Sesamol decreases melanin biosynthesis in melanocyte cells and zebrafish: Possible involvement of MITF via the intracellular cAMP and p38/JNK signalling pathways

Seung-hwa Baek¹ and Sang-Han Lee¹²³

¹Department of Food Science & Biotechnology, Graduate School, Kyungpook National University, Daegu, Korea; ²Department of Nano-Science & Technology, Graduate School, Kyungpook National University, Daegu, Korea; ³Food & Bio-industry Research Institute, Kyungpook National University, Daegu, Korea

Correspondence: Sang-Han Lee, PhD, Department of Food Science & Biotechnology, Kyungpook National University, Daegu 702-701, Korea, Tel.: 82-53-950-7754, Fax: 82-53-950-6772, e-mail: sang@knu.ac.kr

Abstract: The development of antimelanogenic agents is important for the prevention of serious aesthetic problems such as melasma, freckles, age spots and chloasma. The aim of this study was to investigate the antimelanogenic effect of sesamol, an active lignan isolated from Sesamum indicum, in melan-a cells. Sesamol strongly inhibited melanin biosynthesis and the activity of intracellular tyrosinase by decreasing cyclic adenosine monophosphate (cAMP) accumulation. Sesamol significantly decreased the expression of melanogenesis-related genes, such as tyrosinase, tyrosinase-related protein-1,2 (TRP-1,2), microphthalmia-associated transcription factor (MITF) and melanocortin 1 receptor (MC1R). In addition, sesamol also induces phosphorylation of p38 mitogen-activated protein kinase (p38 MAPK) and c-Jun N-terminal kinase (JNK). Moreover, sesamol dose-dependently decreased zebrafish pigment formation, tyrosinase activity and expression of melanogenesis-related genes. These findings indicate that sesamol inhibited melanin biosynthesis by down-regulating tyrosinase activity and melanin production via regulation of gene expression of melanogenesis-related proteins through modulation of MITF activity, which promoted phosphorylation of p38 and JNK in melan-a cells. Together, these results suggest that sesamol strongly inhibits melanin biosynthesis, and therefore, sesamol represents a new skin-whitening agent for use in cosmetics.

Key words: antimelanogenic agents – cyclic adenosine monophosphate – microphthalmia-associated transcription factor – sesamol – tyrosinase – zebrafish

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Introduction

Melanin is synthesized as a UV defense mechanism via a complex process involving a series of enzymes and diverse signal transduction pathways (1). Melanogenesis is initiated by the tyrosinase (Tyr)-catalysed oxidation of tyrosine to dopaquinone via the intermediate 3,4-dihydroxyphenylalanine (2). In the absence of thiols, such as cysteine or glutathione, the second enzyme in the melanin synthesis pathway, tyrosinase-related protein 2 (TRP-2), rapidly converts dopaquinone to dopachrome and subsequently converts dopachrome to 5,6-dihydroxyindole (DHI) or indole 5,6quinone 2-carboxylic acid (DHICA). The last enzyme involved in melanin synthesis, tyrosinase-related protein 1 (TRP-1), catalyses the oxidation of DHICA and forms eumelansins (3). Tyr contains a binuclear copper centre in its active site and catalyses the hydroxylation of phenols to catechols, as well as the oxidation of catechols to quinones (4,5). Additionally, Tyr is a glycoprotein that requires N-glycan with processing by intracellular α-glucosidase and α-mannosidases, and its glycosylation and degradation play crucial roles on the development of anti-melanogenic agents (6).

Melanogenesis is modulated by several factors, including UV irradiation, alpha-melanocyte-stimulating hormone (α-MSH) (7), forskolin, stem cell factor (SCF) (8), wnt-3a (9) and isobutylmethylxanthine (IBMX) (10), within intracellular melanosomes in the melanocytes (11). Stimulation of the melanocortin 1 receptor (MC1R) by α-MSH activates adenyl cyclase through G protein signalling, which subsequently increases cyclic adenosine monophosphate (cAMP) production. cAMP participates in the expression of microphthalmia-associated transcription factor (MITF), a master transcription factor that plays an important role in melanogenesis by transcriptionally regulating Tyr, TRP-1 and TRP-2 expression (12). Mitogen-activated protein kinase (MAPK) signalling is also a key modulator of melanogenesis through its regulation of MITF activation (13). Phosphorylation of extracellular-signal-regulated kinases (ERK), c-Jun N-terminal kinase (JNK) and p38 MAPK lead to MITF ubiquitination and degradation, and thus inhibit melanin production (14–17).

Sesame seeds (Sesamum indicum L.) are widely used as food ingredients in Asian and African countries, and sesame seed oil is more widely used than olive oil in Korea. Sesame oil contains high concentrations of tocopherols, tocotrienols and essential fatty acids, such as linolenic acid and omega-3 fatty acid (18). It has been reported that sesame seed oil produces more health benefits than olive oil (19). Sesame seeds are rich in phytochemicals known as sesame lignans, such as sesamin, sesamolin, sesaminol, pinoresinol and sesamol, which are methane dioxygenyl compounds (20). Sesamin affects hyperpigmentation (21), alters osteogenic activity (22), lowers cholesterol (23), influences lipid metabolism (24), reduces tumorgenesis in the liver (25) and protects neuronal cells against oxidative stress (26). Very recently, it was demonstrated that sesamol exhibited significant antioxidative and anti-Tyr activity (27), but the molecular mechanisms through which sesamol inhibits melanogenesis have not been studied. In this study, we investigated the inhibitory activity of sesamol on melanin biosynthesis in melanocyte systems and zebrafish embryos...
and studied the underlying molecular mechanisms of the effects of sesamol in melan-a cells.

**Materials and methods**

**Materials**

Arbutin, N-phenylthiourea (PTU), tetradecanoyl phorbol acetate (TPA), l-DOPA (3,4-dihydroxyphenylalanine), l-tyrosine, Tyr, sodium hydrosulfoxy (NaOH), thiazolyl blue tetrazolium bromide (MTT), Tween-20, hydrogen chloride (HCl), tricaine methanesulphonate, synthetic melanin, protase inhibitors, TRIS base, dithiothreitol (DTT), bromphenol blue, glycerol, 2-mercaptoethanol, dimethyl sulfoxide (DMSO), isobutylmethylxanthine (IBMX), rosmarinic acid, rutin hydrate, (-)-quinic acid, baicaline, coumaric acid, epicatechin, ferulic acid, sesamol, protocatechuic acid, quercetin, phloretin and esculin were obtained from Sigma-Aldrich Co., (St. Louis, MO, USA). Anti-Tyr (C-19), anti-TRP1 (G-17), anti-TRP2 (D-18), anti-goat IgG-HRP, anti-mouse IgG-HRP or anti-β-actin antibody (Santa Cruz Biotech., Santa Cruz, CA, USA) was used. All other reagents and chemicals were high-grade and commercially available.

**Measurement of Tyr activity**

Tyr activity was determined as described previously, with minor modifications (27). The reaction mixture for the determination of mushroom Tyr (EC 1.14.18.1) activity consisted of 150 μl of 0.1 mM phosphate buffer (pH 6.5), 3 μl of sample, 36 μl of 1.5 mM l-tyrosine and 7 μl of mushroom Tyr (2100 units/ml); 0.05 mM phosphate buffer, pH 6.5, and the reactions were performed in a 96-well microplate (SPL, Pocheon, Korea). The initial absorbance of the mixture was measured at 490 nm using a microplate reader (Fig. 1b; graph). Zymogram sample proteins (30 μg/well) were subjected to 10% SDS-PAGE and gels stained with 20 μM l-DOPA in 0.1 mM sodium phosphate buffer at 37°C for 1 h (Fig. 1b; 1st row). Coomassie blue staining was qualified as the same amounts of protein loading (Fig. 1b; 2nd row).

**Measurement of cAMP**

The cellular cAMP assay was performed with cell lysates using a cAMP direct immunoassay kit (Biovision, San Francisco, CA, USA). Cells were plated into 6-well plates at 5 x 10^5 cells/well and incubated overnight at 37°C under normal culture conditions. Cells were treated with the test compound at various concentrations, and the cells were lysed and processed according to the manufacturer's protocol using 0.1N HCl. The termination of

[Figure 1. Effect of sesamol on melanogenesis, intracellular tyrosinase and expression of melanogenesis-related protein and genes in melan-a cells. (a) Cells (1 x 10^5 cells/ml) were cultured for 24 h, and the medium was replaced with fresh medium containing various concentrations of the sesamol and arbutin for 3 days. The cells were collected and lysed with 1N NaOH. Melanin content was estimated by measuring the absorbance at 405 nm. (b) After incubation under the conditions mentioned above, the cells were collected and lysed. The cell lysates were incubated with 20 μM l-DOPA for 1 h. After quantifying protein levels, tyrosinase activity was determined by l-DOPA zymography. Protein was evaluated by Coomassie blue staining. (c) Total cell lysates were extracted and assayed by Western blotting using antibodies against tyrosinase, TRP-1, TRP-2 and MITF. Equal amounts of protein loading were confirmed using β-actin. The band intensity was normalized to the β-actin band using ImageJ software. Cells (2 x 10^5 cells/ml) were cultured with medium containing various concentrations of the sesamol and arbutin for 24 h. The cells were collected using Trizol. (d) After quantifying RNA levels, mRNA expression was analysed by RT-PCR. The band intensity was normalized to the 18s RNA signal using ImageJ software (d). Each determination was made in triplicate, and the data represent the mean ± SD. * P < 0.05, Student’s t-test.]
Inhibition of melanin synthesis by sesamol

reactions and cAMP measurements were carried out as described in the cAMP kit manual using a microplate reader.

**Western blot analysis**

Cell were cultured with or without sesamol and arbutin in six-well plates, and then prepared using a standard protocol. Sample proteins were Western blot analysis was performed as described (32). The Western blot assays were representative of at least three independent experiments.

**RT-PCR analysis of mRNA expression**

Total RNA was extracted using TRIzol (Invitrogen Co., Carlsbad, CA, USA) according to the manufacturer’s instructions (33). Total RNA (2 µg) was reverse transcribed using reverse transcriptase (MP Biomedicals, Santa Ana, CA, USA) and oligo(dT) primers. cDNA was amplified using a PCR Thermal Cycler Dice TP600 (Takara Bio Inc., Otsu, Shiga, Japan). PCR products were visualized by ethidium bromide staining after electrophoresis (31). The mRNA levels in zebrafish were evaluated as follows: 1 and 2 days after fertilization, embryos (50 per well) were transferred to a 6-well plate with 5 ml of test solution. After 1 day, total RNA was isolated using TRIzol reagent and first-strand cDNA was synthesized using 2 µg of total RNA, 1 µM of oligo-dT18 primer and Omniscript Reverse Transcrip-tase (Qiagen, Valencia, CA, USA). SYBR green-based quantitative PCR was performed using the Applied Biosystems 7500 Fast Real-Time PCR system and Fast SYBR® Green Master Mix (Applied Biosystems, Foster City, CA, USA) as described previously. All reactions were run in triplicate, and data were analysed using the 2^{-ΔΔCT} values method (34). Specific oligonucleotide primers for mouse and zebrafish transcripts were used (Table S1).

**Tyr activity and melanin content in zebrafish**

Compound treatment and phenotype-based evaluation were measured as previously described (35). Briefly, about 200 zebrafish embryos were treated with melanogenic modulators from 24 to 48 hpf and sonicated in Pre-protein extraction solution (iNtRON Biotechnology, Sungnam, Korea). The lysate was clarified by centrifugation at 9,000 × g for 10 min. After protein quantification, 250 µg of total protein in 100 µl of lysis buffer was transferred into a 96-well plate, and 100 µl of 5 mM l-DOPA was added. The control contained 100 µl of lysis buffer and 100 µl of 5 mM l-DOPA. After incubation for 1 h at 37°C, absorbance was measured at 490 nm. The absorbance of the blank sample was subtracted from each absorbance value, and the final activity was expressed as a percentage of that of the water-exposed control group. PTU was used as a positive control. All experiments were performed in triplicate. For determination of melanin content, extract was prepared at 72 hpf. After centrifugation, the pellet was dissolved in 1 ml of 1 N NaOH at 100°C for 10 min. The optical density of the supernatant was measured at 405 nm, and the result was compared with a standard curve of synthetic melanin.

**Statistical analysis**

Data are expressed as the mean ± standard deviation. Statistical significance was determined using Student’s t-test for independent means in Microsoft Excel software (Microsoft Corp., Redmond, WA, USA). The critical level for significance was set at \( P < 0.05 \) or \( P < 0.01 \).

**Results**

**Sesamol has anti-melanogenesis activity**

To search for natural compounds from plant extracts with anti-melanogenesis effects, we screened 12 single compounds that were isolated from food ingredients for inhibitory effects on melanogenesis using in melan-a cells. From the results of these assays, two compounds with good activity without toxicity were identified: sesamol and quercetin (Table S2). Sesamol was chosen for further evaluation as an anti-melanogenic compound. We examined the effect of sesamol on the activity of Tyr, a key enzyme responsible for melanin synthesis, as well as its copper chelation activity. Sesamol produced a potent inhibitory effect on Tyr with a 50% inhibitory concentration (IC\(_{50}\)) of 6.7 µM, and the IC\(_{50}\) of arbutin was about 416.6 µM (Fig. S1). Therefore, sesamol was a powerful Tyr inhibitor under the same conditions in which arbutin produced a similar effect. However, sesamol was not found to act as a copper chelator. These findings demonstrate that sesamol suppressed Tyr activity without copper chelating activity (Table S3). Moreover, the capacity of sesamol to inhibit the formation of melanin in S. biki-niensis was evaluated by measuring inhibition zones on paper discs, and it was found to produce clear inhibitory activity after 48 h. Sesamol potently inhibited melanin biosynthesis in a dose-depen-dent manner without influencing the growth of S. biki-niensis. Sesamol inhibited melanin synthesis at a concentration of 35 µg per disc. Positive control treatment arbutin showed a similar inhibition zone at a concentration of 545 µg per disc. Sesamol had the strongest inhibitory effect on melanin biosynthesis in S. biki-niensis (15-fold greater than arbutin) (Table S4).

**Sesamol decreased melanin content and inhibited intracellular Tyr activity in melan-a cells**

To investigate the melanin content and cytotoxicity of sesamol, after treatment with sesamol at various concentrations for 72 h, cell melanin and viability were measured. Sesamol significantly decreased melanin content in a dose-dependent manner without cytotoxicity (Fig. 1a). Treatment of melan-a melanocyte cells with sesamol (50 µM) or arbutin (1 mM) decreased melanin content to 69 ± 0.5% and 35 ± 3.4%, respectively, than that of the control group (Fig. 1a, 4th and 5th columns). These results indicate that sesamol decreased melanin synthesis without influencing melanocyte viability in melan-a cells. Decreased Tyr activity in melanocytes could be produced either by direct inhibition of Tyr or by repression of Tyr gene expression. Cell lysates prepared from the melan-a cells treated with different concentrations of sesamol were used as a Tyr source. Intracellular Tyr activity was suppressed by treatment with sesamol at concentrations ranging from 12.5 to 50 µM. Sesamol showed a potent anti-Tyr effect with IC\(_{50} = 97 \) µM in melan-a cells (Fig. 1b, top). There seem to be little difference in intracellular Tyr inhibitory activity both L-DOPA zymography and intracellular Tyr activity in melan-a cells (Fig. 1b, bottom).

**Sesamol inhibited protein levels and expression of melanogenesis-related proteins and genes in melan-a cells**

We examined the influence of sesamol on expression of melanogenesis-related proteins Tyr, TRP-1, TRP-2 and MITF, by Western blotting using cell lysates treated with different concentrations of sesamol for 72 h. Sesamol at 50 µM suppressed protein expression of Tyr, TRP-1 and TRP-2 by 65.8 ± 2.8%, 56.4 ± 1.1% and 75.7 ± 4.8%, respectively, in comparison with the control group (Fig. 1c, 1st–3rd rows, 3rd lanes). MITF is a key transcription factor that regulates the expression of the most melanogenesis-related genes (13). Sesamol significantly decreased MITF protein expression in melanocytes (Fig. 1d, 4th row, 1st–3rd lanes, compared to 4th lane). It has been suggested that down-regulation of melanogenesis-related
proteins by sesamol could be associated with MITF signalling. To determine whether inhibition of protein expression was associated with decreased mRNA levels of melanogenesis-related genes, the mRNA levels of Tyr, TRP-1, TRP-2, MITF and MC1R were confirmed using RT-PCR. Sesamol at 50 μM suppressed gene expression of Tyr, TRP-1 and TRP-2, by 74.7 ± 5.6%, 52.9 ± 2.2% and 85.3 ± 4.7%, respectively. Additionally, sesamol significantly decreased mRNA expression of MITF and MC1R (Fig. 1d). Taken together, these results show that the presence of sesamol significantly decreased the expression of Tyr-related genes, suggesting that it worked by down-regulating MITF and MC1R transcription.

**Sesamol induced Tyr lysosomal and proteasomal degradation in melan-a cells**

We next examined whether the decrease in Tyr abundance was due to proteasome-mediated proteolytic degradation using proteolysis inhibitors. To inhibit protein synthesis, cycloheximide (CHX) was added to melan-a cells, which were then pretreated with MG132, a proteasome inhibitor, and/or chloroquine (CQ), a lysosomal proteolysis inhibitor, for 1 h, followed by 6 h of sesamol treatment. As shown in Fig. 2, the decreased Tyr levels that occurred in response to treatment with sesamol were clearly restored by pretreatment with MG132 and CQ. These findings demonstrate that regulation of Tyr protein levels by sesamol results from proteosomal and lysosomal degradation. These results clearly show that sesamol may contribute to the inhibition of melanogenesis by regulating the expression of Tyr-related proteins, as well as MITF, and proteasomal and lysosomal degradation of Tyr.

**Sesamol inhibited cAMP-dependent melanogenesis in melan-a cells**

Cells were treated with sesamol for 12 h, and intracellular cAMP levels were measured using an ELISA kit. Treatment of melan-a cells with sesamol decreased intracellular cAMP levels in a concentration-dependent manner (Fig. 3a). Furthermore, we elucidated the effects of sesamol on melanin formation in melanocyte cells. Cells were treated with sesamol for 12 h, and intracellular cAMP levels were measured using an ELISA kit. Treatment of melan-a cells with sesamol decreased intracellular cAMP levels in a concentration-dependent manner (Fig. 3a). Treatment of melan-a cells with sesamol decreased intracellular cAMP levels in a concentration-dependent manner (Fig. 3a).

**Figure 2.** Effect of sesamol on tyrosinase lysosomal and proteasomal degradation in melan-a cells. Cells (3 × 10⁵ cells/ml) were pretreated with 25 μM of cycloheximide, a protein synthesis inhibitor, for 1 h. Cells were also pretreated with 10 μM MG-132, a proteasomal degradation inhibitor, or 50 μM chloroquine, a lysosomal degradation inhibitor, for 1 h, and then treated with sesamol for 6 h. Whole cell lysates were subjected to Western blot analysis using anti-β-actin antibodies. Equal protein loading was confirmed using anti-β-actin antibodies.

**Sesamol increased phosphorylation of JNK and p38 in melan-a cells**

To elucidate the role of MAPK signalling in melanogenesis, the effects of sesamol on JNK, ERK and p38 MAPK phosphorylation were detected by Western blotting. Sesamol increased phosphorylation of JNK and p38 MAPK without affecting phosphorylation of ERK1/2 and did not affect total protein levels of p38 MAPK, ERK or JNK (Fig. 3a). The levels of phosphorylated JNK and p38 were 10 times and 30 times higher than those of the untreated control cells (Fig. 3c, 1st and 3rd lanes). Interestingly, phosphorylation of p38 MAPK and JNK increased in a concentration-dependent manner in 15 min (Fig. 3d, compare 1st–2nd rows to 3rd–4th rows of 1st, 3rd and 5th lanes). These results show that JNK1/2 and p38 MAPK phosphorylation are associated with the effects of sesamol on melanin formation in melanocyte cells.

**Sesamol inhibited melanogenesis in zebrafish**

Treatment of zebrafish embryos with sesamol for 72 h at concentrations up to 50 μM significantly inhibited skin melanin formation in developed larvae (Fig. 4a). The melanin content of sesamol-treated zebrafish was less than that of untreated zebrafish larvae (Fig. 4b). Additionally, we measured total melanin content, Tyr activity and Tyr quantity using whole extracts of zebrafish larvae.
(Fig. 4c). There were considerable decreases in total melanin pigment and Tyr activity after treatment with sesamol. PTU decreased total melanin content and Tyr activity (Fig. 4d). Sesamol at a concentration of 50 μM down-regulated expression of Tyr, TRP-1, TRP-2 and MITF mRNA (Table S5). The results point out that sesamol inhibited the melanogenesis in vivo.

Discussion

Sesame oil, used extensively as a traditional health food in Asia, is commonly used in Korea. Sesame seeds are rich in methylene dioxyphenyl phytochemicals called lignans. Sesame lignans, which play an important role in plant defense, are unique phenolic antioxidants with many health benefits (36). Sesamol (3,4-methylenedioxyphenol) is a simple phenolic constituent present in sesame oil that reduces free radicals and prevents certain types of cancer, cardiovascular disease, coronary heart disease, stroke, atherosclerosis and other degenerative diseases related to oxidative stress (37). However, no previous study has examined their antimelanogenesis activity in the context of skin pigment disorders. In the present study, sesamol potently inhibited melanin biosynthesis in melanocytes and zebras.

Tyr plays a key role in melanogenesis by catalysing the hydroxylation of tyrosine to L-DOPA and the oxidation of L-DOPA to DOPAquinone. Most skin-whitening agents specifically act to inhibit the production of melanin. The most important transcription factor involved in melanocyte differentiation and pigmentation (13). To determine the mechanism responsible for the inhibitory effect of sesamol, its effect on melanogenic protein expression was measured. Sesamol decreased protein expression levels of melanogenic proteins and inhibited Tyr, TRP-1 and TRP-2 in melan-a cells. Sesamol also induced down-regulation of MITF protein expression and decreased mRNA levels of Tyr-related genes and MC1R (Fig. 1d). α-MSH, which binds to and acts on the MC1R, activates MITF and increases expression of melanogenic proteins, ultimately inducing melanin synthesis (43).

Tyr can be down-regulated by ubiquitin-dependent proteasomal and lysosomal degradation, and Tyr degradation decreases melanogenesis (44,45). Thus, we measured down-regulation of Tyr by sesamol to determine whether it resulted from Tyr degradation. To evaluate this hypothesis, the cells were treated with proteasome inhibitor MG132 and/or lysosomal proteolysis inhibitor CQ. CHX was added to melan-a cells to inhibit protein synthesis, after which the cells were pretreated with MG132 and/or CQ for 1 h followed by 6 h of treatment with sesamol. Tyrosinase protein levels were detected by Western blotting. Changes in Tyr protein abundance in response to treatment with sesamol were restored by pretreatment with MG132 and CQ. Likewise, co-treatment with MG132 and CQ fully blocked Tyr down-regulation (Fig. 2). These results suggest that sesamol leads to Tyr degradation via both lysosomal proteolysis and the ubiquitin–proteasome pathway.

Many reports have suggested that cAMP signalling plays a critical role in the regulation of melanin biosynthesis. Furthermore, cAMP stimulates melanogenesis mainly through activation of MITF and leads to induction of Tyr protein expression (46). Sesamol decreased intracellular cAMP levels in a dose-dependent manner. Furthermore, sesamol inhibited overexpression of melanin biosynthesis by IBMX, which has been shown to increase cellular cAMP and CGMP levels in melan-a cells (Fig. 3b). These results suggest that sesamol decreases melanin biosynthesis via suppression of the MC1R-cAMP-MITF signalling cascade. Moreover, cAMP-induced melanogenesis has been reported to be mediated by a CRE promoter via the binding of CREB family transcription factors that
are activated by protein kinase A (47). Thus, we will study the effect of sesamol on CREB phosphorylation during melanin biosynthesis in future studies. In addition to cAMP signalling, previous studies have shown that MAPKs such as ERK, p38 MAPK and JNK are also involved in melanogenesis in melanocyte cells (48). We studied the regulation of MAPK phosphorylation by sesamol and found no difference in phospho-ERK levels after sesamol treatment in melan-a cells. On the contrary, phosphorylation of p38 MAPK and JNK was increased by sesamol in a dose- and time-dependent manner (Fig. 3 c-d). These results indicate that sesamol inhibits melanogenesis by activating p38 MAPK and JNK signalling followed by down-regulation of melanogenic proteins.

Because in vitro and in vivo experiments may give differing results, zebrafish, which have been established as a new in vivo model for evaluating the activity of melanogenic regulatory compounds, were evaluated after sesamol treatment (49,50). The zebrafish is an advantageous system for pigment cell chemical biology because the embryos are contained in a large number of transparent eggs that are small enough for easy handling (51). Here, we found that exposure of larvae to sesamol after 48 hpf suppressed pigmentation production even at a low dose, without observable toxicity in the exposed zebrafish. In addition, sesamol markedly decreased melanin content and Tyr activity in comparison with arbutin in zebrafish, and sesamol significantly down-regulated melanogenesis-related gene expression in zebrafish embryos (Fig. 4). Taken together, these results confirm that sesamol suppresses melanin biosynthesis in vivo as well as in vitro.

In this study, we evaluated the inhibitory effect of sesamol on melanin synthesis in melan-a cells and zebrafish. The present results indicate that sesamol inhibits melanin synthesis in melan-a cells by regulating cAMP, p38 MAP kinase and JNK MAP kinase signalling to suppress MITF, as well as inducing destruction of Tyr via proteasomal and lysosomal degradation. Additionally, sesamol reduced body pigmentation and inhibited intracellular Tyr and melanogenesis-related mRNA transcription in the zebrafish model. Consequently, we suggest that sesamol can be used as a skin-lightening agent for treating hyperpigmentation disorders, and it may be used as an active ingredient in skin care products for preventing the darkening of the skin.

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Author contribution

S.-H. Baek performed the research. S.-H. Baek and S.-H. Lee designed the research, analysed the data and wrote the paper. S.-H. Lee revised the paper.

Conflict of interests

The authors declare no conflict of interests.

Supporting Information

Additional supporting data may be found in the supplementary information of this article.

Figure S1. Effect of sesamol and arbutin on mushroom tyrosinase.

Figure S2. Effect of sesamol on IBMX-induced melanin formation in melan-a cells.

Figure S3. Effect of sesamol in cAMP-dependent protein signalling in melan-a cells.

Table S1. Primer list used for RT-PCR.

Table S2. Inhibitory activity of various compound from natural plant extracts on melanin biosynthesis in melan-a cells.

Table S3. Effect of sesamol against copper chelating activity.

Table S4. Effect of sesamol and arbutin on melanin production in Streptomyces bikiniiensis.

Table S5. Effect of sesamol on expression of genes related melanogenesis in zebrafish.

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