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Research Paper

Sphingosine-1-phosphate decreases melanin synthesis via microphthalmia-associated transcription factor phosphorylation through the $S1P_3$ receptor subtype

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Abstract

Objectives Previously, we reported that sphingosine-1-phosphate (S1P) reduced melanin synthesis. In this study we have investigated S1P receptor-mediated extracellular signal-regulated protein kinase (ERK) activation and microphthalmia-associated transcription factor (MITF) phosphorylation.

Methods To examine S1P-induced signalling pathways, electron and confocal microscopic studies, reverse transcription-polymerase chain reaction and Western blot analysis were performed.

Key findings S1P phosphorylated MITF at Ser73, which may have resulted in a MITF mobility shift. Furthermore, 90 kDa ribosomal S6 kinase-1 (RSK-1) phosphorylation was observed after S1P treatment. In addition, PD98059 abrogated the S1P-induced MITF mobility shift and RSK-1 activation. In experiments with MITF mutants, it was shown that dual phosphorylation at Ser73 and Ser409 was indispensable for MITF degradation. We investigated further the actions of S1P on its specific receptors. The results showed that pertussis toxin completely abolished the hypopigmentary effects and ERK pathway activation by S1P, suggesting that S1P regulated melanogenesis via its receptor. The use of specific receptor antagonists indicated that the $S1P_3$ receptor was dominantly involved in S1P-induced ERK activation and hypopigmentation.

Conclusions The results suggested that S1P reduced melanin synthesis via $S1P_3$ receptor-mediated ERK and RSK-1 activation, and subsequent MITF dual phosphorylation and degradation.

Keywords extracellular signal-regulated kinase; melanogenesis; microphthalmia-associated transcription factor; S1P receptor; sphingosine-1-phosphate

Introduction

Sphingosine-1-phosphate (S1P) is known to be involved in the regulation of cell growth, differentiation, migration, and apoptosis.[1–5] In the skin, S1P is involved as a bioactive mediator in regulating the proliferation and differentiation of keratinocytes.[6,7] We were the first to show that S1P was linked to melanogenesis of epidermal melanocytes.[8] In addition, we showed that S1P decreased melanogenesis via extracellular signal-regulated protein kinase (ERK) activation and microphthalmia-associated transcription factor (MITF) degradation.[8] However, the mechanism by which S1P controls melanogenesis is unknown.

MITF is a critical transcription factor which regulates melanocyte development, pigmentation, proliferation and survival.[9–11] This basic helix-loop-helix-leucine zipper protein is an important transcriptional regulator of three major pigment enzymes (tyrosinase, tyrosinase-related protein 1 (TRP1), and tyrosinase-related protein 2 (TRP2)).[12–14] In humans, mutations of the MITF gene are known to cause defects in pigment cells of the skin, eye, hair follicles and inner ear, as occurs in Waardenburg syndrome type IIA.[15,16]

The ERK pathway is a well-established signalling cascade and plays a major role in cell proliferation.[17] The small guanine nucleotide binding protein, Ras, acts as an upstream

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positive effector of the Raf-MAPK/ERK kinase (MEK)-ERK-90 kDa ribosomal S6 kinase-1 (RSK-1) pathway.[18,19] In addition, ERK is known to be involved in regulating cell differentiation.[20,21] It has been reported that inhibition of the ERK pathway leads to B16 melanoma cell differentiation and increases tyrosinase activity, suggesting that the ERK signalling pathway is deeply involved in melanogenesis.[22,23] Moreover, it has been shown that activation of ERK by c-Kit stimulation results in phosphorylation of MITF at Ser73, leading to an initial mobility shift, and that MITF phosphorylation at Ser73 is followed by MITF ubiquitination and degradation.[24,25] RSK-1, a downstream kinase of ERK, is known to phosphorylate MITF at Ser409, and this phosphorylation is followed by MITF degradation.[26]

Although S1P is recognized as a new second messenger, it has been proposed that S1P exerts its action as a ligand, binding to high affinity G-protein-coupled receptors (GPCRs) because endothelial differentiation gene-1 (EDG-1) is a S1P receptor.[1,2,27–30] Five members of S1P receptor subtypes have been identified: $S1P_1$–$S1P_5$ (formerly known as $EDG_{1,5,3,6,8}$, respectively).[2] The S1P receptor, however, has not been documented on melanocytes, despite the hypopigmentary effects of S1P.

S1P is known to activate the ERK signalling pathway in many cell types.[31,32] Also, we showed that S1P-activated ERK was involved in the regulation of melanogenesis in melanocytes; however, it is still unknown how the S1P-activated ERK pathway regulates MITF phosphorylation and degradation.[8,33] Therefore, we have determined the effects of S1P on the relationship between the ERK pathway and MITF phosphorylation in Mel-Ab cells. Furthermore, the expression of S1P receptor subtypes has been analysed on melanocytes.

Materials and Methods

Materials

The following products were used in the experiments: S1P and VPC23019 (Avanti Polar Lipids, Alabaster, AL, USA); pertussis toxin (PTX; Alexis, San Diego, CA, USA); LY294002 (Calbiochem, San Diego, CA, USA); PD98059 (Cell Signaling Technology, Beverly, MA, USA); JTE-013 (Tocris, Ellisville, MO, USA); 12-O-tetradecanoylphorbol-13-acetate (TPA), cholera toxin, fatty acid-free bovine serum albumin (BSA), paraformaldehyde, glutaraldehyde, osmium tetroxide, lead citrate, ethidium bromide, Triton X-100, Tris, β-mercaptoethanol, phenylmethylsulfonyl fluoride, synthetic melanin, and L-DOPA (Sigma, St Louis, MO, USA); antibodies recognizing phospho-specific RSK-1 (Thr359/Ser363, #9344S), phospho-specific ERK1/2 (Thr202/Tyr204, #9101S), total (phosphorylated and nonphosphorylated) ERK1/2 (#9102), phospho-specific MEK (Ser217/221, #9121), and total MEK (#9122; Cell Signaling Technology); microphthalmia Ab-3 (C5 + D5, MS-773-P0; NeoMarkers, Fremont, CA, USA), total RSK-1 (C-21, sc-231), and actin (I-19) antibodies (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA); and phospho-specific MITF (Ser73; a kind gift from Dr D. E. Fisher, Harvard Medical School, Boston, MA, USA).

Cell cultures

The Mel-Ab cell line used in this study is a mouse-derived, spontaneously-immortalized melanocyte cell line which produces large amounts of melanin.[34,35] Mel-Ab cells were incubated in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS; Hyclone, Logan, UT, USA), 100 nM TPA, 1 nM cholera toxin, 50 μg/ml streptomycin, and 50 μg/ml penicillin (Hyclone) at 37°C in 5% CO_2. B16 and NIH 3T3 cells were obtained from the Korean Cell Line Bank (Seoul, Korea). The cells were grown in DMEM supplemented with 10% FBS, 50 μg/ml streptomycin and 50 μg/ml penicillin at 37°C in 5% CO_2.

Electron microscopic examination

Cell pellets were fixed in 2.5% paraformaldehyde glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) and post-fixed in 1% osmium tetroxide. The samples were then dehydrated in graded acetone and embedded in Epon 812. Ultrathin sections were double-stained with 4% uranyl acetate and lead citrate solutions, and observed in a transmission electron microscope (TEM, JEM-1200EX; JEOL, Tokyo, Japan).

Confocal microscopic examination

Mel-Ab cells were treated with 10 μM S1P for 10 min to detect phospho-ERK and for 3 h to detect MITF. The cells were then fixed in 10% formaldehyde in phosphate buffered saline (PBS) for 10 min, and permeabilized with 0.1% Triton X-100 in PBS for 5 min at room temperature. After washing with PBS, cells were incubated with p-ERK or MITF antibody overnight at 4°C. The cells were then incubated with FITC-conjugated anti-rabbit IgG to detect p-ERK and FITC-conjugated anti-mouse IgG for 1 h to detect MITF. Cells were observed with a Bio-Rad confocal microscope (MRC 1024; Bio-Rad, Hercules, CA, USA).

Transfection of MITF constructs

Mel-Ab cells were transfected using FuGENE 6 (Boehringer Mannheim, Mannheim, Germany) following the manufacturer's protocol with wild-type MITF vector and its derivative MITF mutants.[26] MITF mutants were kindly provided by Dr D. E. Fisher (Harvard Medical School, Boston, MA, USA). One mutation was from serine to alanine at position 73 (S73A), one mutation was at Ser409 (S409A), and a dual mutation was at both sites (S73/409A).

Measurement of melanin content

The melanin content was measured as described previously, with slight modification.[36] Briefly, Mel-Ab cells were treated with S1P for four days. Cell pellets were dissolved in 1 ml 1 M NaOH at 100°C for 30 min and centrifuged for 20 min at 16 000*g*. Optical densities of the supernatants were measured at 400 nm using an ELISA reader. Standard curves of synthetic melanin (0–300 μg/ml) were prepared in triplicate for each experiment.

Western blot analysis

After serum starvation, S1P was added directly to serum-starved media. Mel-Ab cells were lysed in cell lysis

buffer (62.5 mM Tris-HCl (pH 6.8), 2% SDS, 5% β-mercaptoethanol, 2 mM phenylmethylsulfonyl fluoride, protease inhibitors (Complete; Roche, Mannheim, Germany), 1 mM Na_3VO_4, 50 mM NaF, and 10 mM EDTA). Protein, 10 μg per lane, was separated by SDS-polyacrylamide gel electrophoresis and blotted onto polyvinylidene fluoride (PVDF) membranes, which were then saturated with 5% dried milk in Tris-buffered saline containing 0.4% Tween 20. Blots were incubated with the appropriate primary antibodies at a dilution of 1 : 1000, then further incubated with horseradish peroxidase-conjugated secondary antibody. The bound antibodies were detected using an enhanced chemiluminescence plus kit (Amersham International, Little Chalfont, UK).

Reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was isolated from the cells using an RNeasy Mini Kit (Qiagen, Valencia, CA, USA). Then, 1 μg RNA was reverse-transcribed using an ImProm II Reverse Transcription System (Promega, Madison, WI, USA). The cDNA obtained was amplified with the following primers: MITF (414 bp product) forward, 5′-CCCGTCTCTGGAAACTTGATCG-3′ and reverse, 5′-CTGTACTCTGAGCAGCAGGTG-3′; tyrosinase (568 bp product) forward, 5′- CTCCGCTGGCC ATTTCCCTA-3′ and reverse, 5′-GGTGCTTCATGGGCA AAATC-3′; $S1P_1$ (1286 bp product) forward, 5′-GatatCat CGTCCGGCatTAC-3′ and reverse, 5-acccttcccagtgcat TGTTC-3′; $S1P_2$ (531 bp product) forward, 5′-CACTCAGCA atGTACCTGTTCC-3′ and reverse, 5′-aacacccagtacgat GGTGAC-3′; $S1P_3$ (342 bp product) forward, 5′-gactgctctacc atCTTGCCC-3′ and reverse, 5-GTAGatGACAGGGTTC atGGC-3′; $S1P_4$ (324 bp product) forward, 5′-CTGCTGC CCCTCTACTCCAA-3′ and reverse, 5-atTAatggctgagtt gaacac-3′; and $S1P_5$ (303 bp product) forward, 5′-atCTGTG CGCTCTatGCAAGGA-3′ and reverse, 5-GGTGTAGat GatGGGatTCAGCA-3′. The PCR conditions were 30 cycles for 1 min at 94°C, 1 min at 55°C, 1 min at 72°C (except $S1P_5$), 30 cycles for 1 min at 94°C, 1 min at 59°C, and 1 min at 72°C (for $S1P_5$). The resulting PCR products were visualized by electrophoretic separation on 1.5% agarose gels with ethidium bromide staining. Specific primers for actin or GAPDH were added as a control.

Statistics

The differences between results were assessed by analysis of variance followed by Student's *t*-test. *P*-values < 0.01 were considered significant.

Results

S1P decreases melanogenesis via ERK activation and MITF degradation

Previously, we reported that S1P decreased melanogenesis via ERK activation and subsequent MITF degradation.[8] To verify the effects of S1P, Mel-Ab cells were exposed to 10 μM S1P for four days, and cell morphology was observed using electron microscopy. As shown in Figure 1a, a decreased number of pigmented melanosomes was detected in S1P-treated cells. Moreover, S1P-treated cells had undifferentiated early-stage melanosomes, whereas untreated cells had internal fibrils and dense pigment in melanosomes. We further showed ERK activation and MITF using confocal microscopy after 10 μM S1P treatment (Figure 1b). After 10 min of S1P treatment, the staining intensity of phospho-ERK increased rapidly. MITF was predominantly observed

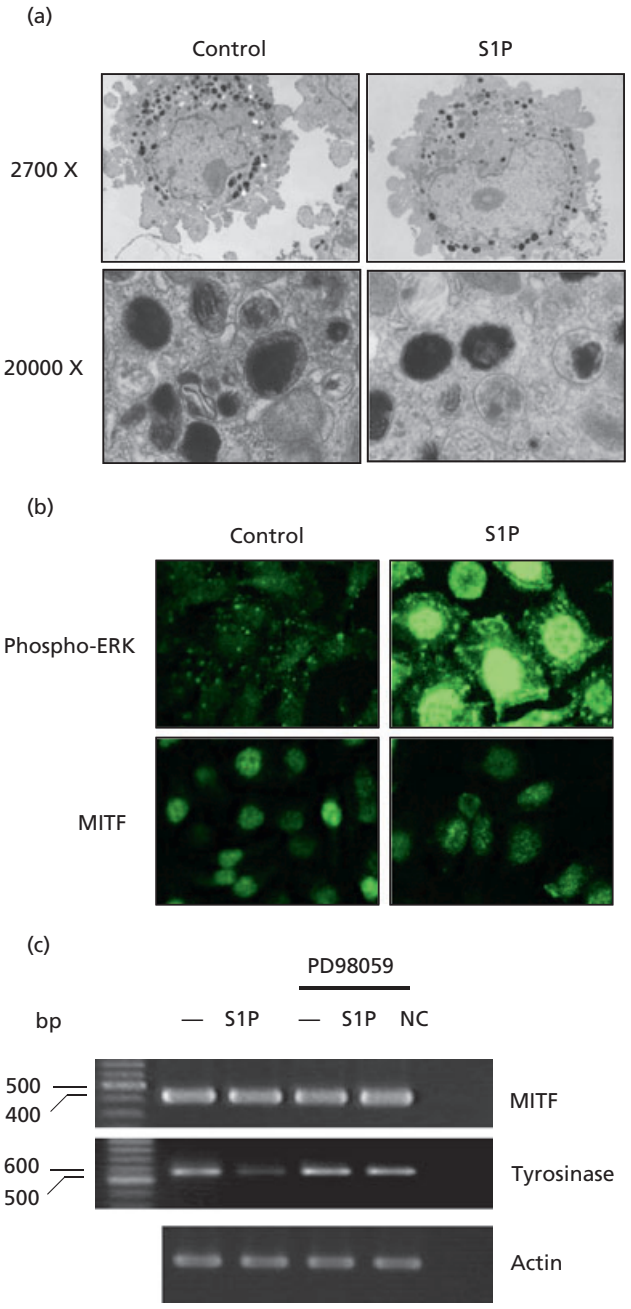

Figure 1 S1P decreased melanogenesis in Mel-Ab cells. (a) Electron microscopic examination. (b) Confocal microscopic examination. Mel-Ab cells were treated with 10 μM S1P for 10 min to detect phospho-extracellular signal-regulated protein kinase (ERK) and for 3 h to detect microphthalmia-associated transcription factor (MITF). (c) RT-PCR analysis of MITF and tyrosinase mRNA. Mel-Ab cells were cultured with 10 μM S1P for 3 h in the presence or absence of 20 μM PD98059. The lane on the left shows markers of the indicated sizes. NC, negative control.

in the nucleus of Mel-Ab cells. S1P-treated cells had decreased fluorescent intensity and a more punctuate distribution of MITF due to its degradation. Furthermore, we performed RT-PCR assays using MITF- or tyrosinase-specific primers. As shown in Figure 1c, S1P had no influence on MITF transcription, while tyrosinase mRNA decreased after S1P treatment. In addition, pretreatment with PD98059, a specific inhibitor of the ERK pathway, prevented S1P-induced tyrosinase mRNA reduction. In conclusion, we suggest that the MITF protein reduction by S1P may have been due to MITF degradation via ERK activation. Thus, MITF degradation resulted in decreased tyrosinase expression, which led to hypopigmentation.

Dual phosphorylation of MITF by S1P results in its degradation

It has been reported that the activation of ERK and RSK-1 is linked to MITF phosphorylation at Ser73 and Ser409, respectively, and that MITF phosphorylation at Ser73 and Ser409 is intimately involved in MITF degradation.[24,26] Thus, we first tested whether S1P could mediate phosphorylation of MITF at Ser73. As shown in Figure 2a, S1P induced phosphorylation of MITF at Ser73. Previously, we had shown that S1P induced the sustained activation of ERK.[8] The kinetics of ERK activation and MITF phosphorylation after S1P treatment showed similar temporal patterns. Furthermore, we found that RSK-1 was similarly activated by 10 μM S1P (Figure 2a). As we have shown previously, the mobility shift of MITF was observed 10 min after S1P treatment (Figure 2b). In this study, PD98059, a specific inhibitor of the ERK pathway, inhibited S1P-induced activation of RSK-1, a downstream kinase of ERK (Figure 2b), and the S1P-dependent mobility shift of MITF (Figure 2b). In addition, we examined the effect of LY294002, a phosphatidylinositol 3-kinase inhibitor, which blocks the Akt signalling pathway. However, LY294002 blocked neither the mobility shift of MITF nor the activation of RSK-1 (Figure 2b).

We examined the relationship between the mobility shift and the degradation of MITF. Mel-Ab cells were transfected with wild-type MITF, single serine-to-alanine mutants at positions 73 or 409 (S73A and S409A, respectively), and a double mutant at both Ser73 and Ser409 (S73/409A). As shown in Figure 2c, ERK and RSK-1 were activated 10 min after S1P treatment under each condition. As expected, wild-type MITF showed the S1P-dependent mobility shift of MITF. The S73A single point mutant and the S73/409A double mutant did not show the mobility shift, whereas the S409A single point mutant showed the mobility shift (Figure 2c). These results indicated that the phosphorylation of Ser73 was responsible for the mobility shift of MITF. Previously, we had reported that S1P induced the mobility shift and degradation of MITF protein over the course of several hours.[8] As shown in Figure 2d, wild-type MITF showed S1P-induced MITF degradation. However, the two single-point mutants (S73A and S409A) and the double mutant (S73/409A) did not show MITF degradation (Figure 2d). From these results, we propose that dual phosphorylation at Ser73 and Ser409 was required for MITF degradation.

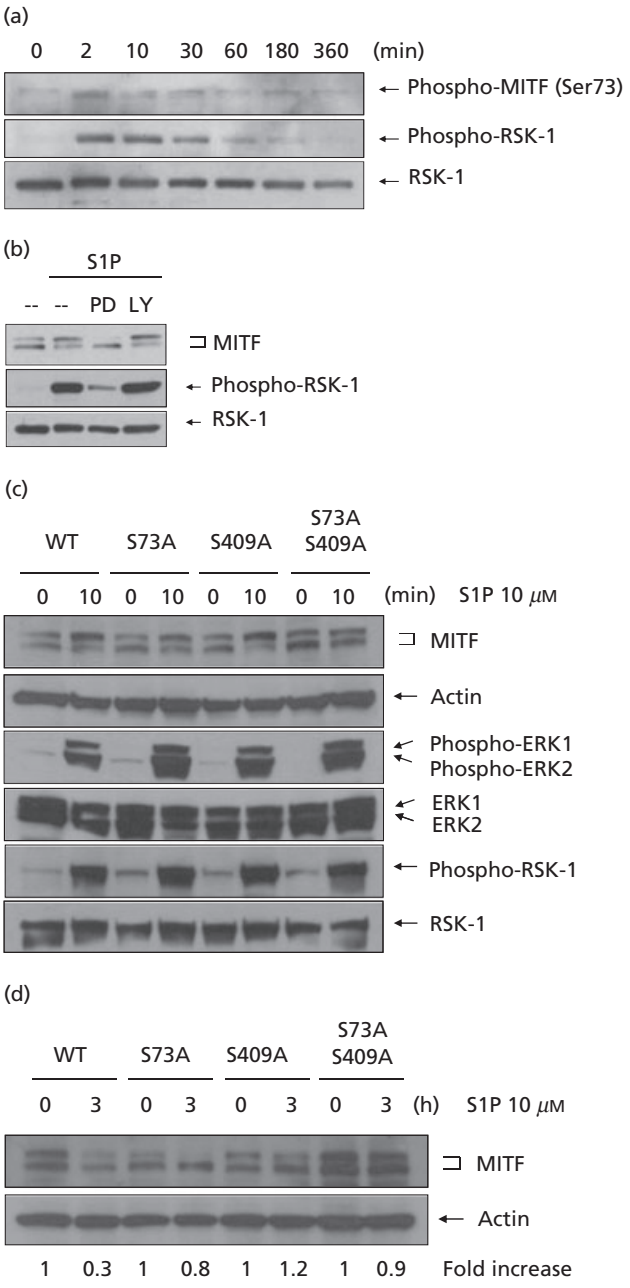

Figure 2 S1P induced dual phosphorylation and degradation of microphthalmia-associated transcription factor (Western blot analyses). (a) S1P-induced phosphorylation of RSK-1 and of microphthalmia-associated transcription factor (MITF) at Ser73. (b) Mobility shift in MITF 10 min after S1P activation, accompanied with RSK-1 phosphorylation and inhibited by 20 μM PD98059 (PD), but not by 20 μM LY294002 (LY). (c) Phosphorylation of MITF at Ser73, but not Ser409 was necessary for MITF mobility shift induced by S1P. ERK and RSK-1 were activated uniformly 10 min after S1P treatment. MITF mobility shift was observed only for wild-type (WT) MITF and S409A mutant (serine-to-alanine at position 409) with preserved Ser73. (d) Degradation of WT MITF and lack of degradation of S73A and S409A MITF mutants indicating that both (73 and 409) serine residues must have been phosphorylated for MITF degradation. The fold increase over each control was determined by densitometric analysis and is shown below each lane.

S1P exerts hypopigmentary effects in a pertussis toxin-sensitive manner

We determined whether the hypopigmentary effects of S1P were mediated through specific receptors. Thus, we first investigated the hypopigmentary effects of S1P using PTX, which inactivates the α-subunit of Gi/Go proteins. In the presence or absence of PTX, Mel-Ab cells were treated with S1P for four days, the melanin content was then measured (Figure 3a). Our results showed that pre-incubation with PTX almost completely abrogated the hypopigmentary effects by S1P (Figure 3a). We further examined whether PTX had an influence on S1P-mediated ERK activation, and found that PTX clearly blocked the S1P-induced activation of MEK (MAPK/ERK kinase), ERK, and RSK-1 in Mel-Ab cells (Figure 3b). Moreover, PTX inhibited the S1P-induced mobility shift of MITF. Taken together, these results indicated that a GPCR was also involved in S1P-mediated signalling pathways and S1P-induced hypopigmentation.

S1P mediates its action on ERK and melanin synthesis via the $S1P_3$ receptor subtype

We examined which S1P receptor subtypes were expressed on Mel-Ab cells. As shown in Figure 4a, $S1P_2$, $S1P_3$, and $S1P_5$ were dominantly expressed on Mel-Ab cells. By way of comparison, B16 mouse melanoma cells express $S1P_2$, $S1P_4$, and $S1P_5$ (Figure 4a). On the basis of these studies, it was of interest to determine via which receptor S1P regulated the ERK pathway and melanin synthesis. For this purpose, we used JTE-013 (a $S1P_2$ receptor antagonist) and VPC23019 (a $S1P_{1/3}$ receptor antagonist) to block each receptor. As shown in Figure 4b, abrogation of $S1P_2$ using JTE-013 decreased melanin synthesis, and JTE-013 and S1P showed additive hypopigmentary effects. In contrast, VPC23019 prevented S1P-induced hypopigmentation. Mel-Ab cells did not express $S1P_1$ (Figure 4a), therefore we reasoned that S1P mediated its effects via $S1P_3$. To confirm this hypothesis, Mel-Ab cells were pretreated with VPC23019, then ERK activation and the mobility shift of MITF were checked after S1P treatment. As shown in Figure 4d, VPC23019 was shown to inhibit the S1P-induced activation of ERK and the S1P-dependent mobility shift of MITF.

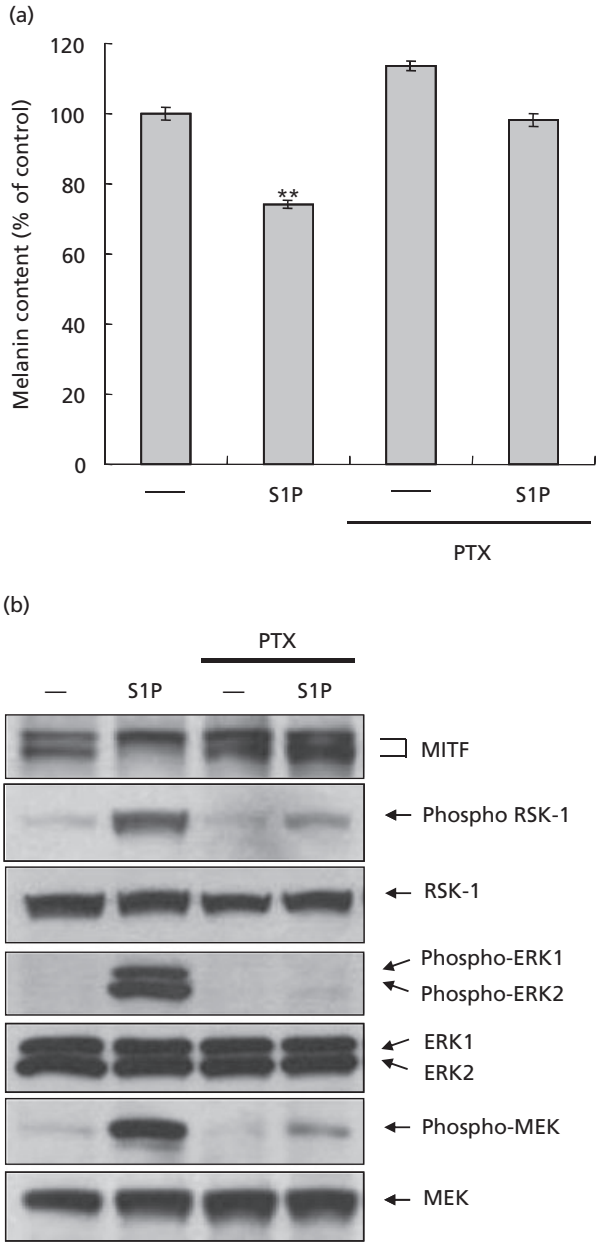

Figure 3 S1P-mediated signalling and hypopigmentation were pertussis toxin-sensitive . Mel-Ab cells were pretreated with 100 ng/ml pertussis toxin (PTX) for 3 h and then cultured with 10 μM S1P for four days (a) PTX almost completely abrogated the hypopigmentary effects by S1P. Melanin content determination was made in triplicate; the data shown represent the mean ± SD. $^{**}P < 0.01$ compared with control. (b) Cells were cultured with 10 μM S1P for 10 min in the presence or absence of PTX. Western blot analysis showed that PTX blocked the S1P-induced microphthalmia-associated transcription factor (MITF) mobility shift as well as activation of MEK, ERK and RSK-1.

Discussion

It has been reported that S1P reduces melanogenesis by ERK activation and subsequent MITF degradation.[8] In this study, we first verified the effects of S1P on melanogenesis using electron microscopy. We demonstrated a decreased number of melanosomes in S1P-treated cells. Although we did not measure the ratio of eumelanin and pheomelanin, reduced melanogenesis could result from the switch of eumelanogenesis to pheomelanogenesis. Furthermore, ERK activation and MITF degradation were observed after S1P treatment by confocal microscopy.

MITF is the major transcriptional regulator of tyrosinase, and thus plays a central role in melanin synthesis.[12,37–39] Decreased MITF gene expression is related to the downregulation of melanogenic enzymes, including tyrosinase, TRP1 and TRP2.[40,41] It has been demonstrated that ERK activation leads to the phosphorylation of MITF at Ser73, and phosphorylated MITF at Ser73 is responsible for MITF degradation.[24,25] Furthermore, RSK-1, a downstream kinase of ERK, was found to phosphorylate MITF at serine 409, and this phosphorylation was followed by MITF degradation.[26] In this study, we found that MITF was phosphorylated at Ser73 by

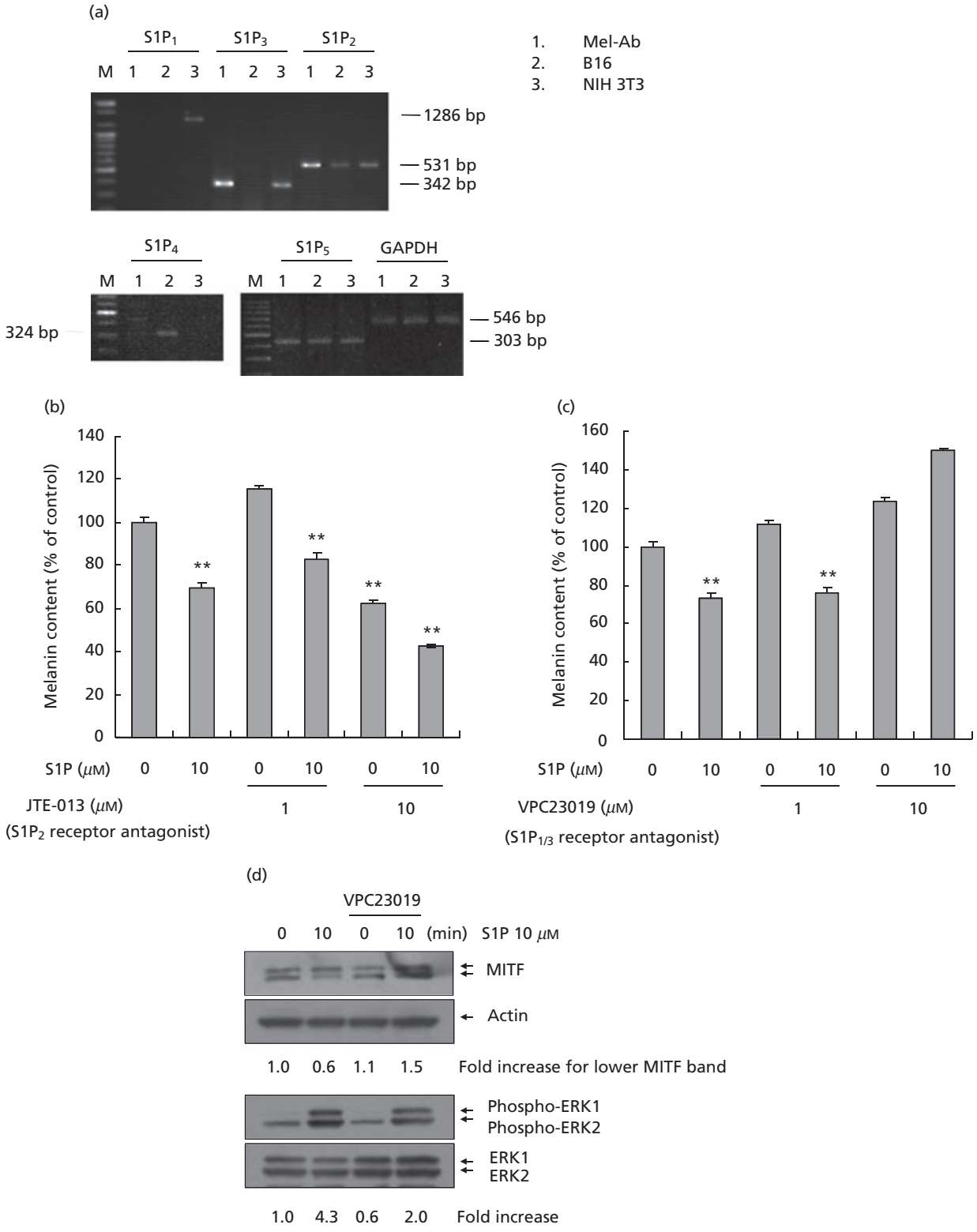

Figure 4 S1P induced hypopigmentation via the $S1P_3$ receptor subtype. (a) RT-PCR analysis of S1P receptor mRNA was performed with total RNA isolated from the Mel-Ab, B16 melanoma, and NIH 3T3 cells. GAPDH primers were used as a control to ensure the even loading of the target cDNA. The left-hand lane shows markers of the indicated size. Mel-Ab cells were pretreated with 1–10 μM JTE-013 (b) or VPC23019 (c) for 30 min and then cultured with 10 μM S1P for four days. Melanin content determination was made in triplicate; the data shown represent the mean ± SD. $^{**}P < 0.01$ compared with control. (d) Western blot analysis showed that VPC23019 inhibited the S1P-induced activation of ERK and mobility shift of microphthalmia-associated transcription factor (MITF). Fold increases over the control were determined by densitometric analysis, and are shown below each lane.

S1P. Moreover, RSK-1 was also activated by S1P. We found that PD98059 treatment markedly inhibited S1P-induced activation of RSK-1 and the S1P-dependent mobility shift of MITF. Our results indicated that phosphorylation of MITF at Ser73 was responsible for the mobility shift of MITF. In addition, neither the two single-point mutations (S73A and S409A) nor the double mutation (S73/409A) resulted in MITF degradation. Thus, it could be concluded that dual phosphorylation of MITF at Ser73 and Ser409 was essential for MITF degradation. In this study, S1P was used to stimulate Mel-Ab cells, whereas TPA or steel factor was used to stimulate NIH 3T3 or 501 Mel cells in another study.[26] In contrast to our study, it has been reported that two single-point mutations were all degraded after steel factor treatment.[26] As we showed, S1P decreased melanin synthesis, whereas TPA or steel factor is known to increase melanogenesis. Thus, it is not easy to simply compare the effects of S1P and steel factor in terms of MITF phosphorylation.

Previously, we reported that the sphingolipid sphingosylphosphorylcholine (SPC), reduced melanogenesis in human melanocytes via ERK and RSK-1 activation, leading to MITF downregulation.[33] Furthermore, we found that SPC-induced MITF downregulation may have been mediated by the ERK pathway. However, we did not observe a mobility shift of MITF, although ERK was clearly activated by SPC. In another study, we found that MITF at Ser73 was not phosphorylated by SPC, although ERK was activated.[23] Lysophosphatidic acid (LPA) is a lysophospholipid, which is structurally related to S1P. We observed that LPA decreased melanogenesis without a MITF mobility shift and degradation, although LPA was also shown to activate RSK-1. Instead, we found that LPA inhibited α-melanocyte stimulating hormone-induced MITF promoter activity.[42] These findings showed that ERK activation does not always result in MITF phosphorylation and that MITF can be degraded without being phosphorylated. To clarify the relationship between the ERK pathway and MITF downregulation, further investigation is needed.

Our results showed that S1P-induced ERK activation and MITF phosphorylation was blocked by PTX. Furthermore, we confirmed the inhibitory effects of PTX against the S1P-induced hypopigmentary effects. This study provides clear evidence that $G\alpha_i$-coupled S1P receptors were involved in S1P-mediated hypopigmentation. Although five members of S1P receptor subtypes have been identified, expression of the S1P receptor has not been studied on melanocytes.[2] In this study, RT-PCR analysis demonstrated that $S1P_2$, $S1P_3$ and $S1P_5$ were expressed on Mel-Ab cells. VPC23019 is known to be a competitive antagonist at the $S1P_1$ and $S1P_3$ receptors.[43] We found that Mel-Ab cells did not express $S1P_1$ and so VPC23019 was used as a $S1P_3$ antagonist in this study. We observed that VPC23019 significantly abrogated S1P-induced hypopigmentation. Furthermore, VPC23019 was found to inhibit S1P-induced activation of ERK and the S1P-dependent mobility shift of MITF. VPC23019 showed partial effects on the ERK signal transduction pathway and so we do not think that the $S1P_3$–mediated pathway is the only pathway to regulate melanogenesis. Although involvement of the $S1P_5$ receptor in melanogenesis remains to be elucidated, these results indicated that S1P mediated its effects at least via $S1P_3$.

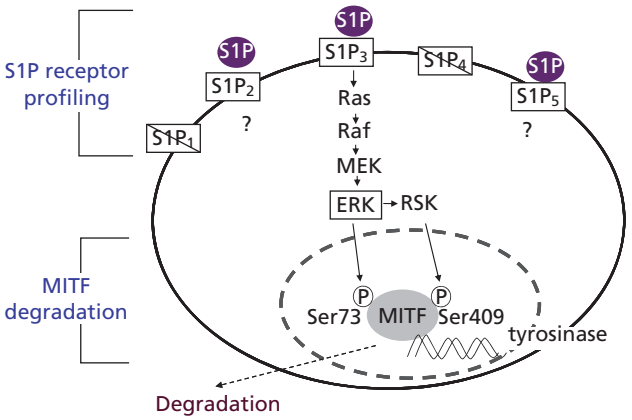

Figure 5 The mechanism of sphingosine-1-phosphate-induced hypopigmentation. ERK, extracellular signal-regulated protein kinase; MEK, MAPK/ERK kinase; MITF, microphthalmia-associated transcription factor; RSK, ribosomal S6 kinase-1; S1P, sphingosine-1-phosphate; Ser73, serine 73; Ser409, serine 409.

JTE-013, a $S1P_2$ receptor antagonist, decreased melanin synthesis (Figure 4b), demonstrating that the $S1P_2$ receptor was not involved in S1P-induced hypopigmentation. Moreover, JTE-013 and S1P exhibited additive hypopigmentary effects. This result suggested that S1P may have increased the likelihood to bind to the $S1P_3$ receptor under $S1P_2$ receptor blockade by JTE-013.

Conclusions

We demonstrated that S1P reduced melanin synthesis via $S1P_3$ receptor-mediated ERK and RSK-1 activation, which in turn was responsible for subsequent MITF dual phosphorylation and degradation (Figure 5).

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

Funding

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