Acta Biochim Biophys Sin 2011, 43: 763–770 | © The Author 2011. Published by ABBS Editorial Office in association with Oxford University Press on behalf of the Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences. DOI: 10.1093/abbs/gmr078.
Advance Access Publication 6 September 2011

Original Article

Sesamin induces melanogenesis by microphthalmia-associated transcription factor and tyrosinase up-regulation via cAMP signaling pathway

Zequn Jiang[1,2†*], Shasha Li[1†], Yunyi Liu[1†], Pengyi Deng[1], Jianguo Huang[1], and Guangyuan He[1*]

[1]China-UK HUST-RRes Genetic Engineering and Genomics Joint Laboratory, The Genetic Engineering International Cooperation Base of Chinese Ministry of Science and Technology, the Key Laboratory of Molecular Biophysics of Chinese Ministry of Education, College of Life Science and Technology, Huazhong University of Science and Technology, Wuhan 430074, China
[2]The Pre-clinical Medicine College, Nanjing University of Chinese Medicine, Nanjing 210046, China
[†]These authors contributed equally to this work.
*Correspondence address. Tel: +86-27-87792271; Fax: +86-27-85811930; E-mail: jiangjzq@sina.com (Z.J.)/hegy@hust.edu.cn (G.H.)

In this study, we confirmed that sesamin, an active lignan isolated from sesame seed and oil, is a novel skin-tanning compound. The melanin content and tyrosinase activity were increased by sesamin in a dose-dependent manner in B16 melanoma cells. The mRNA and protein levels of tyrosinase were also enhanced after the treatment with sesamin. Western blot analysis revealed that sesamin induced and sustained up-regulation of microphthalmia-associated transcription factor (MITF). Sesamin could activate cAMP response element (CRE) binding protein (CREB), but it had no effect on the phosphorylation of p38 mitogen-activated protein kinase (MAPK) or Akt. Moreover, sesamin activated protein kinase A (PKA) via a cAMP-dependent pathway. Consistent with these results, sesamin-mediated increase of melanin synthesis was reduced significantly by H-89, a PKA inhibitor, but not by SB203580, a p38 MAPK inhibitor or by LY294002, a phosphatidylinositol-3-kinase (PI3K) inhibitor. Sesamin-mediated phosphorylation of CREB and induction of MITF and tyrosinase expression were also inhibited by H-89. These findings indicated that sesamin could stimulate melanogenesis in B16 cells via the up-regulation of MITF and tyrosinase, which was, in turn, due to the activation of cAMP signaling.

Keywords B16 melanoma; cAMP; CREB; melanin; MITF

Received: April 6, 2011 Accepted: June 2, 2011

Introduction

Melanogenesis is a physiological process that results in the synthesis of melanin pigment and has many functions in living systems. It serves not only as the determinant of skin and hair color, but also as the natural protective agent against UV radiation. It also prevents sun-induced skin damage as well as skin cancer development [1,2].

In melanocytes and melanoma cells, melanin is synthesized via an enzymatic cascade regulated at the level of tyrosinase. This enzyme catalyses the rate-limiting steps of melanogenesis: namely, the hydroxylation of tyrosine to 3,4-dihydroxyphenylalanine (DOPA) and the oxidation of DOPA to dopaquinone. Thus, up-regulation of tyrosinase is proposed to be responsible for increased melanin production [3]. The most important transcription factor in the regulation of tyrosinase is the microphthalmia-associated transcription factor (MITF), which is involved in the pigmentation, proliferation, and survival of melanocytes [4,5]. MITF has been reported to bind to the M-box within the tyrosinase promoter and thus up-regulate tyrosinase gene expression [6].

Cyclic AMP (cAMP) is a key factor involved in the signal transduction pathways of melanogenesis. The principle intracellular target for cAMP in mammalian cells is the protein kinase A (PKA), which may phosphorylate serine and threonine residues on target proteins, such as the cAMP responsive element binding protein (CREB) and CREB binding protein (CBP). Phosphorylated CREB can interact with CBP to activate MITF, which stimulates tyrosinase gene expression to allow melanin synthesis [7,8].

The p38 mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K) signaling have also been suggested to be involved in the regulation of melanogenesis. The activation of p38 MAPK pathway has been recently demonstrated to activate MITF expression and promote tyrosinase transcription, which leads to the stimulation of melanin synthesis [9]. It has been reported that inhibition of PI3K/Akt signaling enhances melanogenesis by MITF phosphorylation and the following stimulation of tyrosinase expression [10–12].

Sesamin 2,6-(3,4-methylenedioxyphenyl)-cis-2,7-dioxabicyclo-[3.3.0]octane (**Fig. 1**) is a natural lignan extracted

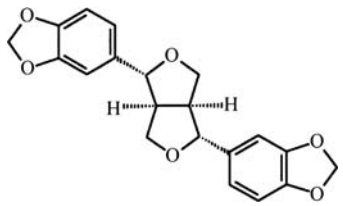

Figure 1 Chemical structure of sesamin

from sesame seed and oil. It has a surprisingly wide range of beneficial properties that include: cholesterol-lowering, anti-hypertensive, lipid-lowering and liver-protective activities [13–16]. During the course of our screening program for the development of pigmenting agents, we found that sesamin was a potent stimulator of melanogenesis, however, the mechanisms by which sesamin promotes melanogenesis remain unknown. In the present study, we tried to investigate the melanogenesis-inducing activities of sesamin and the mechanism of action in B16 melanoma cells.

Materials and Methods

Reagents

Triton X-100, L-dihydroxyphenylalanine (L-DOPA), α-melanocyte stimulating hormone (α-MSH), and forskolin were purchased from Sigma (St. Louis, USA). Tyrosinase (C-19), MITF, and actin antibodies were from Santa Cruz (Santa Cruz, USA). Antibodies against phospho-CREB (pCREB, Ser133), phospho-p38 MAPK (p-p38 MAPK, Thr180, and Tyr182), phospho-Akt (pAkt, Ser473) and CREB, p38 MAPK, Akt were acquired from Cell Signaling Technology (Danvers, USA). H-89, SB203580, and LY294002 were purchased from Beyotime Institute of Biotechnology (Shanghai, China). Sesamin (purity＞99%) was obtained from Qingze Biotechnology (Nanjing, China). Other reagents were of the highest quality available. Before use, sesamin, forskolin, α-MSH, H-89, SB203580, and LY294002 were all dissolved in DMSO and stored at −20°C.

Cell culture and treatment

Murine B16 melanoma cell line (B16F1) was obtained from CCTCC (China Center for Type Culture Collection, Wuhan, China). The B16F1 cells were cultured in Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin in a 5% CO_2 incubator at 37°C. In experiments using inhibitors such as H-89, SB203580, and LY294002 (the specific inhibitors of PKA, p38 MAPK, and PI3K, respectively), B16 melanoma cells were serum-starved for 24 h, and then either pre-treated with or without the inhibitor (10 μM) for 1 h before sesamin was applied for the indicated time at 10 μM.

Measurement of melanin content and microscopy

Melanin contents in cultured B16 melanoma cells were measured according to the method of Tsuboi *et al.* [17] with a slight modification. Briefly, B16 melanoma cells were plated at a density of 10^4 cells per well in 24-well culture dishes. After 1 day of culture, cells were treated with the indicated concentrations of sesamin or α-MSH (1 μM) for 48 h, and harvested by centrifugation for 10 min at 10,000 *g*. The cell pellets were then solubilized in 500 ml of 1 N NaOH at 80°C for 1 h, and spectrophotometric analysis of melanin content was performed at 400 nm absorbance. Before measuring the melanin content, the cells were observed under a phase contrast microscope (Olympus Optical Co., Japan) and photographed using a $CoolSNAP_{cf}$ digital video camera system supported by RS Image software (Pixera Penguin 150CL, USA).

Cell number counting and tyrosinase activity assays

After incubation with the indicated concentrations of sesamin for 48 h, the cells were harvested and the number of viable cells was counted using a hemocytometer. Analysis of tyrosinase activity by zymography was performed as reported by Laskin and Piccinini [18]. Briefly, B16 melanoma cells were treated with the indicated concentrations of sesamin or α-MSH (1 μM) for 24 h. Equal amounts of the cell lysates were mixed with Laemmli sample buffer without β-mercaptoethanol. Boiling was avoided and then proteins were resolved by 10% SDS–polyacrylamide gel electrophoresis (PAGE). After electrophoresis, gels were washed with 100 mM sodium phosphate buffer (PBS, pH 6.8) for 1 h by gentle shaking, and then incubated with 5 mM L-DOPA substrate at 37°C for 1 h. Protein bands that contained tyrosinase activity were visualized in the gels as dark bands.

Reverse transcription–polymerase chain reaction and western blot analysis

Total RNA was isolated from B16 cells using the Trizol reagent (GibcoBRL Life Technologies, Carlsbad, USA) according to the manufacturer's protocol. After preparing cDNA from the extracted RNA using oligo$(dT)_{16}$ as a reverse transcriptase primer, polymerase chain reaction (PCR) amplification was performed. The specific primers used for PCR were designed according to the report by Kim *et al.* [19]. *Tyrosinase*: upstream, 5′-GGCCAGCTTT CAGGCAGAGGT-3′; downstream, 5′-TGGTGCTTCATG GGCAAAATC-3′. *Actin*: upstream, 5′-CGAGCGGGAAA TCGTGCGTGACATTAAGGAGA-3′; downstream, 5′-C GTCATACTCCTGCTTGCTGATCCACATCTGC-3′. Amplification conditions were 94°C (60 s), 56°C (60 s),

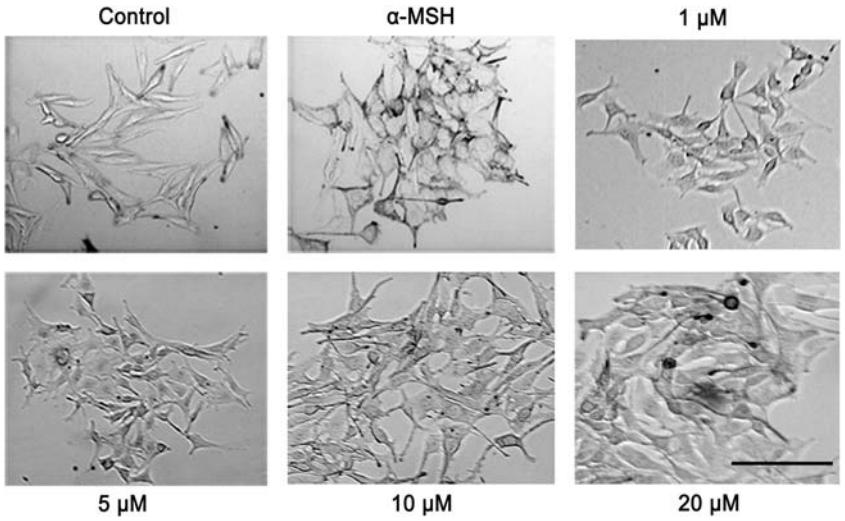

Figure 2 Sesamin-induced morphological changes in B16 melanoma cells Control cells were treated with DMSO, which at the highest dose (0.2%), or cultured with sesamin (1–20 μM) or α-MSH (1 μM) for 48 h. Pictures were taken under a phase contrast microscope using a digital video camera system supported by RS Image software. α-MSH was employed as a positive control.

and 72°C (60 s) for 26 cycles [19]. PCR products were analyzed by electrophoresis on 1.5% agarose gels stained with ethidium bromide.

For western blot analysis, cells were lysed with PBS containing 0.1% Triton X-100, 1 mM phenylmethylsulfonyl fluoride (PMSF), 1 mg/ml aprotinin, and 10 mg/ml leupeptin, followed by centrifugation at 12,000 rpm for 10 min. The total protein content of each supernatant was determined with a Bio-Rad protein assay kit. About 20 μg of protein per lane was then separated by SDS–PAGE and blotted onto nitrocellulose membranes. These membranes were subsequently blocked overnight with 5% non-fat dry milk in Tris-buffered saline (TBS) containing 0.1% Tween 20 (TBST). After three washes with TBST, membranes were incubated with an appropriate dilution of specific primary antibodies in TBST overnight at 4°C, and then further incubated with horseradish peroxidase-conjugated secondary antibodies. Protein bands were detected using an enhanced chemiluminescence plus kit (Pierce, Rockford, USA).

PKA activity assay and cyclic AMP measurement

The PKA activity was determined using PepTag non-radioactive PKA assay kit (Promega, Madison, USA) according to the manufacturer's instructions. Briefly, after induction with sesamin for 1 h, cells were lysed to release PKA and then PKA reaction solution was added. After incubating at 30°C for 30 min, the mixture was placed in a boiling water bath for 10 min to stop the reaction. Then the samples were separated on a 0.8% agarose gel at 100 V for 15 min. The intact agarose gel which reflected the activity of PKA can be analyzed in a scanning densitometer using a wavelength of ~570 nm.

Intracellular cAMP was measured using cAMP-Glo™ assay kit (Promega) according to the manufacturer's instructions. Briefly, after induction with sesamin or forskolin (1 μM) for 1 h, cells were lysed to release cAMP, and then the cAMP detection solution that contains PKA was added. The kinase reagent was added to terminate the reaction and detected the remaining ATP via a luciferase reaction. Plates were read using a microplate-reading luminometer. Luminescence could be correlated to the cAMP concentrations by using a cAMP standard curve.

Statistical analysis

Data were expressed as mean ± SD. Each experiment was performed in triplicate. The differences between results were assessed for significance using Student's *t*-test. $P < 0.05$ was considered statistically significant.

Results

Sesamin induces morphological changes in B16 melanoma cells

To determine the effect of sesamin on melanogenesis in B16 melanoma cells, cells were treated with sesamin at 1–20 μM. As shown in **Fig. 2**, sesamin induced dendritogenesis after 48 h when compared with untreated cells. Similar morphological changes were observed in B16 melanoma cells that were treated with α-MSH, a known melanoma differentiating agent.

Effect of sesamin on melanin amount and tyrosinase activity in B16 cells

Then we measured melanin contents in B16 cells after sesamin treatment. As shown in **Fig. 3(A)**, sesamin

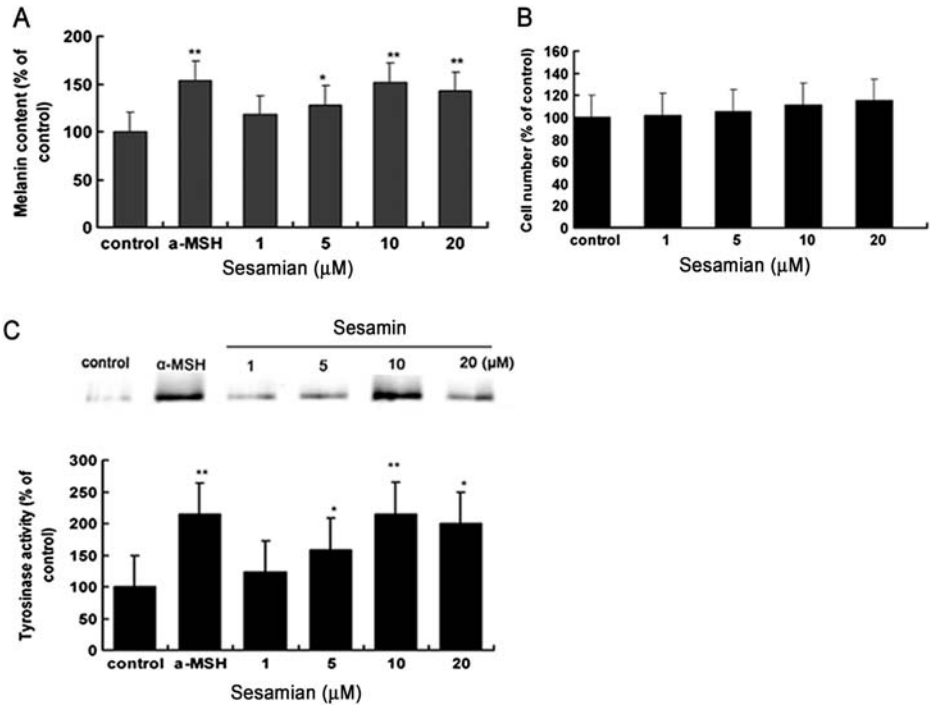

Figure 3 Effects of sesamin on melanogenesis in B16 cells After incubation of cells with various concentrations (1–20 μM) of sesamin for 48 h, melanin content (A) and cell number (B) were measured. α-MSH (1 μM) was employed as a positive control in melanin content assay. Control cells were treated with 0.2% DMSO only. (C) Effect of sesamin on tyrosinase activity in B16 cells. After B16 cells were treated with sesamin (1–20 μM) or α-MSH (1 μM) for 48 h, tyrosinase activity was determined by L-DOPA zymography. $*P < 0.05$, $**P < 0.01$, compared with untreated controls.

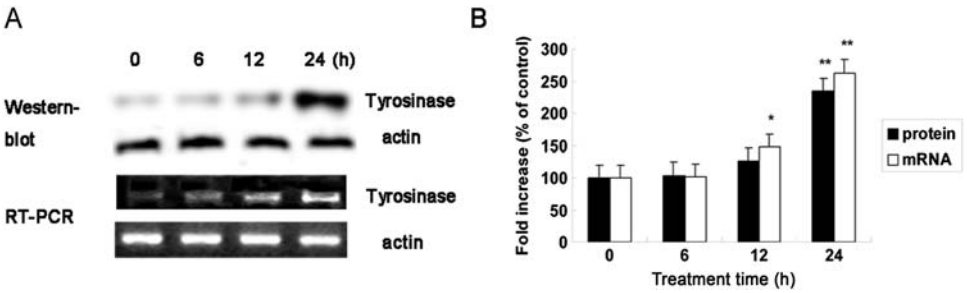

Figure 4 Effect of sesamin on tyrosinase expression in B16 cells After incubation of B16 melanoma cells with sesamin (10 μM) for the indicated time period (0–24 h), tyrosinase protein and mRNA expression were detected by western blot and RT-PCR, while β-actin was used as an internal control for comparable loading. Control cells were treated with 0.1% DMSO.

increased melanin content in a dose-dependent manner. To exclude the possibility that upsurges in melanin content may have been induced by the cell proliferating effect of sesamin, the cell number was calculated. No significant effect was found on cell proliferation at a concentration of 1–20 μM [**Fig. 3(B)**].

Moreover, we found sesamin at the same concentrations resulted in increasing degrees of tyrosinase activity in B16 cells [**Fig. 3(C)**]. We also noticed that beyond 10 μM of sesamin, melanin content, and tyrosinase activity did not increase significantly than that of 10 μM [**Fig. 3(A,C)**], and so we use 10 μM as an effective concentration of sesamin in the subsequent experiments.

Effect of sesamin on tyrosinase expression in B16 cells

In order to clarify the mechanism of tyrosinase activation by sesamin, the levels of tyrosinase expression in B16 cells were examined by reverse transcription (RT)-PCR and western blot analysis. As expected, it was found that mRNAs of tyrosinase were clearly increased after 12–24 h of sesamin (10 μM) treatment and tyrosinase protein levels were also up-regulated in a time-dependent manner (**Fig. 4**).

Sesamin induces the persistent activation of cyclic AMP response element binding protein (CREB) and increases MITF protein levels

As sesamin increased melanin synthesis and tyrosinase expression, we further hypothesize that sesamin may affect

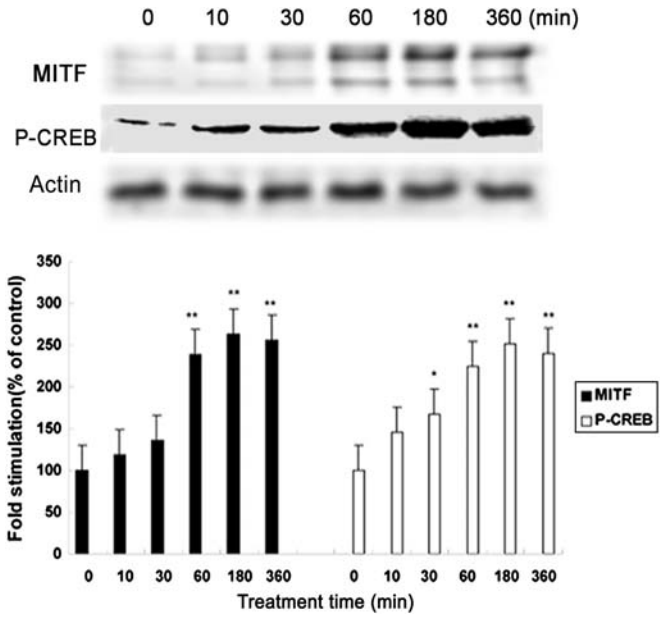

Figure 5 Sesamin induces CREB activation, and up-regulates MITF After incubation of B16 cells with sesamin (10 μM) for the indicated time period (0−360 min), whole-cell lysates were subjected to western blot analysis using antibodies against MITF, phospho-specific CREB (P-CREB), and β-actin. P-CREB antibody recognizes specifically CREB when phosphorylated on serine 133. Fold increases over the control were determined by densitometric analysis. $*P < 0.05$, $**P < 0.01$, compared with untreated controls.

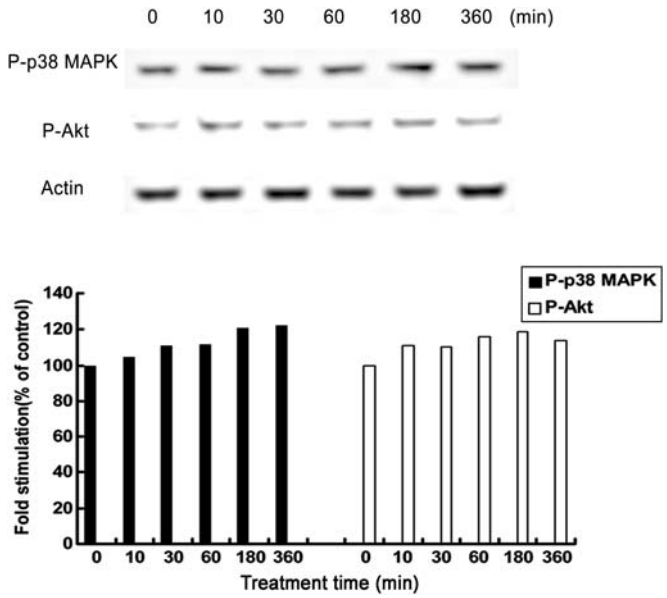

Figure 6 Effect of sesamin on p38 MAPK or Akt phosphorylation in B16 cells Cells were treated with 0.1% DMSO or with 10 μM sesamin for the indicated time period (0−360 min), and the phosphorylation of p38 MAPK and Akt were measured by western blot analysis. Fold increases over the control were determined by densitometric analysis. $*P < 0.05$, $**P < 0.01$, compared with untreated controls.

the expression of MITF, which plays an important role in tyrosinase gene expression and melanogenesis. To test this hypothesis, we measured MITF levels after sesamin (10 μM) treatment. Our results showed that MITF protein was clearly enhanced from 60−360 min after sesamin addition (**Fig. 5**).

To investigate whether sesamin activates cAMP signaling in B16 cells, western blot analysis was carried out to detect the phosphorylation level of CREB, which is known to be involved in tyrosinase activation through up-regulation of MITF gene expression. As shown in **Fig. 5**, sesamin increased the phosphorylation level of CREB in a time-dependent manner, indicating the involvement of sesamin in cAMP signaling. Moreover, the change trend of MITF corresponded to strong CREB phosphorylation. Similar results have also been found in forskolin treatment of NHM, which induced a rapid increase in phosphorylated CREB and transcription of MITF [20]. Collectively, all these results revealed that activated CREB confered cAMP responsiveness to the MITF promoter [21,22].

Sesamin has no effect on p38 MAPK or Akt phosphorylation in B16 cells

Besides cAMP signaling, activation of p38 MAPK or inhibition of PI3K has also been reported to induce melanogenesis. Therefore, in this study, we designed an experiment to elucidate whether sesamin could induce the phosphorylation of p38 MAPK, or whether it is capable of stimulating a reduction of Akt phosphorylation in B16 melanoma cells. As shown in **Fig. 6**, sesamin neither induced p38 MAPK phosphorylation, nor inhibited Akt phosphorylation. These results suggested that sesamin might induce melanogenesis through cAMP signaling, not through p38 MAPK activation or PI3K inhibition.

Sesamin stimulated cAMP production and PKA activation

The cAMP signaling pathway leading to melanin synthesis is known to be represented by cAMP accumulation followed by PKA activation. In order to confirm this, we performed cAMP production and PKA activity assays. As shown in **Fig. 7**, sesamin increased intracellular cAMP concentration and PKA activity in a dose-dependent manner in B16 cells. In addition, H-89, a PKA inhibitor, was shown to clearly inhibit the increase of cAMP and activation of PKA. In some reports, treatment of cells with H89 also caused a decrease in cAMP accumulation. For example, it has been reported that the rise of cAMP induced by leptin or gAd is also inhibited by H89 in murine macrophages [23]. The only explanation is that H89 may act by inhibiting adenylyl cyclase (AC) or activating phosphodiesterase (PDE), the upstream enzymes of cAMP.

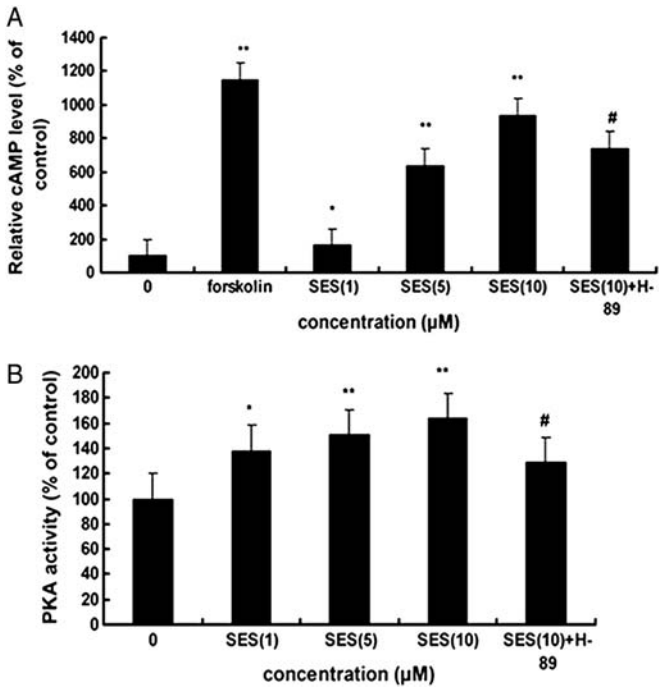

Figure 7 Sesamin activates PKA and increases cAMP levels in B16 cells Cells were pre-treated or not with 10 μM H-89 for 1 h before sesamin (SES, 1–10 μM) was applied for 1 h. Control cells were treated with 0.1% DMSO. (A) cAMP levels and (B) PKA activity were measured. Forskolin (1 μM) was employed as a positive control in intracellular cAMP assay. $^{*}P < 0.05$, $^{**}P < 0.01$, compared with untreated controls; $^{\#}P < 0.05$, compared with SES(10) group.

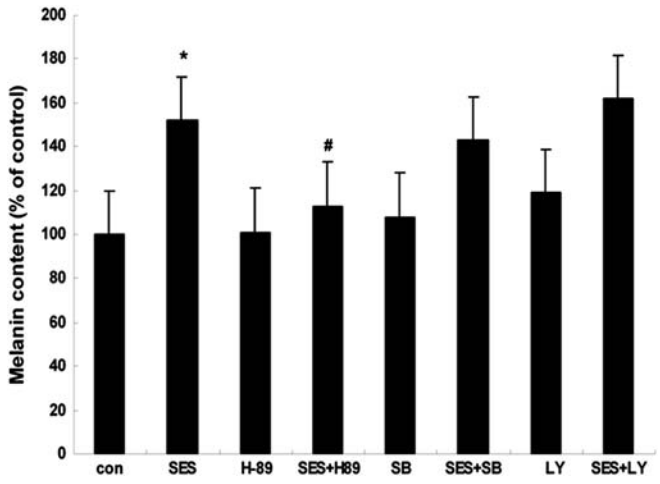

Figure 8 Effects of PKA, p38 MAPK, and PI3K inhibitors on sesamin-induced melanogenesis in B16 cells After 24 h serum starvation, cells were pre-treated with or without H-89 (H-89, 10 μM), SB203580 (SB, 10 μM), and LY294002 (LY, 10 μM) for 1 h before sesamin (10 μM) was applied for 48 h. Melanin contents were measured. Control cells were treated with 0.1% DMSO. $^{*}P < 0.05$, $^{**}P < 0.01$, compared with untreated controls; $^{\#}P < 0.05$, compared with '*' marked group.

Sesamin-induced melanin synthesis is attenuated by H-89, a PKA inhibitor

To confirm the link between the cAMP pathway and sesamin-induced melanogenesis, we used several inhibitors, including H-89 (PKA inhibitor), SB203580 (p38 MAPK inhibitor), and LY294002 (PI3K inhibitor), to perform melanin content assay. We found that the increase of melanin content by sesamin was completely attenuated by H-89, while SB203580 and LY294002 had no such effect (**Fig. 8**). Then, a western blot for CREB phosphorylation, MITF, and tyrosinase expression was performed using H-89. As shown in **Fig. 9(A)**, H-89 clearly diminished sesamin-induced phosphorylation of CREB. The parallel up-regulation of MITF and tyrosinase expression was also abrogated by H-89 [**Fig. 9(B)**]. Therefore, we concluded that it is through the cAMP–PKA pathway that sesamin induced melanogenesis in B16 cells.

Discussion

Many research groups are investigating the regulation of skin pigmentation with the goal of developing tanning cosmetics to reduce skin cancer risk, and also to elucidate the mechanisms of pigmentary disorders to cure or prevent those diseases. In this study, we found that sesamin, a potent melanogenic stimulator, could induce melanin synthesis and dendritogenesis by the cAMP–PKA signaling pathway in B16 melanoma cells.

It was concluded that the dendric morphology of the melanoma cells treated with sesamin became more pronounced when compared with the untreated cells, suggested that melanoma cell differentiation had been induced. Melanin content and tyrosinase activity were increased in response to treatment with sesamin in a concentration-dependent manner, and these changes were accompanied by an increase in the mRNA and protein levels of tyrosinase expression. Thus, it was suggested that one of the mechanisms for sesamin-induced melanogenesis might attribute to tyrosinase activation upon the signaling pathway regulating tyrosinase levels.

On the other hand, many reports have suggested that the cAMP signaling pathway plays a critical role in the regulation of melanogenesis. It has been reported that cAMP-activating agents, such as ACTH and a-MSH, are involved in regulating melanogenesis or melanocyte dendrite formation through the activation of cAMP/PKA [24,25]. Forskolin, another cAMP elevating agent, by manipulation of the cAMP pathway, could induce melanin in the epidermis of a fair-skinned mouse [26]. Moreover, cAMP stimulates melanogenesis mainly via activation of MITF, and leads to induction of tyrosinase expression [22]. Besides, PKA has been reported to be involved in cAMP-induced melanogenesis through the activation of CREB phosphorylation, which consequently raises the expression level of MITF [24].

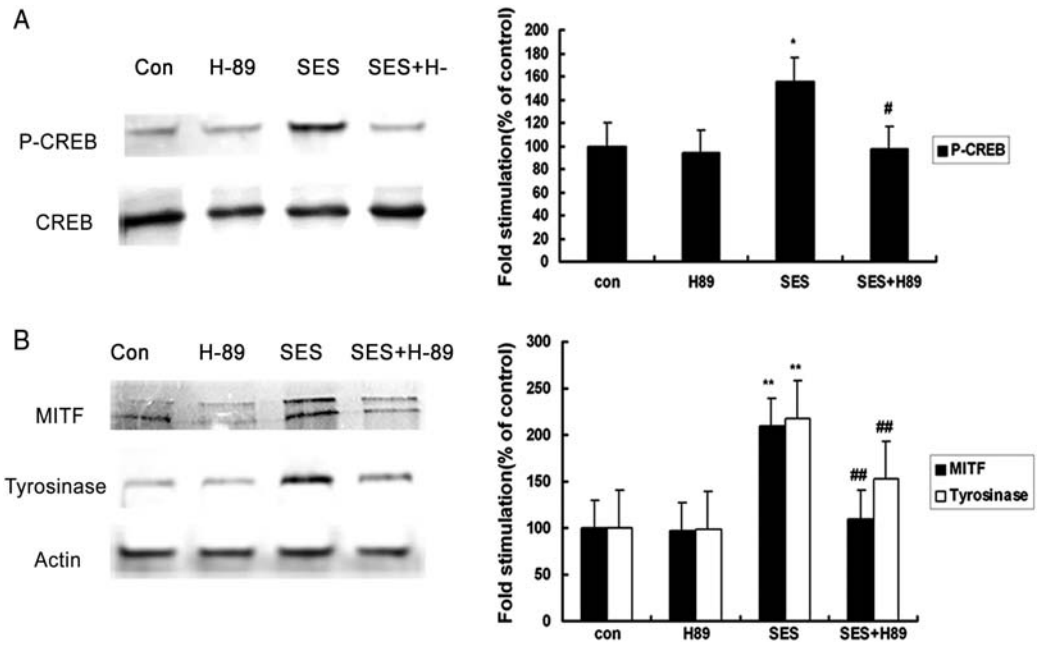

Figure 9 Effect of H-89 on the expression of MITF and tyrosinase and CREB phosphorylation in sesamin-induced B16 cells Cells were starved in serum-free medium for 24 h, and then pre-treated with or without H-89 (10 μM) for 1 h before sesamin (SES) was applied for 24 h at 10 μM. Control cells were treated with 0.1% DMSO. The expression levels of MITF and tyrosinase (B) and CREB phosphorylation (A) were examined by western blot. Equal protein loading was confirmed by actin expression. $^{*}P < 0.05$, $^{**}P < 0.01$, compared with untreated controls; $^{\#}P < 0.05$, compared with '*' marked group; $^{\#\#}P < 0.01$ compared with '**' marked group.

In agreement with these studies, we found that sesamin triggered an increase in MITF protein level and CREB phosphorylation, indicating the involvement of sesamin in cAMP signaling. This was further strengthened by the result that a dose-dependent increase in cAMP level and PKA activity was induced by sesamin. We also found that H-89 (a PKA inhibitor) inhibited the increase of cAMP and PKA activity induced by sesamin. The level of intracellular cAMP is regulated by two types of enzyme: AC and the cyclic nucleotide PDE. Thus, it can be hypothesized that sesamin may elevate cytoplasmic cAMP levels and mediate PKA activation by directly activating AC or inhibiting PDE. It is also possible that H89 facilitates the phosphorylation of AC or dephosphorylation of PDE to inhibit cAMP synthesis. These needs further investigation, however.

A previous study has revealed that glycyrrhizin induces melanogenesis by cAMP signaling, which can be blocked by H-89, a PKA inhibitor [27]. In our present study, H-89 was also found to significantly attenuate the sesamin-induced increase in melanin content. The increase of sesamin-induced CREB phosphorylation, MITF, and tyrosinase gene expression was abolished by H-89. All these results indicated the involvement of cAMP signaling in sesamin-induced melanogenesis in B16 melanoma cells.

It was suggested that sesamin-induced melanin synthesis via activation of the cAMP–PKA–CREB signaling cascade and consequent up-regulation of MITF and tyrosinase expression. In addition to cAMP signaling, previous studies have shown that p38 MAPK or PI3K signaling is also related to melanogenesis in B16 melanoma cells. We have reported that activation of p38 MAPK plays an important role in THSG-induced B16F1 melanogenesis by up-regulating MITF and tyrosinase expression [28]. PI3K/Akt activation by PPC has recently been found to reduce melanin synthesis by suppressing MITF and its downstream signal pathway including tyrosinase and TRPs in a-MSH-induced melanogenesis [28]. However, we found no difference in phospho-p38 MAPK or phospho-Akt levels after sesamin treatment in B16 cells, which suggested that neither the p38 MAPK nor the PI3K/Akt pathway plays any role in the sesamin-induced stimulation of melanogenesis.

In summary, this is the first study to demonstrate the pigmentation effect of sesamin and identify the molecular mechanism by which sesamin induces melanogenesis. Until recently, it was thought that the higher the melanin content existed, the less chance of DNA damage resulted from UV-R exposure. Another role of melanin in the skin is to neutralize the ROS generated by a variety of factors, including UV-R [29], and therefore functions like a natural sunscreen. In this sense, if sesamin really could increase melanin levels in skin, it might be very useful as a sunscreen without inflicting any damage to the skin or any undesirable changes in the metabolism. Therefore, shedding light on the molecular mechanisms underlying hyperpigmentation

induced by sesamin could be applied to various ends, to enhance photoprotection and consequently to decrease photoaging and photocarcinogenesis.

Funding

This study was supported by grants from the Ministry of Science and Technology of China (2005CCA1900) and the National Natural Science Foundation of China (30640043).

References

1 Sturm RA. Skin colour and skin cancer-MC1R, the genetic link. Melanoma Res 2002, 12: 405–516.
2 Eller MS, Yaar M and Gilchrest BA. DNA damage and melanogenesis. Nature 1994, 372: 413–414.
3 Hearing V and Tsukamoto K. Enzymatic control of pigmentation in mammals. FASEB J 1991, 5: 2902–2909.
4 Hodgkinson CA, Moore KJ, Nakayama A, Steingrímsson E, Copeland NG, Jenkins NA and Arnheiter H. Mutations at the mouse microphthalmia locus are associated with defects in a gene encoding a novel basic-helix-loop-helix-zipper protein. Cell 1993, 74: 395–404.
5 Steingrímsson E, Moore KJ, Lamoreux ML, Ferré-D'Amaré AR, Burley SK, Zimring DC and Skow LC, *et al*. Molecular basis of mouse microphthalmia (mi) mutations helps explain their developmental and phenotypic consequences. Nat Genet 1994, 8: 256–263.
6 Bentley N, Eisen T and Goding C. Melanocyte-specific expression of the human tyrosinase promoter: activation by the microphthalmia gene product and role of the initiator. Mol Cell Biol 1994, 14: 7996–8006.
7 Saito H, Yasumoto KI, Takeda K, Takahashi K, Yamamoto H and Shibahara S. Microphthalmia associated transcription factor in the wnt signaling pathway. Pigment Cell Res 2003, 16: 261–265.
8 Widlund HR and Fisher DE. Microphthalamia-associated transcription factor: a critical regulator of pigment cell development and survival. Oncogene 2003, 22: 3035–3041.
9 Hirata N, Naruto S, Ohguchi K, Akao Y, Nozawa Y, Iinuma M and Matsuda H. Mechanism of the melanogenesis stimulation activity of (-)-cubebin in murine B16 melanoma cells. Bioorg Med Chem 2007, 15: 4897–4902.
10 Khaled M, Larribere L, Bille K, Aberdam E, Ortonne JP, Ballotti R and Bertolotto C. Glycogen synthase kinase 3 is activated by cAMP and plays an active role in the regulation of melanogenesis. J Biol Chem 2002, 277: 33690–33697.
11 Khaled M, Larribere L, Bille K, Ortonne JP, Ballotti R and Bertolotto C. Microphthalmia associated transcription factor is a target of the phosphatidylinositol-3-kinase pathway. J Invest Dermatol 2003, 121: 831–836.
12 Lee J, Jung K, Kim YS and Park D. Diosgenin inhibits melanogenesis through the activation of phosphatidylinositol-3-kinase pathway (PI3K) signaling. Life Sci 2007, 81: 249–254.
13 Hirose N, Inoue T, Nishihara K, Sugano M, Akimoto K, Shimizu S and Yamada H. Inhibition of cholesterol absorption and synthesis in rats by sesamin. J Lipid Res 1991, 32: 629–638.
14 Hirata F, Fujita K, Ishikura Y, Hosoda K, Ishikawa T and Nakamura H. Hypocholesterolemic effect of sesame lignan in humans. Atherosclerosis 1996, 122: 135–136.
15 Akimoto K, Kitagawa Y, Akamatsu T, Hirose N, Sugano M, Shimizu S and Yamada H. Protective effects of sesamin against liver damage caused by alcohol or carbon tetrachloride in rodents. Ann Nutr Metab 1993, 37: 218–224.
16 Ashakumary L, Rouyer I, Takahashi Y and Ide T. Sesamin, a sesame lignan, is a potent inducer of hepatic fatty acid oxidation in the rat. Metabolism 1999, 48: 1303–1313.
17 Tsuboi T, Kondoh H, Hiratsuka J and Mishima Y. Enhanced melanogenesis induced by tyrosinase gene transfer increases boron uptake and killing effect of boron neutron capture therapy for amelanotic melanoma. Pigment Cell Res 1998, 11: 275–282.
18 Laskin J and Piccinini L. Tyrosinase isozyme heterogeneity in differentiating B16/C3 melanoma. J Biol Chem 1986, 261: 16626–16635.
19 Kim HJ, Cho YD, Leem KH, Lee DN, Kim EH, Kim MG and Kim DK, *et al*. Effects of ephedrae herba on melanogenesis and gene expression profiles using cDNA microarray in B16 melanocytes. Phytotherapy Res 2006, 20: 748–754.
20 Newton RA, Cook AL, Roberts DW, Leonard JH and Sturm RA. Post-transcriptional regulation of melanin biosynthetic enzymes by cAMP and resveratrol in human melanocytes. J Invest Dermatol 2007, 127: 2216–2227.
21 Price ER, Horstmann MA, Wells AG, Weilbaecher KN, Takemoto CM and MW L. Alpha-Melanocyte-stimulating hormone signaling regulates expression of microphthalmia, agenedeficientin Waarden-burgsyndrome. J Biol Chem 1998, 273: 33042–33047.
22 Busca R and Ballotti R. Cyclic AMP a key messenger in the regulation of skin pigmentation. Pigment Cell Res. 2000, 13: 60–69.
23 Zhao T, Hou M, Xia M, Wang Q, Zhu H, Xiao Y and Tang Z, *et al*. Globular adiponectin decreases leptin-induced tumor necrosis factor-[alpha] expression by murine macrophages: Involvement of cAMP-PKA and MAPK pathways. Cell Immunol 2005, 238: 19–30.
24 Sassone-Corsi P. Coupling gene expression to cAMP signalling: role of CREB and CREM. Int J Biochem Cell Biol 1998, 30: 27–38.
25 Hunt G, Todd C, Cresswell J and Thody A. Alpha-melanocyte stimulating hormone and its analogue Nle4DPhe7 alpha-MSH affect morphology, tyrosinase activity and melanogenesis in cultured human melanocytes. J Cell Sci 1994, 107: 205–211.
26 D'Orazio JA, Nobuhisa T, Cui R, Arya M, Spry M, Wakamatsu K and Igras V, *et al*. Topical drug rescue strategy and skin protection based on the role of Mc1r in UV-induced tanning. Nature 2006, 443: 340–344.
27 Lee J, Jung E, Park J, Jung K, Park E, Kim J and Hong S, *et al*. Glycyrrhizin induces melanogenesis by elevating a cAMP level in B16 melanoma cells. J Invest Dermatol 2005, 124: 405–411.
28 Jang JY, Lee JH, Jeong SY, Chung KT, Choi YH and Choi BT. Partially purified Curcuma longa inhibits alpha melanocyte stimulating hormone stimulated melanogenesis through extracellular signal regulated kinase or Akt activation mediated signalling in B16F10 cells. Exp Dermatol 2009, 18: 689–694.
29 Sarna T. New trends in photobiology: properties and function of the ocular melanin—a photobiophysical view. J Photochem Photobiol B 1992, 12: 215–258.