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Sargaquinoic acid ameliorates hyperpigmentation through cAMP and ERK-mediated downregulation of MITF in α-MSH-stimulated B16F10 cells

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ABSTRACT

Hyperpigmentation disorders of the skin adversely influence the quality of life. We previously demonstrated the hypopigmenting properties of the ethanolic extract from *Sargassum serratifolium* and identified sargaquinoic acid (SQA) as an active component. The current study aims to investigate the hypopigmenting action of SQA in α-melanocyte stimulating hormone (α-MSH)-stimulated B16F10 cells. SQA attenuated cellular melanin synthesis by inhibiting the expression of the melanogenic enzymes, including tyrosinase (TYR), tyrosinase-related protein 1 (TRP1), and TRP2. SQA also inhibited cellular TYR activity in a dose-dependent manner. Reduced intracellular cAMP accumulation by SQA treatment resulted in the suppressed phosphorylation of cAMP-responsive element-binding protein (CREB), leading to the downregulation of microphthalmia-associated transcription factor (MITF) in α-MSH-stimulated B16F10 cells. SQA increased the phosphorylation of extracellular signal-regulated kinase (ERK)1/2 and MITF (Ser73), inducing proteasomal degradation of MITF. SQA showed high binding affinity to the cAMP binding domain of PKA; the direct binding of SQA to PKA may exert an additional inhibitory effect on the PKA-dependent CREB activation. Our data demonstrated that SQA suppressed melanin production through the cAMP/CREB- and ERK1/2-mediated downregulation of MITF in α-MSH-stimulated B16F10 cells and SQA has a potential therapeutic agent for the treatment of skin hyperpigmentation disorders.

1. Introduction

Hyperpigmentation is the over-production of melanin in different parts of the body and causes visible skin pigmentation disorders such as melasma, freckles, moles, and lentigo [1]. Undesirable hyperpigmentation is primarily associated with solar radiation, inflammation, genetic predisposition, hormonal factors, drugs, and cosmetics [2,3]. Hypopigmentation treatment has become a major concern for Asian women to maintain skin whitening. Thus, natural skin whitening compounds having efficacy and safety are constantly attracted in the cosmetic and pharmaceutical industries.

Various intracellular signaling pathways are associated with the regulation of skin pigmentation. Among them, cyclic adenosine monophosphate (cAMP)/cAMP-responsive element-binding protein (CREB) signaling [4], mitogen-activated protein kinase kinase (MEK)/extracellular signal-regulated kinase (ERK), phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt)/glycogen synthase kinase 3β (GSK3β) [5], Akt/GSK3β/β-catenin [6], p38 mitogen-activated protein kinase (MAPK) [7], and c-Jun N-terminal kinase (JNK) [8] signaling pathways are well-characterized hyperpigmentation pathways. In melanogenic signaling pathways, the microphthalmia-associated transcription factor (MITF) plays a central role in the expression of melanogenic enzymes, including tyrosinase (TYR), tyrosinase-related protein 1 (TRP1), and TRP2 [9]. MITF is also considered as an essential regulator for the differentiation, proliferation, and survival of melanocytes [9]. cAMP is a vital player in the modulation of MITF expression. Increased cAMP activates protein kinase A (PKA) and stimulates the CREB-mediated transcriptional activation of MITF. cAMP also modulates the post-translational degradation of MITF. The intracellular elevation of cAMP activates ERK, leading to direct phosphorylation of Ser73 in MITF or

Abbreviations: α-MSH, α-melanocyte stimulating hormone; BISA, bisabolangelone; cAMP, cyclic adenosine monophosphate; CREB, cAMP-responsive element-binding protein; DOPA, 3, 4-dihydroxyphenylalanine; ESS, ethanolic extract of *Sargassum serratifolium*; ERK, extracellular signal-regulated kinase; GSK3β, glycogen synthase kinase 3β; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; MEK, mitogen-activated protein kinase kinase; MITF, microphthalmia-associated transcription factor; PI3K, phosphoinositide 3-kinase; PKA, protein kinase A; PKA(Cα), catalytic subunit α of PKA; PKA (RIIβ), regulatory subunit IIβ of PKA; RSK, ribosomal S6 kinase; SQA, sargaquinoic acid; TRP, tyrosinase-related protein; TYR, tyrosinas

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indirect phosphorylation of Ser409 in MITF via activation of ribosomal S6 kinase (RSK), leading to proteasomal degradation of both phosphorylated MITF [10]. cAMP also regulates MITF through the inhibition of PI3K/Akt phosphorylation, leading to the activation of GSK3β. Activated GSK3β phosphorylates MITF on Ser298; this modification increases MITF binding to the TYR promoter to stimulate melanogenesis [11]. MITF is therefore the ultimate target of multiple signaling pathways and is recognized as the master regulator of melanogenic enzymes.

Sargassum serratifolium is an edible perennial brown alga of Sargassaceae family found throughout the coast of Korea and Japan. Brown algae are reported to be a rich source of many health beneficial compounds, including phlorotannins [12–14] and meroterpinoids [15]. Recently, we investigated the hypopigmenting properties of the ethanolic extract of *S. serratifolium* [16] and identified sargaquinoic acid (SQA), a meroterpenoid compound, as a potent anti-melanogenic component in *S. serratifolium*. SQA has several biological activities including anti-inflammatory [17–19], anti-carcinogenic [20], anti-adipogenic [21], and neuroprotective [22] activities. However, no study has yet described its hypopigmenting mechanisms in vitro model. Here, we investigated the hypopigmentation mechanisms of SQA in α-melanocyte stimulating hormone (α-MSH)-treated mouse melanoma cells B16F10.

2. Materials and methods

2.1. Materials

SQA (Fig. 1A) was isolated from the brown alga, *S. serratifolium* as described previously [18] and its purity (> 98%) was determined by HPLC. Dulbecco's modified Eagle's medium (DMEM) was purchased from WELGENE Inc. (Gyeongsan-si, South Korea). Fetal bovine serum (FBS), penicillin-streptomycin and trypsin-EDTA were from GenDEPOT Inc. (Barker, TX, USA). Arbutin, α-MSH, L-3, 4-dihydroxyphenylalanine (L-DOPA), and dimethyl sulfoxide (DMSO) were from Sigma-Aldrich, Co. (St. Louis, MO, USA). CellTiter 96® AQueous One Solution Cell Proliferation assay kit was bought from Promega (Madison, WI, USA). BCA protein assay kit, NE-PER nuclear and cytoplasmic extraction reagents, and enhanced chemiluminescence (ECL) detection kit were from Thermo Scientific (Rockford, IL, USA). MEK/ERK-specific inhibitor PD98059, protein G Sepharose, primary antibodies for PKA (RIIβ) and MITF were purchased from Abcam (Cambridge, MA, USA). The primary antibodies for p-Akt (Ser473), p-ERK1/2, p38 MAPK, p-p38 MAPK, PKA (Cα) and proteasome inhibitor, MG-132 were from Cell Signaling Technology Inc. (Beverly, MA, USA). The primary antibody against p-MITF (Ser73) was purchased from Assay BioTech (Sunnyvale, CA, USA). Other primary antibodies and HRP conjugated secondary antibodies were collected from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA).

2.2. Cell culture and viability assay

The B16F10 mouse melanoma cells were obtained from American Type Culture Collection (Manassas, VA, USA). Cells were cultured at 37 °C in high glucose DMEM, supplemented with 10% FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin in a humidified atmosphere containing 5% CO_2 in air. Cells were pre-treated with SQA for 1 h before stimulation with 1.0 μM of α-MSH. Cytotoxicity of SQA was determined using 3-(4,5-Dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay with a CellTiter 96® AQueous One Solution Cell Proliferation assay kit according to the manufacturer's instructions. Briefly, B16F10 cells were seeded in a 96-well plate at 10^4 cells/well and incubated for 24 h in DMEM supplemented with 10% FBS. Then, culture medium was replaced with FBS-free DMEM and incubated for 4 h. After that, cells were treated with different concentrations of SQA for 24 h in DMEM containing 2% FBS. The culture medium was replaced with 95 μL of FBS-free DMEM and 5 μL of MTS solution. After 1 h of incubation, the absorbance was measured at 490 nm by a microplate reader (Promega Corporation Instrument, USA).

2.3. Melanin content assay

Intracellular melanin content was determined according to the procedure described previously with minor modifications [23]. Briefly, B16F10 cells were seeded in a 24-well plate at 2×10^4 cells/well and incubated for 24 h in DMEM containing 10% FBS. Cells were pre-treated with SQA for 1 h and stimulated with α-MSH for 72 h. The cells were washed with PBS and dissolved in 1 N NaOH containing 10% DMSO by boiling at 80 °C for 30 min. The cell lysates were centrifuged at 14,000 rpm for 10 min and absorbance of the supernatant was measured at 405 nm. The melanin content was determined by normalizing the absorbance with total protein content. Arbutin (500 μM) was used as a positive control.

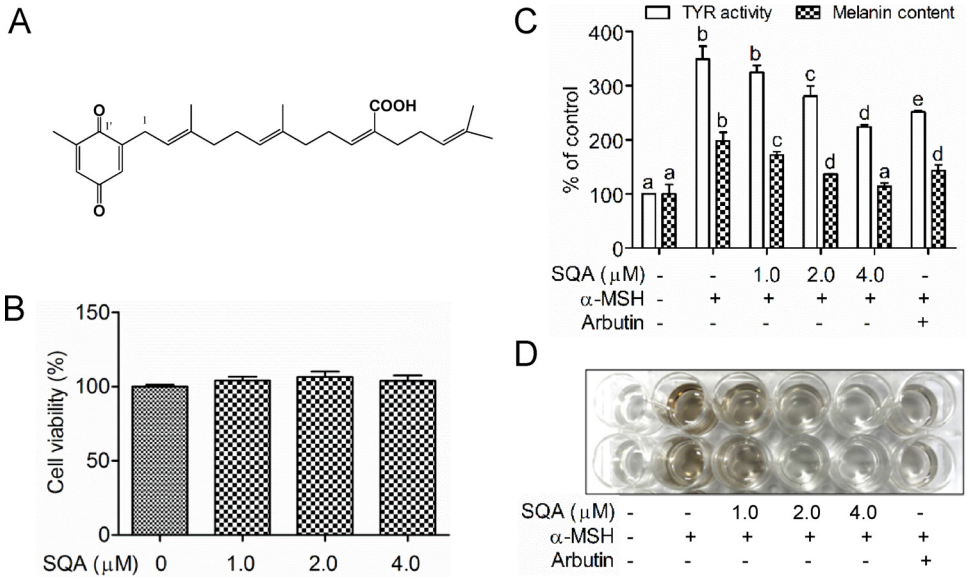

Fig. 1. SQA attenuates melanin synthesis in B16F10 mouse melanoma cells. (A) The structure of SQA isolated from *S. serratifolium*. (B) Cell viability was determined using an MTS assay kit after treatment of the cells with 1.0–4.0 μM SQA for 24 h. (C) The cellular TYR activity and melanin contents were measured after pre-treatment of the cells with SQA for 1 h followed by exposure to α-MSH (1.0 μM) for 72 h in the presence or absence of SQA. Arbutin (500 μM) was used as positive control. (D) Extracellular melanin content in the cell culture medium after 72 h of SQA treatment. Data represent the mean ± standard deviation (SD) ($n = 3$). Different letters indicate the treatment groups with significant ($p < 0.05$) differences (C).