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Original artcle

Fargesin inhibits melanin synthesis in murine malignant and immortalized melanocytes by regulating PKA/CREB and P38/MAPK signaling pathways

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ABSTRACT

Background: Fargesin is commonly used in the treatment of allergic rhinitis, inflammation, sinusitis and headache.
Objective: The aim of the study is to investigate a new function of fargesin against melanin production and its underlying molecular mechanism.
Methods: B16F10 mouse melanoma cells, Melan-a and human epidermal melanocytes were treated with different concentrations of fargesin for the indicated time. The extracellular and cellular melanin content was detected by spectrometry at 490 nm and 405 nm, respectively. RT-qPCR and Western blot analysis were used to exam the expression of melanogenic enzymes and the activities of PKA/CREB and p38 MAPK pathway components. Zebrafish was used as an *in vivo* model for studying the function of fargesin in regulating melanogenesis.
Results: Fargesin effectively inhibited melanin production at moderate dose in mouse B16F10 melanoma cells, normal melanocyte cell lines and zebrafish. The expression of microphthalmia-associated transcription factor (*MITF*), its downstream melanogenic enzymes and tyrosinase activity were also strongly reduced by fargesin. Moreover, the increase of melanin production induced by UVB and forskolin could be fully reversed by fargesin treatment. Fargesin also effectively inhibited the activation of PKA/CREB and p38 MAPK as well as their interactions, which in turn is responsible for the expression of MITF and melanogenic enzymes.
Conclusions: These results show that fargesin can function as an anti-melanogenic agent, at least in part, by inhibiting PKA/CREB and p38/MAPK signaling pathways. Therefore, fargesin and its derivatives may potentially be used for preventing hyperpigmentation disorders in the future.

1. Introduction

Melanogenesis is a complex process, which serves as the determinant of skin and hair color. In human, skin hyperpigmentation is physiologically stimulated by UV radiation in order to protect skin against harmful UV injury. However, excess melanin production or abnormal distribution can cause skin hyperpigmentation. In melanocytes and melanoma cells, melanin production is mainly regulated by melanogenic enzymes such as tyrosinase (TYR), tyrosinase-related protein 1 (TRP-1), and TRP-2. MITF, a basic helix–loop–helix leucine zipper transcription factor, is important for melanocyte differentiation, survival and proliferation [1]. The expression of critical melanogenic enzymes is mainly regulated by MITF in melanocytes.

Cyclic AMP (cAMP) is a key factor involved in the signal transduction pathways of melanogenesis. CREB, a cellular transcription factor, can be activated by the cAMP signaling pathway through protein kinase A (PKA). PKA is an important group of Ser/Thr kinase family and can phosphorylate CREB (p-CREB) at serine 133 [2]. Once activated, CREB protein directly binds to the *MITF* promoter region and stimulates MITF transcription [3]. In addition to PKA, protein kinase C (PKC) has been shown to be able to phosphorylate CREB [4]. Thus, PKC is able to stimulate human

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melanogenesis by activating tyrosinase in pigment cells [5]. P38 MAPK is also involved in melanogenesis induced by UV irradiation. A previous study reported that p38 also phosphorylated CREB and promoted its binding to the *MITF* promoter [6].

Skin is a target organ for melanin-production regulating proopiomelanocortin (POMC)-derived neuropeptides, such as alpha-melanocyte stimulating hormone (α-MSH), β-endorphin, and adrenocorticotropic hormone (ACTH) [7]. The expression of α-MSH receptor (MC1R) and POMC as well as the secretion of α-MSH, β-endorphin and ACTH are upregulated by UVB stimulation [8,9]. Clearly, melanogenesis is under complex regulatory control via pathways activated by receptor-dependent and -independent mechanisms [10]. At present, a comprehensive understanding of the specific molecular mechanisms regulating melanogenesis is still lacking.

The lignans are a large group of polyphenols found in plants. Fargesin, a bioactive lignan from Flos Magnoliae, is commonly used in the treatment of allergic rhinitis, inflammation, sinusitis and headache [11]. It is reported that fargesin could increase basal glucose uptake and GLUT4 translocation in L6 myotubes [12]. Fargesin also has antihypertensive effects in 2K1C hypertensive rats via inhibiting oxidative stress and promoting NO release [13]. Sesamin is also a lignan in sesame seeds and sesame seed oil. A previous study reported that sesamin was a potent stimulator of melanogenesis in B16 cells [14], while another study showed that sesamin blocked melanin induction and melanogenic enzyme production [15]. Although the anti-inflammatory and antioxidant properties of fargesin have been elucidated [16,17], its function in depigmentation is still unclear.

In this study, we aim to investigate the inhibitory effects of fargesin on melanin synthesis in murine melanoma cells, normal melanocytes and zebrafish embryos. Moreover, we further explored the underlying molecular mechanisms by which fargesin plays its role. Our results demonstrate that PKA/CREB and p38 MAPK are the important targets of fargesin as an anti-melanogenic agent.

2. Materials and methods

2.1. Materials

L -3,4-dihydroxyphenylalanine (L-Dopa) and kojic acid were purchased from Sigma–Aldrich (St Louis, USA). Antibodies against TYR, TRP1, TRP2 and MITF were purchased from Abcam (Cambridge, UK). Antibody against β–actin, PKA, P-CREB, CREB were purchased from Cell Signaling Technology (Danvers, USA). Forskolin and H89 diHydrochloride were purchased from Shanghai Target Molecule Corp. (Target Mol, Shanghai, China).

2.2. Cell culture

The mouse B16F10 melanoma cell line (Shanghai Key Laboratory of Regulatory Biology, China) and Melan-a cells, an immortalized mouse melanocyte cell line derived from C57BL6 mice, were cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco, MA, USA) containing 10% fetal bovine serum and 1% penicillin/streptomycin (Wisent, Montreal, Canada). Human epidermal melanocytes (HEM) were purchased from Sciencell (USA) and cultured in melanocyte medium (Sciencell, USA). Culturing was done in a humidified atmosphere with 5% CO_2 at 37°C.

2.3. Cell viability assay

The viability of B16F10 and Melan-a cells was determined by the CCK8 (QiHai, China) assay. Specifically, cells were seeded on 96-well plates, and after 24-h growth, the medium was removed and fresh medium containing various concentrations of fargesin (0–100 μM) was added. Forty eight hours later, 20 μL of CCK8 was added to each well. After incubating for 30 min, the absorbance was measured at 450 nm using a SPECTRA MAX 190 spectrometer (Molecular Devices, CA, USA).

2.4. Melanin content measurement

B16F10 cells (1.2×10^5 /well in 6-well plates), Melan-a cells (1.2×10^5 /well in 6-well plates) and HEM cells (1.0×10^5 /well in 6-well plates) were maintained in phenol red-free DMEM (Gibco, MA, USA) that contains 104 mg/L L-Tyrosine disodium salt dehydrate and treated with fargesin (0–100 μM) for 72 h. Phenol red-free DMEM without fargesin served as a control. Extracellular melanin in the medium secreted by B16F10 cells was measured at 490 nm.

To measure the cellular melanin content, cells were washed with PBS and 1 M NaOH containing 10% DMSO was then added. The cells pellets were heated at 80 °C for 1 h, and intracellular melanin release was measured at 405 nm.

2.5. TYR activity assay

B16F10, Melan-a, and HEM cells were incubated with various concentrations of fargesin for 48 h at 37°C and then lysed with 1% Triton X-100 at −80°C for 30 min in 24-well plates. Cell extracts were centrifuged at 12,000 rpm for 10 min at 4°C, and then the supernatants were used for TYR activity assay, where 100 μL of 4 mM L–Dopa and the test supernatants were incubated in a 96-well plate for 1 h at 37 °C. Colorimetric measurement was performed at 490 nm.

2.6. Quantitative RT-PCR (RT-qPCR)

Total cellular RNA was prepared from B16F10 and Melan-a cells treated with fargesin by using RNAiso plus (TaKaRa, Dalian, China). cDNA was synthesized using PrimeScript RT Master Mix (TaKaRa, Dalian, China) according to the manufacturer's instructions. Quantitative RT-PCR was performed using SYBR Green for 40 cycles using the following conditions: denaturation 30 s at 95°C, annealing 30 s at 55°C, and extension 30 s at 72°C. Primer sequences were shown in Supplemental Table 1. Expression levels were determined in triplicates and normalized to the expression of β-actin.

2.7. Western blot analysis

Intracellular proteins from B16F10 cells treated with or without fargesin were lysed with RIPA lysis buffer containing phosphatase inhibitor and protease inhibitor. Protein concentration was measured using BCA kit assay. Proteins were separated by 10% SDS-polyacrylamide gel electrophoresis and then transferred onto a nitrocellulose membrane. The membrane was blocked with 5% skim milk for 1 h and incubated at 4 °C overnight with primary antibody at 1:1000 dilutions. Goat anti-mouse or rabbit IgG with fluorescent monomer was added in a solution containing the target protein and measured using the LI-COR Odyssey imaging system (LI-COR, Inc).

2.8. Fargesin treatment of zebrafish embryos

Zebrafish embryos were obtained from natural spawning (School of life sciences, East China normal University, Shanghai, China) according to the ethical and scientific standards set by the Animal Center at East China Normal University. After 8-h post-fertilization

(h.p.f), 5 to 10 zebrafish embryos were transferred into 24-well plates and various concentrations of fargesin were added to the embryo-containing medium. Kojic acid (1 mM) was used as a standard positive control. Zebrafish embryos were maintained under the following conditions: 28.5 °C, with a 14/10 h light/dark cycle. Live embryos at 72 h.p.f were classified and used for imaging analysis.

2.9. Statistical analysis

The data are presented as means ± SD. Paired student *t*-test or one-way ANOVA was used to compare the differences in data between the groups. $P < 0.05$ is considered to be significant.

3. Results

3.1. Fargesin exhibits lower toxicity effects on B16F10, Melan-a and HEM cells

Fargesin is a component of Flos Magnoliae, which belongs to the class of furanoid lignans with a structure that contains either a tetrahydrofuran ring, a furan ring, or a furoxan ring system (Fig. 1A). CKK8 assay was performed to evaluate the cytotoxic effects of fargesin on B16F10, Melan-a and HEM cells. As shown in Fig. 1B and C, no apparent cytotoxicity was observed for B16F10 and Melan-a cells after fargesin treatments at the concentrations of 10, 20, 30, 50, 80 and 100 μM. Cell viability was still above 95% even with the highest concentration of 100 μM (Fig. 1B and C). Importantly, viability of HEM cells was also not affected by fargesin at the concentrations of 50 and 100 μM. In addition, the morphology of B16F10 and HEM cells was similar before and after fargesin treatments (Fig. S1A and 1B). Therefore, we conclude that fargesin is not toxic to B16F10, Melan-a and HEM cells at the concentrations below 100 μM.

3.2. Fargesin reduces the intracellular production as well as extracellular secretion of melanin in B16F10 cells

By visual observation of supernatant color or biochemical measurement of the melanin content, we found that melanin production detected at 490 nm (OD 490) was remarkably decreased in the culture medium when B16F10 cells were treated with fargesin at the concentration of 50 μM (Fig. 2A). When 400 nm wavelength was used for spectroscopic estimation of melanin concentration in the media, similar results were obtained (Supplemental Fig. 2). In addition, cellular melanin was also greatly reduced by 50 μM fargesin treatment (Fig. 2B). Surprisingly, the higher concentration treatments (80 and 100 μM) exhibited less inhibition on melanin production than 50 μM fargesin treatment (Fig. 2A and B).

To further confirm the effects of fargesin on melaningenesis, we used UVB irradiation to induce melanin production. Indeed, UVB at the dose of 30 mJ/cm^2 markedly increased melanin content both within the cells and in the culture medium (Fig. 2C and D). Importantly, the increase of melanin induced by UVB could be fully reversed after fargesin treatment at a concentration of 50 μM (Fig. 2E). These results suggest that fargesin is a potent agent for reducing melanin production in B16F10 cells.

3.3. Fargesin inhibits the expression of melanogenic enzymes in B16F10 cells

MITF is a master regulator of melanogenesis and can upregulate melanogenic genes (*Tyrosinase*, *TRP-1* and *TRP-2*) via binding to the M-box motif in their promoter regions. Thus, we examined the effects of fargesin on melanogenic enzyme production using RT-qPCR and Western blot analysis. As shown in Fig. 3A and B, the mRNA and protein expression levels of MITF and tyrosinase in B16F10 cells were significantly reduced after 50 μM fargesin treatment. In the UVB-irradiated cells, the UVB-induced increase of MITF and tyrosinase at the mRNA and protein levels were also effectively inhibited by fargesin at 50 μM (Fig. 3C and D). Similar effects were also found in the mRNA expression of *TRP-1* and *TRP-2* (Fig. 3C). These results indicate that fargesin inhibits the expression of melanogenic enzymes.

3.4. Fargesin inhibits melanin production and melanogenic enzyme expression in normal melanocytes

Given that B16F10 is a tumor cell line, next we used mouse and human normal melanocytes (Melan-a and HEM) to determine whether fargesin also inhibits melanin production in non-malignant cells. As shown in Fig. 4A and B, cellular melanin content in Melan-a and HEM cells was markedly reduced after fargesin treatment at 50 μM. RT-qPCR analysis also revealed dramatic decreases of *MITF* and *tyrosinase* mRNA

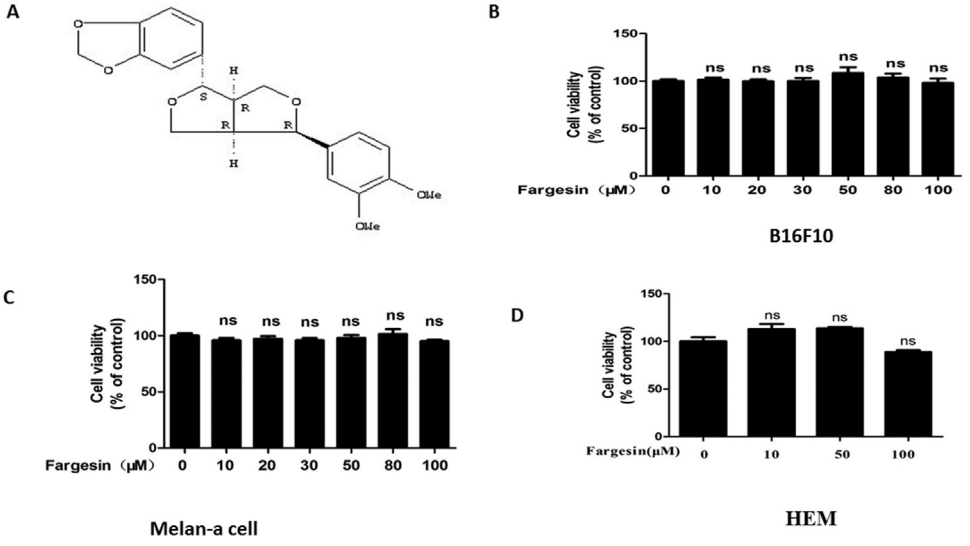

Fig. 1. Cell cytotoxicity was analyzed in B16F10 cells and Melan-a cells after fargesin treatments. A. Chemical structure of fargesin. B–D. The effects of fargesin on the viability of B16F10 (B), Melan-a (C), and HEM (D) cells. Cells were treated with various concentrations of fargesin as indicated. Data are presented as means ± SEM of three independent experiments.* $p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

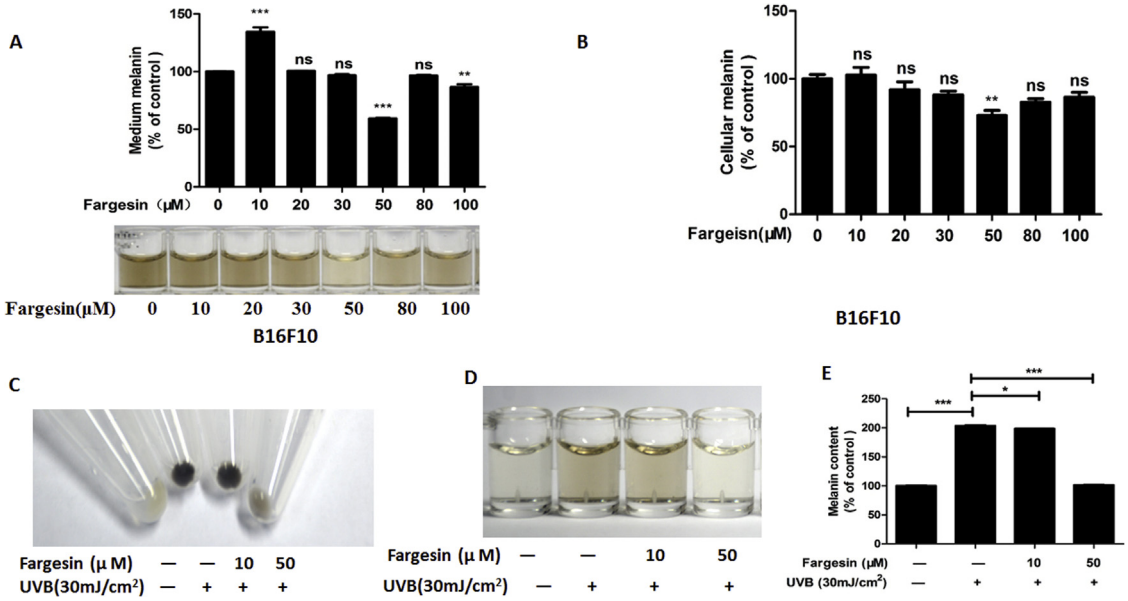

Fig. 2. Fargesin reduces melanin production in B16F10 cell. A&B. Measurement of melanin contents in the medium (A, at 490 nm) and cells (B, at 405 nm) after treatments with various concentrations of fargesin (μM) for 72 h. C. Images of B16F10 cell pellets with or without UVB (30 mJ/cm^2) and/or fargesin (10 or 50 μM). D. Supernatant color of B16F10 samples from (C). E. Medium melanin contents from (C). Data are presented as means ± SEM of three independent experiments.* $p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

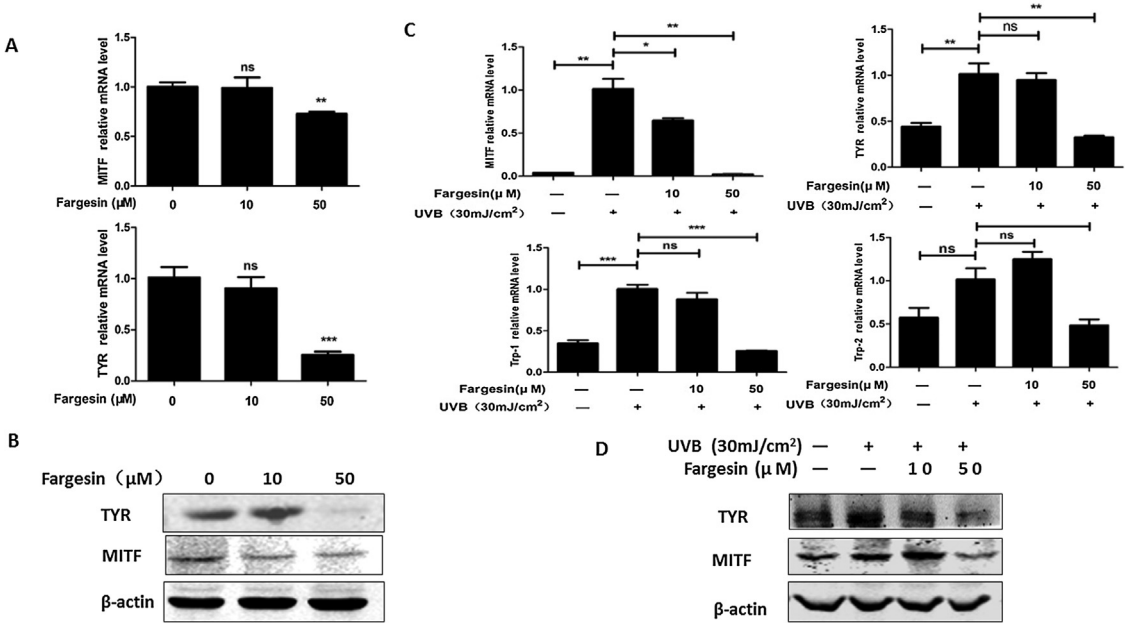

Fig. 3. Fargesin inhibits the expression of melanogenic enzymes in B16F10 cells. A. RT-qPCR analysis of MITF and TYR. B. Western blotting analysis of tyrosinase and MITF protein expression. C. B16F10 cells were treated with or without UVB for 30 mJ/cm^2 and then various concentrations of fargesin for 48 h. The mRNA levels of *TYR*, *MITF*, *TRP1* and *TRP2* from B16F10 cells were detected using RT-qPCR. D. Tyrosinase and MITF protein levels were analyzed in B16F10 cells treated with 30 mJ/cm^2 UVB followed by fargesin of 10 or 50 μM for 48 h. Data are presented as means ± SEM of three independent experiments.* $p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

expression in fargesin-treated Melan-a cells (Fig. 4C). Consistent with the PCR results, the MITF and tyrosinase protein levels in fargesin-treated Melan-a cells were significantly lower compared to the untreated controls (Fig. 4D). Thus, fargesin is also effective in inhibiting melanin production in normal melanocytes.

3.5. Fargesin inhibits the activity of tyrosinase in B16F10 and Melan-a cells

Tyrosinase activity and its expression are equally important for controlling melanin synthesis. Therefore, we further asked whether fargesin treatment affects tyrosinase activity. In B16F10 cells, treatment with 50 μM fargesin led to a ~2-fold decrease in tyrosinase activity (Supplemental Fig. 3A). Consistent with Fig. 2A and B, the inhibitory effects of fargesin were weaker at 80 and 100 μM than at 50 μM.

In Melan-a cells, a 30% decrease in tyrosinase activity was detected after fargesin treatment at a concentration of 50 μM (Supplemental Fig. 3B), but 80 μM fargesin showed better effects than both 50 and 100 μM. In HEM cells, fargesin at 50 μM also inhibited tyrosinase activity by 30% (Supplemental Fig. 3C). Taken together, these data suggest that fargesin inhibits tyrosinase activity in both melanoma cells and normal melanocytes.

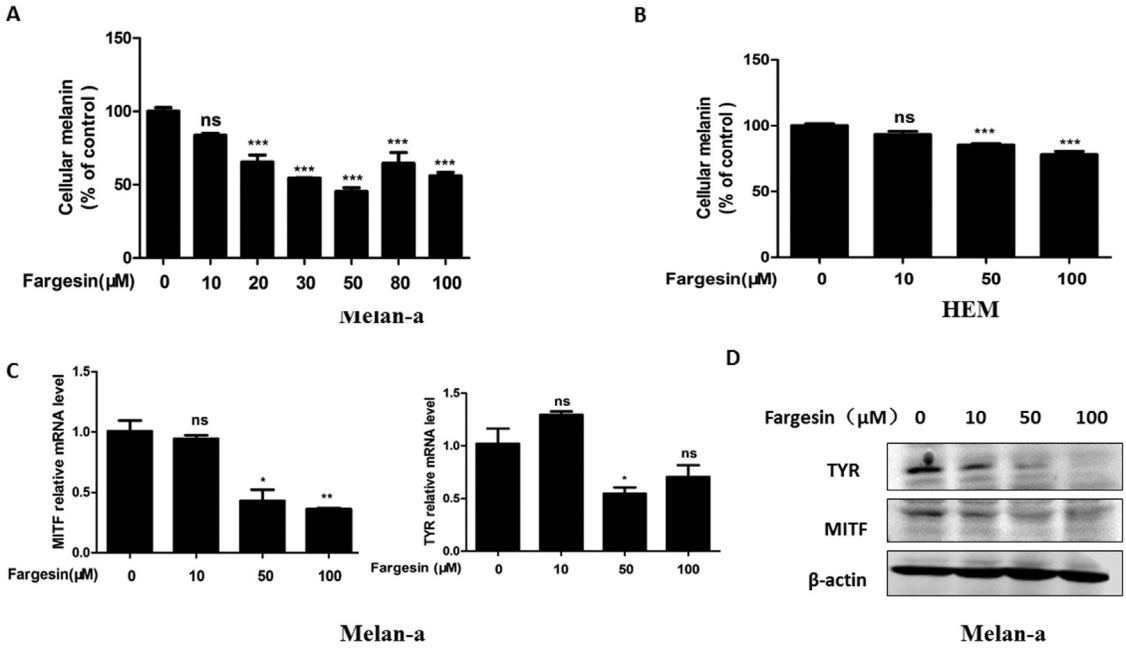

Fig. 4. Fargesin inhibits melanin production and melanogenic enzyme expression in normal melanocytes. A&B. Measurement of melanin contents in Melan-a (A) and HEM (B) cells after treatments with various concentrations of fargesin (μM) for 72 h. C. Melan-a cells were treated with various concentrations of fargesin for 48 h. The mRNA levels of *TYR* and *MITF* were detected using RT-qPCR. D. TYR and MITF protein levels were analyzed in Melan-a cells after treatments with fargesin for 48 h by Western blot analysis. Data are presented as means ± SEM of three independent experiments.* $p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

3.6. Fargesin inhibits melanin production and melanogenic enzyme expression in zebrafish embryos

Zebrafish has recently been established as a model for evaluating the depigmenting activity of melanogenic regulatory compounds [18]. Thus, we used zebrafish to evaluate the effects of fargesin on melanogenesis. As shown in Fig. 5A, zebrafish embryos treated with fargesin for 72 h at a concentration of 300 μM showed significant decrease in skin melanin formation at developing larvae stage. In contrast, a lower dosage of fargesin (100 μM) did not significantly affect zebrafish body pigmentation. Importantly, the inhibitory efficiency of 300 μM fargesin was better than kojic acid, which is routinely used as a positive control for depigmentation (Fig. 5A). Additionally, the total melanin content in zebrafish, measured by visual observation of supernatant color or biochemical methods, was markedly decreased after treatment with 300 μM fargesin (Fig. 5B). Consistently, the mRNA levels of *TYR* and *MITF* in the whole extract of zebrafish were also greatly inhibited by 300 μM fargesin (Fig. 5C). Collectively, these results demonstrate an *in vivo* inhibitory effect of fargesin on zebrafish pigmentation.

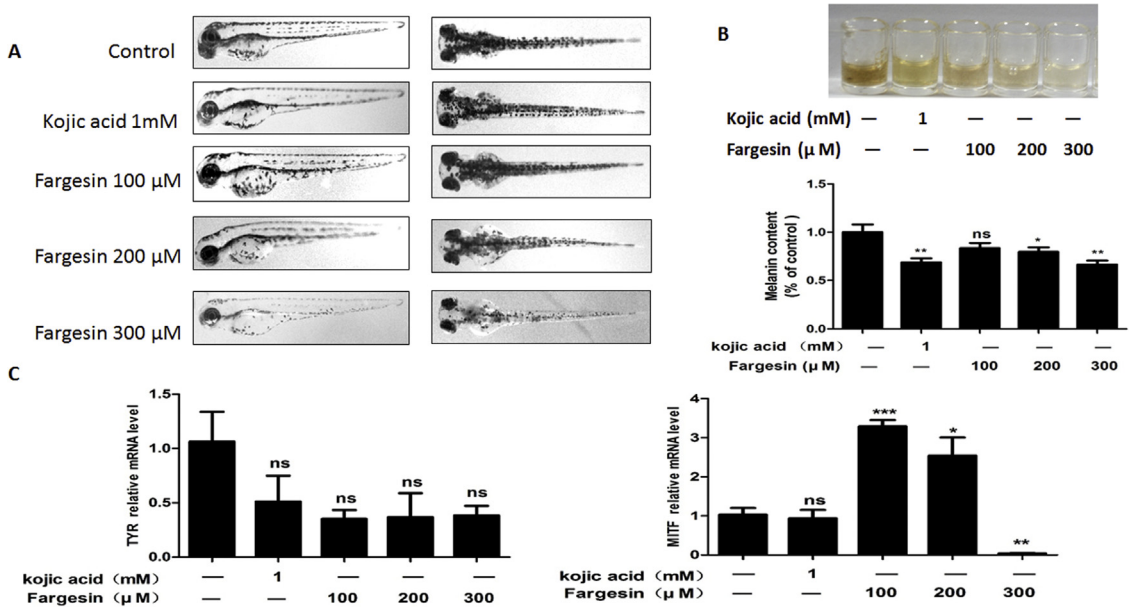

Fig. 5. Fargesin inhibits melanin production and melanogenic enzyme expression in zebrafish embryos. A. The effects of fargesin on the pigmentation of zebrafish were observed under a stereomicroscope. B. zebrafish embryos were collected and dissolved in 1 N NaOH. Total melanin content was measured. C. zebrafish embryos were collected and mRNA levels of *TYR* and *MITF* were detected using RT-qPCR. Data are presented as means ± SEM of three independent experiments.* $p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

3.7. Fargesin inhibits the activities of PKA and p38 in B16F10 cells

Since the role of cAMP pathway during melanogenesis is well-documented and the activation of p38 MAPK pathway has been shown to induce MITF and tyrosinase expression, we proceeded to study the effect of fargesin on cAMP/PKA/CREB and MAPK signaling pathways with Western blotting analysis. PKA and p-CREB levels in B16F10 cells were greatly decreased after treatment with 50 μM fargesin (Fig. 6A). In addition, fargesin at this concentration markedly inhibited the phosphorylation of p38 MAPK but not of ERK or JNK (Fig. 6B). Notably, cAMP activator (forskolin)-stimulated melanin production was effectively inhibited by 50 μM fargesin (Fig. 6C). Consistently, forskolin-induced increases of MITF and tyrosine expression were fully reversed to normal level after the treatment of 50 μM fargesin (Fig. 6D). Interestingly, we found that forskolin also induced the phosphorylation of p38 in addition to PKA activation in B16F10 cells, and this effect was reversed by 50 μM fargesin (Fig. 6E). Thus, fargesin likely inhibits melanin production in part by blocking cAMP/PKA/CREB and p38/MAPK signaling pathways.

4. Discussion

Fargesin is widely used as an anti-hypertensive and anti-inflammatory agent. In this study, we found that fargesin inhibits melanin production and melanogenic enzyme expression in murine melanoma cells and melanocytes, normal human melanocytes, and zebrafish embryos. Thus, fargesin may potentially be used as a skin-whitening compound for preventing skin darkening in skin care products in the future.

cAMP is a key second messenger in regulating skin pigmentation. Activation of PKA is known to induce CREB phosphorylation and stimulates MITF transcription [19]. Consistent with this previous finding, we observed that forskolin treatment markedly increased, while H89 inhibited, melanin production in B16F10 cells. Notably, simultaneous treatments with forskolin and fargesin fully blocked the increase of melanin content and melanogenic enzyme expression induced by forskolin. Moreover, fargesin treatment can effectively inhibit PKA and p-CREB activation in B16F10 cells. These data together indicate that fargesin may play a role in anti-melanogenesis by blocking the cAMP/PKA signaling pathway. It is worth emphasizing that fargesin is able to reverse the forskolin-induced increase of melanin content to near-normal level. In zebarfish, fargesin even showed stronger effects for depigmentation than kojic acid, a compound that is well-recognized as a skin-lightening cosmetic product. Consequently, fargesin may be an effective candidate agent for inhibiting melanin synthesis through regulating the cAMP/PKA signaling pathway. Interestingly, we found that 50 μM fargesin exerted a higher suppressive effect on melanogenesis than 100 μM in B16F10 and Mel Melan-a cells but not in HEM cells. This cell-specific phenomenon may be due to the possible compensatory pathways induced by higher concentration of fargesin which partly counteracted its anti-melanogenic effects.

It has been demonstrated that α-MSH binds to melanocortin-1 receptor (MC1R) on the cell surface and activates adenylate cyclase, which leads to the upregulation of cAMP/PKA signaling pathway and the induction of MITF expression [20]. Both L-tyrosine and L-DOPA act as positive regulators of melanogenesis by stimulating MSH receptor activities [21]. Therefore, a potential effect of fargesin on MSH receptor and MC1R should be investigated in the future.

Ultraviolet radiation (UVR) can stimulate melanin production in human epidermal melanocytes. It is reported that the MC1R-cAMP pathway plays an important role in UVR-induced tanning [22]. Similar to the UVR-induced effect, p38 MAPK signaling is involved in stress-induced melanogenesis. A previous study reported that phosphorylation at Ser301 of MITF by p38 results in the stimulation of MITF transcriptional activity [3]. On the other hand, another study showed that p38 regulates pigmentation in a manner that is dependent on proteasomal degradation of tyrosinase [23]. In this study, we show that fargesin not only

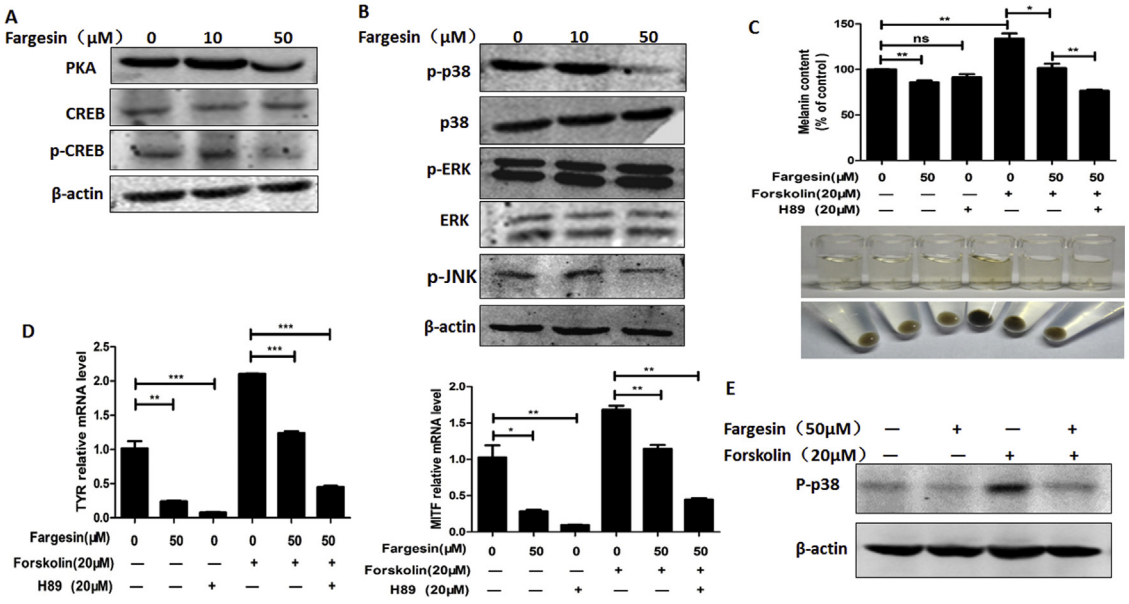

Fig. 6. Fargesin inhibits the activities of PKA and p38 signaling pathway components in B16F10 cells. A. B16F10 cells were treated with fargesin of 10 μM and 50 μM for 48 h, and the expression levels of proteins including PKA, CREB and p-CREB were detected by using Western blot analysis. B. B16F10 cells were cultured with fargesin of 10 μM and 50 μM for 48 h. Whole-cell lysate was subjected to Western blot analysis using antibodies against p-p38, p38, p-ERK, ERK, and p-JNK. Equal protein loading was confirmed using a β-actin antibody. C. Changes in melanin content in B16F10 cells upon treatments with fargesin (50 μM), forskolin (20 μM), H89 (20 μM) or forskolin plus fargesin. D. Changes in mRNA levels of *TYR* and *MITF* in B16F10 cells upon treatments with fargesin (50 μM), forskolin (20 μM), H89 (20 μM) or forskolin plus fargesin. E. P-p38 changes in B16F10 cells upon treatments with fargesin (50 μM), forskolin (20 μM) or forskolin plus fargesin, assayed by Western blotting. Data are presented as means ± SEM of three independent experiments.* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

greatly inhibits UVB-induced melanin synthesis and melanogenic enzyme expression, but also effectively decrease the activity of p38 kinase in murine melanoma cells. These data suggest that p38 is also likely a target molecule of fargesin in blocking melanin production. Consistent with our findings, activation of p38 MAPK pathway was reported to contribute to the melanogenic property of apigenin (a natural product belonging to the flavone class) in B16F10 cells [24].

The detailed mechanism by which fargesin regulates cAMP/PKA and p38 MAPK signaling pathways is still unclear and remains to be elucidated. It is reported that p38-MAPK can be activated by cAMP in a cell-specific manner [25,26]. In B16F10 cells, we found that forskolin markedly increased p38 activity. We thus suspect that fargesin also has the ability to interrupt their interaction, and this may be why melanin synthesis induced by forskolin or UVB can both be effectively inhibited by fargesin. A previous study showed that MITF expression can also be initiated by lymphoid-enhancing factor-1 (LEF1) [27]. Thus, fargesin may also regulate MITF phosphorylation via Wnt signaling pathway, which requires further investigation.

TYR is exclusively critical for melanogenesis, and tyrosinase is regulated at both expression and activity levels to increase melanin production. Interestingly, fargesin can directly lower the catalytic activity of tyrosine in addition to downregulating tyrosine gene expression. A previous study reports that sesamol, a bioactive lignan with strong antioxidative activity, can cause the lysosomal and proteasomal degradation of tyrosinase in melan-a cells [28]. Thus, the possible influence of fargesin on tyrosinase stability also needs to be studied in the future.

Together, this study suggests a model where fargesin decreases melanogenesis in murine melanoma cells, normal melanocytes and zebrafish not only by downregulating the expression of *MITF* and its downstream melanogenic enzyme-coding genes, but also by inhibiting the catalytic activity of tyrosinase. We also demonstrate that fargesin inhibits the activation of PKA/CREB and p38 MAPK signaling pathways, unveiling the possible molecular mechanisms underlying the anti-melanogenic function of fargesin (Supplemental Fig. 2). Our study underscores fargesin and its derivatives as potential agents to be used for preventing hyperpigmentation disorders in the clinic in the future.

Conflict of interest

The authors have no conflict of interest to declare.

Acknowledgements

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