Syntenin regulates melanogenesis via the p38 MAPK pathway

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Abstract. Melanogenesis is the synthesis of the skin pigment melanin, which serves a critical role in the study of pigmentary skin diseases. Syntenin has been identified as a melanosome protein, but its role in melanogenesis is not completely understood. The present study aimed to investigate the effects and mechanisms underlying syntenin on melanogenesis in immortalized human melanocytes. Depletion of syntenin expression increased both tyrosinase (Tyr) activity and melanin content. Syntenin silencing also increased the protein expression levels of Tyr, pre-melanosomal protein and microphthalmia-associated transcription factor. In addition, the results indicated that syntenin regulated melanogenesis by upregulating the phosphorylation of p38 mitogen-activated protein kinase (p38 MAPK). Taken together, these findings suggested that the regulation of melanogenesis by syntenin may be mediated by the activation of p38 MAPK and that syntenin might provide new insights into the pathogenesis of pigmented diseases.

Introduction

Melanin is synthesized by melanocytes and determines the color of fur, hair and skin. The melanosome, in which melanin is synthesized and stored, is a functionally specialized membrane-encased organelle (1). The melanosome maturation process involves a number of proteins, including tyrosinase (Tyr), pre-melanosomal protein (Pmel), tyrosinase-related protein 1 (Tyrp1), Tyrp2 and ocular albinism type 1 protein (2-4). Among these proteins, Tyr is a crucial catalytic enzyme component and it is irreplaceable in melanogenesis (5). Pmel is one of the structural proteins that serves a key role in the formation of intraluminal fibrils, eventually leading to the deposition of melanin in melanosomes (6). Tyr and Pmel play an important role in melanogenesis, and their expression is regulated by microphthalmia-associated transcription factor (MITF) (7).

Syntenin, also known as melanoma differentiation-related gene-9 (mda-9), is an evolutionarily conserved intracellular adaptor protein involved in many important physiological and pathological aspects, such as development, immunity and cancer metastasis (8). Syntenin was first identified in melanoma research and previous studies have demonstrated that it is expressed in a number of normal and tumoral tissues, as well as normal melanocytes (9-11); however, to the best of our knowledge, the effects of syntenin on melanin synthesis and corresponding molecular mechanisms have not yet been reported.

Therefore, the present study aimed to clarify the role of syntenin in melanogenesis, the effect of depletion of syntenin on melanin production and expression of melanogenic molecules Tyr, Pmel and MITF in melanocytes. In addition, the effect of syntenin on the phosphorylation of p38 mitogen-activated protein kinase (p38 MAPK) was also determined.

Materials and methods

Antibodies and reagents. Rabbit anti-syntenin (cat. no. ab133267), anti-Pmel (cat. no. ab137078), anti-MITF (cat. no. ab20663) and anti-β-actin (cat. no. ab8227) antibodies were purchased from Abcam. Horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG (cat. no. ab205719) and HRP-conjugated goat anti-rabbit IgG (cat. no. ab205718) secondary antibodies were purchased from Abcam. The mouse anti-Tyr monoclonal antibody (cat. no. AT4426a) was purchased from Abgent, Inc. The rabbit anti-p38 (cat.

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no. 9212) and anti-phosphorylated p38 (p-p38; cat. no. 9211) monoclonal antibodies were purchased from Cell Signaling Technology, Inc. SB203580 (p38 MAPK inhibitor) was purchased from Beyotime Institute of Biotechnology. L-3,4-dihydroxyphenylalanine (L-DOPA) was purchased from MedChemExpress.

Cell culture. The immortalized human melanocyte cell line PIG1 was a gift from Professor Caroline Le Poole (Department of Dermatology, University of Cincinnati, USA). Cells were maintained in Medium 254 supplemented with 5% fetal bovine serum and human melanocyte growth supplement (cat. no. S-002-5), all from Gibco (Thermo Fisher Scientific, Inc.), at 37˚C in a humidified atmosphere of 5% CO_2.

Following syntenin-siRNA transfection for 24 h, co-treatment with 10 μM p38 MAPK inhibitor SB203580 at 37˚C for 2 h, the melanin content and Tyr activity of the cells were detected.

Transfection of small interfering (si)RNAs. For the targeted knockdown of syntenin, a mixture of three pairs of syntenin-siRNAs were designed and synthesized by Invitrogen (Thermo Fisher Scientific, Inc.), according to a previously described method (12). The nucleotide sequences of the siRNAs were as follows: Syntenin-homo-612, sense 5'-GGG ACCAAGUACUUCAGAUTT-3', antisense 5'-AUCUGAAGU ACUUGGUCCCTT-3'; Syntenin-homo-398, sense 5'-GCA AGACCUUCCAGUAUAATT-3', antisense 5'-UUAUACUGG AAGGUCUUGCTT-3'; Syntenin-homo-839, sense 5'-GGU CUUCUCACGGAACAUATT-3', antisense 5'-UAUGUU CCGUGAGAAGACCTT-3', respectively. Negative control (NC)-siRNAs with the nucleotide sequences of sense 5'-UUC UCCGAACGUGUCACGUTT-3' and antisense 5'-ACGUGA CACGUUCGGAGAATT-3' were also used. A total of $5x10^4$ melanocytes were seeded in 24-well plates and grown for one day to reach 30-50% confluence. A total of 15 pmol of the three aforementioned syntenin-siRNA pairs or 15 pmol NC-siRNA were then transfected into the cells using 1.5 μl Lipofectamine® RNAi-MAX (Invitrogen; Thermo Fisher Scientific, Inc.). Untreated cells were used as blank. Cells were cultured in Medium 254 at 37˚C with 5% CO_2. At 24 and 48 h post-transfection, transfection efficiency was determined by western blotting.

Cell viability assay. The cell viability assay was performed using a Cell Titer-Blue H Cell Viability assay kit (Promega Corporation) according to the manufacturer's instructions. Cells were seeded at a density of $5x10^3$ cells/well in 96-well plates and incubated over night at 37˚C. After 24 and 48 h of syntenin-targeted siRNA transfection, cell viability was determined by adding cell titer blue (20 μl/well) as an indicator and further incubated at 37˚C for 4 h, fluorescence was subsequently measured at a wavelength of 560/590 nm using a Gen™ spectrophotometer (• BioTek Instruments, Inc.).

Western blotting. Whole cell protein extracts prepared using the RIPA buffer (Beyotime Institute of Biotechnology) and quantified using the BCA Protein assay kit (Beyotime Institute of Biotechnology). Protein (20 μg per lane) was separated by 12% SDS-PAGE and then transferred to a nitrocellulose membrane (Pharmacia; GE Healthcare). After blocking with 5% skimmed milk at room temperature for 1 h, the membrane was incubated with diluted primary antibody at 4˚C overnight. The primary antibodies used were anti-Pmel (1:300), anti-syntenin (1:500), anti-Tyr (1:200), anti-MITF (1:500), anti-β-actin (1:1,000), anti-p38 (1:500) and p-p38 (1:500). Then the membrane was incubated with HRP-labeled secondary antibodies at room temperature for 2 h. Protein bands were visualized using ECL reagents (Roche Diagnostics GmbH). Protein expression levels were semi-quantified using Gel-Pro Analyzer software (version 4.0; Media Cybernetics, Inc.) with β-actin as the loading control.

Melanin content determination. The total content of melanin was measured as previously described by Hosoi *et al* (13). Cells were seeded ($3x10^5$ cells/well) in 6 wells plate and incubated over night at 37˚C. After 24 or 48 h of transfection with syntenin-siRNA, cells were washed twice with PBS and the cell pellets were dissolved in 1 N NaOH (1 ml) at 100˚C for 30 min and centrifuged at 16,000 x g for 20 min at room temperature. The optical density of the supernatant was measured at 405 nm using an ELISA microplate reader.

Tyr activity assay. Tyr activity was estimated using a modification of a previously reported method (14). Cells were seeded ($5x10^3$ cells/well) in 96 wells plate and incubated overnight at 37˚C. After 24 or 48 h of syntenin-siRNA transfection, cells were washed twice with PBS and homogenized in 200 μl of 0.1 M sodium phosphate buffer (pH 6.8) containing 1 M phenylmethylsulfonyl fluoride and 1% Triton X-100. A total of 50 μl of the supernatant, 50 μl of 0.1% L-DOPA and 100 μl of 0.1 M sodium phosphate buffer (pH 6.8) were combined and incubated at 37˚C for 15 min. The formation of dopachrome was monitored by detection of the absorbance at a wavelength of 475 nm with an ELISA microplate reader. Each treatment was repeated three times.

Statistical analysis. Data were analyzed using the SPSS 19.0 statistical software (IBM Corp). Data are expressed as the mean ± SEM. All experiments were repeated at least three times. One-way ANOVA followed by the Tukey's post hoc test was used for multiple comparison tests. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Syntenin-siRNA significantly downregulates syntenin protein levels in immortalized human melanocytes. Western blot analysis was used to determine whether the syntenin siRNA transfections successfully inhibited the expression of syntenin protein in PIG1 cells. At 24 and 48 h after transfection, the expression of syntenin protein was effectively reduced in syntenin-siRNA-transfected melanocytes compared with NC-siRNA transfected melanocytes (Fig. 1A). Syntenin-siRNA transfection had no significant effect on cell viability over 48 h (Fig. 1B). These results demonstrated that syntenin-siRNA transfection effectively depleted expression of syntenin.

siRNA-induced silencing of syntenin increases melanin content and Tyr activity. Melanin content and Tyr enzyme

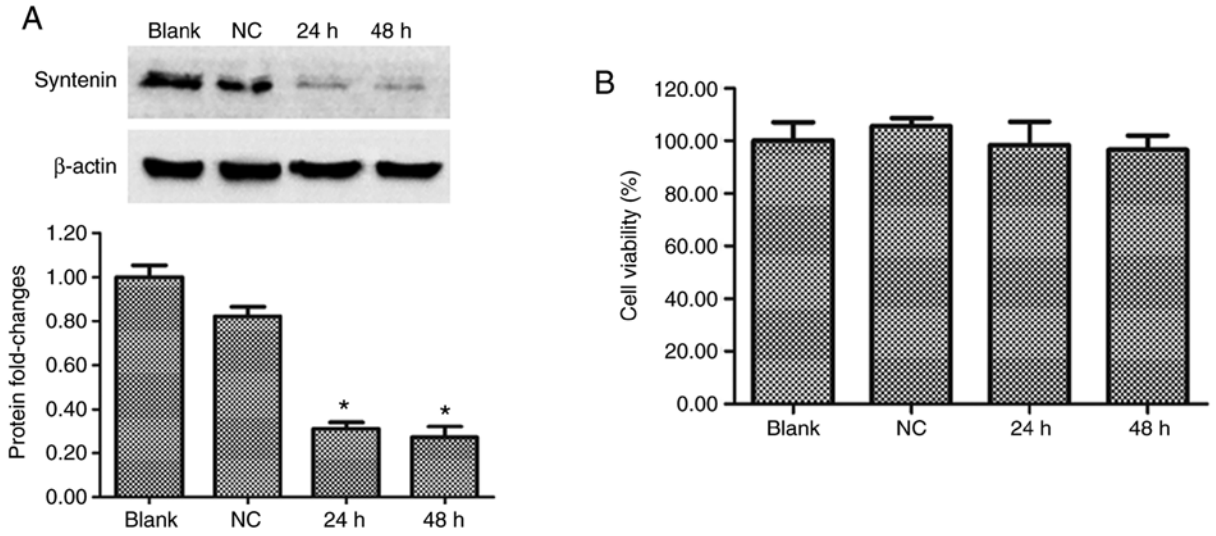

Figure 1. Syntenin-siRNA transfection downregulated the expression of syntenin without affecting cell viability. PIG1 immortalized human melanocytes were either left untreated (Blank), transfected with NC-siRNA or transfected with syntenin-siRNA. (A) Syntenin protein expression levels were downregulated following syntenin-siRNA transfection after 24 and 48 h, as determined by western blotting. Results were normalized to β-actin expression. (B) The viability of the melanocytes was assessed using a Cell Titer-Blue H Cell Viability assay kit. *P<0.05 vs. Blank. NC, negative control; siRNA, small interfering RNA.

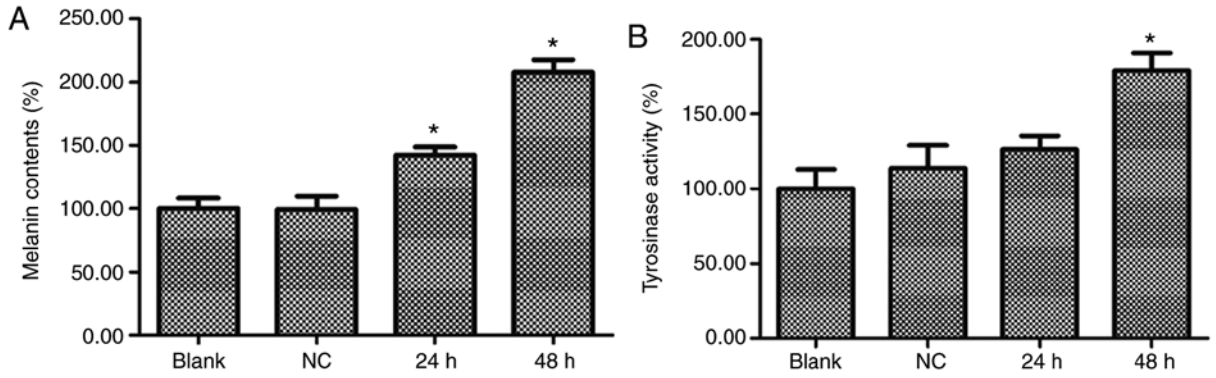

Figure 2. Silencing of syntenin increases melanin content and tyrosinase activity in immortalized human melanocytes. (A) Melanin content and (B) tyrosinase enzyme activity were examined after transfection with small interfering RNAs targeting syntenin. *P<0.05 vs. Blank. NC, Negative control.

activity were detected at 24 and 48 h after PIG1 cells were transfected with syntenin-siRNA. Compared with the NC-siRNA-transfected group, the syntenin-siRNA-transfected cells demonstrated a significant increase in melanin content and Tyr activity (Fig. 2A and B, respectively). These results indicated that depletion of syntenin induced melanogenesis in immortalized human melanocytes.

Syntenin silencing increases the expression of melanogenesis-related proteins in PIG1 cells. As the depletion of syntenin was found to increase melanin production and Tyr activity, the study sought to determine the effects of syntenin depletion on the expression of melanogenesis-related proteins Tyr, Pmel and MITF. At 24 and 48 h after syntenin-siRNA transfection, a significant increase in the protein expression levels of Tyr, Pmel and MITF, alongside a decreased expression of syntenin were observed compared with the control group (Fig. 3).

Silencing of syntenin stimulates melanin synthesis through activation of p38 MAPK. The phosphorylation of p38 MAPK was reported to be one of the signaling pathways involved in hyperpigmentation (15). Therefore, western blot analysis was performed to determine the effects of syntenin on p38 phosphorylation. As shown in Fig. 4A, phosphorylation of p38 MAPK was significantly increased after transfection of syntenin-siRNA in PIG1 cells compared with the control group. To confirm that the p38 MAPK is involved in syntenin-mediated melanogenesis, a melanin content assay and a cellular Tyr activity assay were performed. After syntenin-siRNA transfection for 24 h, co-treatment with 10 μM p38 MAPK inhibitor SB203580 at 37˚C for 2 h significantly reduced syntenin-siRNA triggered melanin content and Tyr activity (Fig. 4B and C). These observations revealed that the p38 MAPK signaling may be involved in the melanogenesis pathway mediated by syntenin.

Discussion

Syntenin, also known as mda-9, was originally cloned from human melanoma cells (9). As a molecule containing a PDZ domain, syntenin recuits membrane receptors and cytoplasmic signaling proteins into functional complexes via

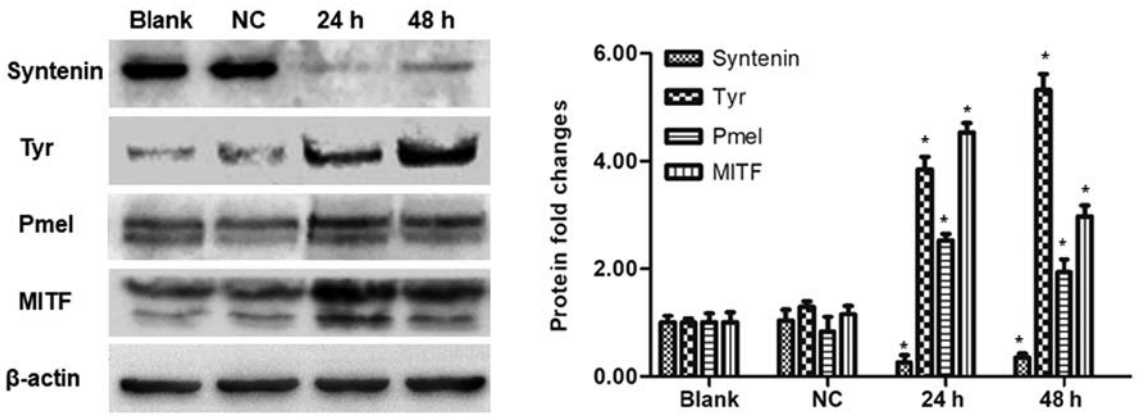

Figure 3. Syntenin-siRNA transfection increases the expression of melanogenesis-related proteins in PIG1 immortalized human melanocytes. After transfection with syntenin-siRNA, western blotting was performed to examine the protein expression levels of catalytic component Tyr, melanosome structural protein Pmel and transcription factor MITF. Results were normalized against β-actin expression. *P<0.05 vs. Blank. MITF, microphthalmia-associated transcription factor; NC, negative control; Pmel, pre-melanosomal protein; siRNA, small interfering RNA; Tyr, tyrosinase.

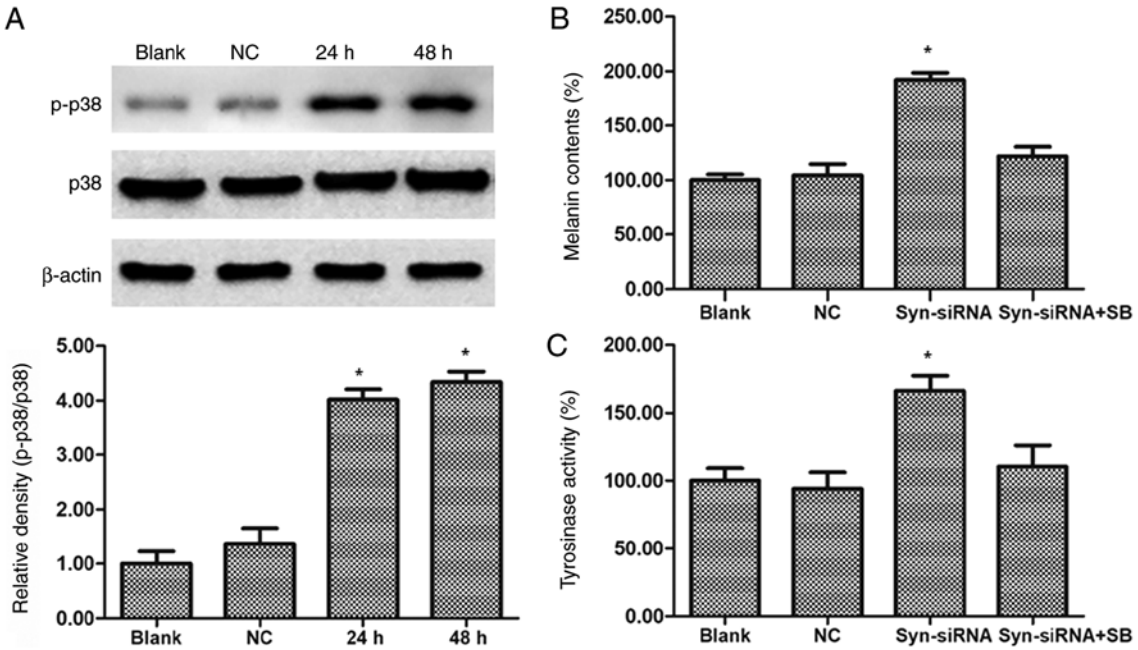

Figure 4. Syntenin silencing activates the p38 MAPK signaling pathway. (A) Phosphorylation of p38 MAPK was significantly increased after transfection of syntenin-siRNA in melanocytes. (B) Melanin content and (C) tyrosinase activity were significantly increased after transfection of syntenin-siRNA, but this effect was abolished with co-treatment with p38 MAPK inhibitor SB203580. *P<0.05 vs. Blank group. NC, Negative control; p, phosphorylated; p38, p38 mitogen-activated protein kinase; SB, SB203580; siRNA, small interfering RNA; syn, syntenin.

its PDZ domains, allowing for fast and efficient signal transduction and membrane transport processes in responses to external stimuli. Thereby, syntenin serves a number of roles and regulates a variety of physiological processes (8-10). Syntenin has been identified as a melanosome protein, but its role in melanin biosynthesis is still unclear (16). The present study focused on how syntenin affected melanosome formation. The data demonstrated an increase in melanogenesis after syntenin was silenced by siRNAs,. The findings reveal a novel mechanism by which syntenin dysfunction can regulate melanogenesis.

The synthesis of melanin is essential for the research and treatment of pigmentary skin diseases (17). Tyr is a key enzyme that regulates the synthesis of melanin; the expression and enzymatic activities of Tyr affect melanogenesis and the total melanin content of cells (5,18). Pmel is a melanosome-specific type 1 transmembrane glycoprotein that directly initiates the formation of pro-melanosome proteins in polyvesicular bodies, which are important for Tyr sorting into melanosomes and ultimately pigmentation (19). The present study results showed that syntenin gene silencing led to increased expression of Tyr and the melanosome structural protein Pmel. These results also demonstrated that melanin synthesis was increased following depletion of syntenin, which was consistent with the Tyr activity assay.

The transcription factor MITF has an important role in the formation and transport of melanosomes (20). MITF belongs to the family of helix-loop-helix leucine zipper transcription

factors, specifically binding to M-box (AGTCATGTGCT) and E-box (CATGTG) motifs, regulating the transcription of more than 25 pigment-related genes, including enzyme components and structural protein components that have key roles in melanogenesis (21). Tyr is mediated via activation of MITF production (22). In addition, decreased MITF expression induces downregulation of melanocyte differentiation markers and inhibits melanogenesis (23). The results of the present study demonstrated that the protein expression levels of the key enzyme component Tyr and the melanosome structural protein Pmel were increased, whereas the expression level of MITF, which regulates the expression of these two proteins was also increased; this indicated that silencing of syntenin increased the expression of melanogenic-related proteins that were dependent on MITF transcription factors, and also suggested a possible mechanism for syntenin-based silencing of melanin.

MAPKs are key signaling molecules involved in the regulation of melanogenesis, including the stress-activated protein kinase/JNK, extracellular signal-regulating kinase (ERK) and the p38 MAPK signaling cascades (24). Previous studies have shown that the JNK and ERK stress-activated protein kinase pathways led to downregulated melanin synthesis (25,26). By contrast, the phosphorylation of p38 MAPK activates MITF to ultimately stimulate melanogenesis (17,18). In the present study, silencing of syntenin significantly promoted p38 phosphorylation in PIG1 melanocytes. To verify whether the p38 MAPK signaling molecules are responsible for syntenin-induced melanogenesis, co-incubation of the p38 MAPK inhibitor SB203580 with syntenin-siRNA significantly abolished syntenin-stimulated tyrosinase activity and melanin content. These results suggested that the p38 MAPK may be responsible for the pigmentation process mediated by depletion of syntenin in melanocytes among the upstream pathways involved in melanogenesis.

In conclusion, syntenin has an effect on melanin synthesis in PIG1 immortalized human melanocytes. Silencing of syntenin enhanced melanogenesis through activation of the p38 MAPK signaling pathways. In future studies, we aim to further verify the function of syntenin in primary melanocytes instead of relying on studies conducted in immortalized cell lines. This study has demonstrated that syntenin may represent a promising candidate of treatment for pigmented diseases.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

LS, XX and JH conceived and designed the study. LS, CG and LY performed the experiments. HL and JS analyzed the data. XH interpreted the data and critically revised the manuscript for important intellectual content. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. d'Ischia M, Wakamatsu K, Napolitano A, Briganti S, Garcia-Borron JC, Kovacs D, Meredith P, Pezzella A, Picardo M, Sarna T, *et al*: Melanins and melanogenesis: Methods, standards, protocols. Pigment Cell Melanoma Res 26: 616-633, 2013.
2. Olivares C and Solano F: New insights into the active site structure and catalytic mechanism of tyrosinase and its related proteins. Pigment Cell Melanoma Res 22: 750-760, 2009.
3. Cortese K, Giordano F, Surace EM, Venturi C, Ballabio A, Tacchetti C and Marigo V: The ocular albinism type 1 (OA1) gene controls melanosome maturation and size. Invest Ophthalmol Vis Sci 46: 4358-4364, 2005.
4. Bissig C, Rochin L and van Niel G: PMEL amyloid fibril formation: The bright steps of pigmentation. Int J Mol Sci 17: pii: E1438, 2016.
5. Paterson EK, Fielder TJ, MacGregor GR, Ito S, Wakamatsu K, Gillen DL, Eby V, Boissy RE and Ganesan AK: Tyrosinase depletion prevents the maturation of melanosomes in the mouse hair follicle. PLoS One 10: e0143702, 2015.
6. Berson JF, Harper DC, Tenza D, Raposo G and Marks MS: Pmel17 initiates premelanosome morphogenesis within multivesicular bodies. Mol Biol Cell 12: 3451-3464, 2001.
7. Yasumoto K, Yokoyama K, Takahashi K, Tomita Y and Shibahara S: Functional analysis of microphthalmia-associated transcription factor in pigment cell-specific transcription of the human tyrosinase family genes. J Biol Chem 272: 503-509,1997.
8. Sarkar D, Boukerche H, Su ZZ and Fisher PB: mda-9/syntenin: Recent insights into a novel cell signaling and metastasis-associated gene. Pharmacol Ther 104: 101-115, 2004.
9. Lin J, Jiang H and Fisher P: Characterization of a novel melanoma differentiation associated gene, mda-9, that is down regulated during terminal cell differentiation. Mol Cell Differ 4: 317-333, 1996.
10. Kegelman TP, Das SK, Emdad L, Hu B, Menezes ME, Bhoopathi P, Wang XY, Pellecchia M, Sarkar D and Fisher PB: Targeting tumor invasion: The roles of MDA-9/Syntenin. Expert Opin Ther Targets 19: 97-112, 2015.
11. Xiao D, Ohlendorf J, Chen Y, Taylor DD, Rai SN, Waigel S, Zacharias W, Hao H and McMasters KM: Identifying mRNA, microRNA and protein profiles of melanoma exosomes. PLoS One 7: e46874, 2012.
12. Zhang P, Liu W, Zhu C, Yuan X, Li D, Gu W, Ma H, Xie X and Gao T: Silencing of GPNMB by siRNA inhibits the formation of melanosomes in melanocytes in a MITF-independent fashion. PLoS One 7: e42955, 2012.
13. Hosoi J, Abe E, Suda T and Kuroki T: Regulation of melanin synthesis of B16 mouse melanoma cells by 1 alpha, 25-dihydroxyvitamin D3 and retinoic acid. Cancer Res 45: 1474-1478, 1985.
14. Martínez-Esparza M, Jiménez-Cervantes C, Solano F, Lozano JA and García-Borrón JC: Mechanisms of melanogenesis inhibition by tumor necrosis factor-alpha in B16/F10 mouse melanoma cells. Eur J Biochem 255: 139-146, 1998.

15. Zhou J, Shang J, Ping F and Zhao G: Alcohol extract from Vernonia anthelmintica (L.) willd seed enhances melanin synthesis through activation of the p38 MAPK signaling pathway in B16F10 cells and primary melanocytes. J Ethnopharmacol 143: 639-647, 2012.
16. Basrur V, Yang F, Kushimoto T, Higashimoto Y, Yasumoto K, Valencia J, Muller J, Vieira WD, Watabe H, Shabanowitz J, *et al*: Proteomic analysis of early melanosomes: Identification of novel melanosomal proteins. J Proteome Res 2: 69-79, 2003.
17. Jung E, Kim JH, Kim MO, Jang S, Kang M, Oh SW, Nho YH, Kang SH, Kim MH, Park SH and Lee J: Afzelin positively regulates melanogenesis through the p38 MAPK pathway. Chem Biol Interact 254: 167-172, 2016.
18. Makpol S, Jam FA, Rahim NA, Khor SC, Ismail Z, Yusof YA and Wan Ngah WZ: Comparable down-regulation of TYR, TYRP1 and TYRP2 genes and inhibition of melanogenesis by tyrostat, tocotrienol-rich fraction and tocopherol in human skin melanocytes improves skin pigmentation. Clin Ter 165: 39-45, 2014.
19. Watt B, van Niel G, Raposo G and Marks MS: PMEL: A pigment cell-specific model for functional amyloid formation. Pigment Cell Melanoma Res 26: 300-315, 2013.
20. Vachtenheim J and Borovanský J: 'Transcription physiology' of pigment formation in melanocytes: Central role of MITF. Exp Dermatol 19: 617-627, 2010.
21. Pan L, Ma X, Wen B, Su Z, Zheng X, Liu Y, Li H, Chen Y, Wang J, Lu F, *et al*: Microphthalmia-associated transcription factor/T-box factor-2 axis acts through Cyclin D1 to regulate melanocyte proliferation. Cell Prolif 48: 631-642, 2015.
22. Khaled M, Larribere L, Bille K, Aberdam E, Ortonne JP, Ballotti R and Bertolotto C: Glycogen synthase kinase 3beta is activated by cAMP and plays an active role in the regulation of melanogenesis. J Biol Chem 277: 33690-33697, 2002.
23. Jiménez-Cervantes C, Martínez-Esparza M, Pérez C, Daum N, Solano F and García-Borrón JC: Inhibition of melanogenesis in response to oxidative stress: Transient downregulation of melanocyte differentiation markers and possible involvement of microphthalmia transcription factor. J Cell Sci 114: 2335-2344, 2001.
24. D'Mello SA, Finlay GJ, Baguley BC and Askarian-Amiri ME: Signaling pathways in melanogenesis. Int J Mol Sci 17: pii: E1144, 2016.
25. Kim DS, Jeong YM, Park IK, Hahn HG, Lee HK, Kwon SB, Jeong JH, Yang SJ, Sohn UD and Park KC: A new 2-imino-1,3-thiazoline derivative, KHG22394, inhibits melanin synthesis in mouse B16 melanoma cells. Biol Pharm Bull 30: 180-183, 2007.
26. Bu J, Ma PC, Chen ZQ, Zhou WQ, Fu YJ, Li LJ and Li CR: Inhibition of MITF and tyrosinase by paeonol-stimulated JNK/SAPK to reduction of phosphorylated CREB. Am J Chin Med 36: 245-263, 2008.