MSH and 0.1 mM of IBMX for 48 h. Western blot analysis shows that MITF protein expression was decreased. Western blot detection of α-tubulin was used as a protein loading control for each lane

A number of tyrosinase inhibitors are available from natural and synthetic sources, but only a few are used as skin-whitening agents because many inhibitors have drawbacks such as safety concerns and low activity [27]. To overcome these limitations, natural compounds derived from plant extracts were investigated. Ar-turmerone is an abundant component of turmeric, which has been traditionally used in cooking, medicines, cosmetic formulations, and fabric dying for over 2,000 years in Asia. Ar-turmerone has been shown to be highly biologically active and possesses anti-oxidant, anti-inflammatory, and anti-tumor properties. The safety of ar-turmerone is certified by traditional usage as well as our results (Fig. 1a). Thus, ar-turmerone can be employed as an agent in functional cosmetics to develop safe and effective skin-whitening treatments. Consistent with our results, partially purified *C. longa* has been reported to suppress α-MSH-stimulated melanogenesis [17]. In this study, utilizing B16F10 melanoma cell lines, the data presented here show that ar-trumerone suppressed α-MSH, IBMX-induced protein kinase A signaling in α-MSH, IBMX-induced melanin synthesis via tyrosinase, TRP-1 and TRP-2 expression. Nevertheless, further studies are needed to investigate the anti-melanogenic effect of ar-trumerone in animal models.

α-MSH and IBMX are crucial cAMP-elevating agents; these compounds exhibit differences in their mechanism of activity. α-MSH combines with its receptor, melanocortin 1 receptor (MC1R), and activates adenylate cyclase, which can increase the intracellular cAMP concentration. Compared with α-MSH, IBMX inhibits cAMP phosphodiesterase, increasing the intracellular cAMP concentration. Tyrosinase is a key enzyme in melanin synthesis and production and is primarily regulated by tyrosinase expression and activation. In this study, we detected an inhibitory effect of ar-turmerone on melanin synthesis and tyrosinase activity induced by α-MSH and IBMX. To rule out the possibility that the effect of ar-turmerone on α-MSH and IBMX stimulates melanin synthesis as a consequence of its cytotoxic effect, non-lethal concentrations (≤20μM) of ar-turmerone were used. A proper incubation time was essential in evaluating the effect of ar-turmerone on α-MSH- and IBMX-stimulated melanin synthesis. We incubated cells treated with varying concentrations of α-MSH and IBMX for various durations (data not shown). As shown in Fig. 1, ar-turmerone inhibits melanin synthesis and tyrosinase activity. To further explore the exact mechanism of ar-turmerone inhibition on α-MSH- and IBMX-induced melanogenesis, we performed western blotting and real-time RT-PCR to determine tyrosinase, TRP-1, and TRP-2 expression at the protein and mRNA levels. We found that ar-turmerone reduces mRNA and protein induction for tyrosinase, TRP-1, and TRP-2 in response to α-MSH and IBMX. Curcumin, well known for its antioxidative and anti-melanogenic effects, was used as a comparison control, and it significantly inhibited melanin synthesis and tyrosinase activity at a concentration of 20 μM. However, under these conditions, ar-turmerone is more effective than curcumin.

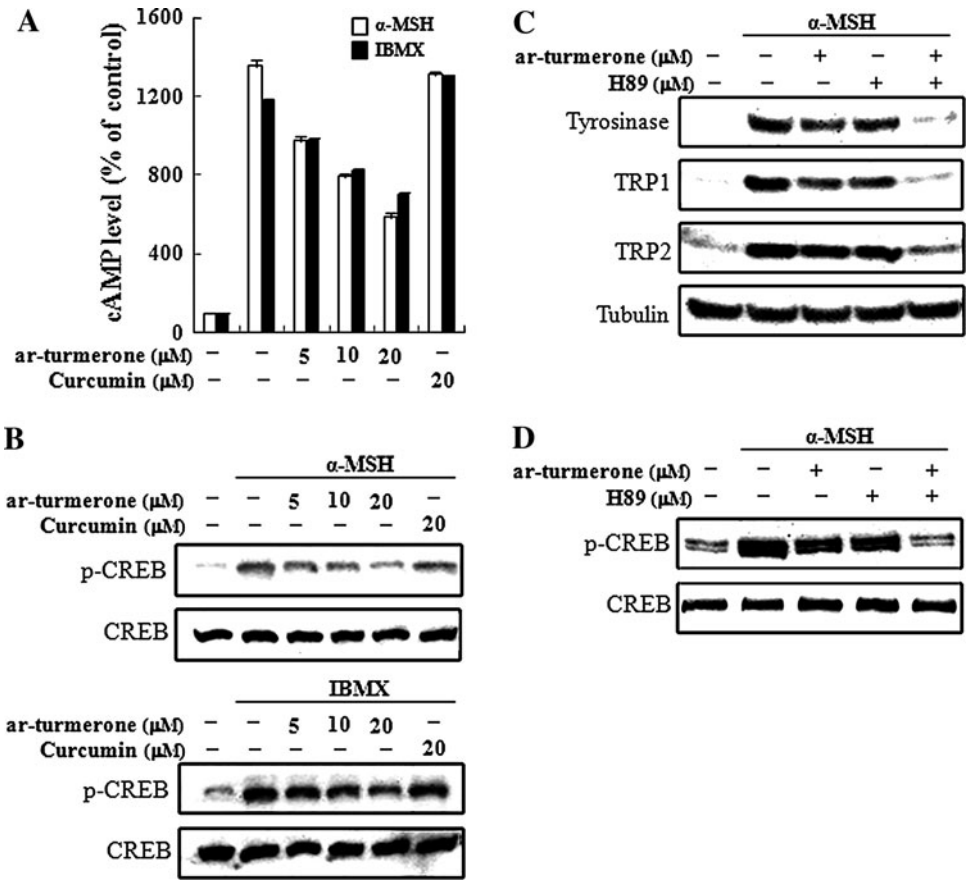

Fig. 4 Effect of ar-turmerone on CREB phosphorylation in α-MSH- and IBMX-induced B16F10 cells. **a** Cells pretreated with ar-turmerone were stimulated with 0.5 μM of α-MSH and 0.1 mM of IBMX for 0.5 h. The cAMP concentration was measured as described in "Materials and methods". **b** Cells were incubated with various concentrations of ar-turmerone or curcumin (20 μM) for 1 h, and then stimulated with 0.5 μM of α-MSH and 0.1 mM of IBMX for 0.5 h. Expression levels of phospho-CREB and CREB were examined by western blot analysis. Equal protein loading was confirmed by α-tubulin expression. **c** Cells were treated with H89 or ar-turmerone for 1 h, and then stimulated with 0.5 μM of α-MSH for 48 h. Expression levels of tyrosinase, TRP-1 and TRP-2 were examined by western blot analysis. **d** Cells were incubated with H89 or ar-turmerone for 1 h, and then stimulated with 0.5 μM of α-MSH for 0.5 h. Expression levels of phospho-CREB and CREB were examined by western blot analysis

MITF is a transcription factor present in numerous cell types and is an essential regulator in the tyrosinase gene family. Promoters of the *Tyr*, *Trp1*, and *Trp2* genes contain an MITF consensus E-box sequence. MITF shows high binding activity toward the E-box/M-box within the tyrosinase promoter and thus upregulates *Tyr* gene expression [21]. The MITF promoter contains DNA-binding sites for SOX10, PAX3, TCF/LEF, and CREB. In the MITF pathway, expression of MITF is modulated by several signaling pathways, such as the MAPK/ERK and PI3/AKT pathways. We further investigated the mechanism of MITF transcriptional regulation by ar-turmerone. We found that ar-turmerone significantly suppresses α-MSH- and IBMX-induced MITF mRNA and protein levels. In the MITF pathway, CREB phosphorylation activates MITF transcription, resulting in increased melanin synthesis. In addition, we found that treatment with curcumin does not significantly affect CREB phosphorylation and cAMP levels, whereas ar-turmerone decreases CREB phosphorylation and cAMP levels induced by α-MSH and IBMX in B16F10 melanoma cells. Thus, ar-turmerone inhibits α-MSH- and IBMX-stimulated melanogenesis at the transcriptional level for tyrosinase, TRP-1, TRP-2, and MITF, and influences cAMP dependent pathways.

In conclusion, ar-turmerone inhibits melanin synthesis in α-MSH- and IBMX-induced melanocytes by suppressing CREB activation and expression of MITF, tyrosinase, TRP-1, and TRP-2. On the basis of our results, we suggest that ar-turmerone is a potent hypopigmenting agent with implications in various dermatologic hyperpigmentation disorders, such as freckles and melasma, and has beneficial effects in whitening cosmetics.

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