

# S1PR1 mediates anti-apoptotic/pro-proliferative processes in human acute myeloid leukemia cells

XIAO-QIAN XU<sup>1\*</sup>, CHONG-MEI HUANG<sup>1\*</sup>, YI-FAN ZHANG<sup>2</sup>, LI CHEN<sup>1</sup>, HUI CHENG<sup>1</sup> and JIAN-MIN WANG<sup>1</sup>

<sup>1</sup>Department of Hematology, Changhai Hospital, Second Military Medical University, Shanghai 200433;

<sup>2</sup>Department of Nuclear Medicine, Ruijin Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai 200025, P.R. China

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**Abstract.** Sphingosine-1-phosphate receptor 1 (S1PR1), the G protein-coupled receptor 1 of sphingosine 1-phosphate (S1P), is crucial in the progression of various types of solid tumor through the positive regulation of anti-apoptotic/pro-survival responses by S1P-S1PR1 signaling. However, the role of S1PR1 in the progression of human acute myeloid leukemia (AML) remains to be fully elucidated. In the present study, the correlation between the expression level of S1PR1 and the growth and apoptosis of human AML cells was investigated. Using over-expression and RNA interference knockdown assays, it was shown that the expression of S1PR1 in several human myeloid leukemia cell lines contributed to the suppression of cell apoptosis and promotion of cell proliferation. S1PR1 inhibited the expression level of B cell lymphoma-2-associated X protein, a mitochondrion-associated apoptosis promoter, and prevented caspase-3 cleavage, indicating that S1PR1 interfered with the mitochondrion-associated apoptotic processes in AML cells. In addition, the present study demonstrated that S1PR1 suppressed the generation of reactive oxygen species (ROS) in AML cells, and that the apoptosis mediated by the down-regulation of S1PR1 was partially reversed by treatment with N-acetyl-L-cysteine, a ROS scavenging agent, suggesting that the S1PR1-induced resistance of cell apoptosis resulted, at least partially, from the suppression of ROS generation. Additionally, S1PR1 was able to interfere with the signaling of c-Jun N-terminal kinase, which may have also contribute to the suppression of cell apoptosis. By contrast, extracellular signal-regulated kinase (ERK) signaling appeared to be positively modulated by the S1PR1-induced enhancement

of mitogen-activated protein kinase (MAPK) kinase 1 (MKK1) activation, suggesting that S1PR1 may promote cell survival and proliferation through enhancing the activation of MKK1-ERK signaling. In conclusion, S1PR1 likely serves as an anti-apoptotic/pro-proliferative protein in AML cells through the inhibition of mitochondrion-associated apoptosis and ROS generation, and via the regulation of multiple MAPK signaling cascades.

## Introduction

Sphingosine-1-phosphate receptor 1 (S1PR1) belongs to the G protein-coupled receptor family for sphingosine 1-phosphate (S1P), a biologically active lipid. S1P-S1PR1 signaling is vital in a variety of developmental processes, including cell growth, survival and migration (1). However, S1PR1 signaling also promotes tumor progression and tumor angiogenesis (2,3). In myeloid cells derived from tumors, including activated B cell-like diffuse large B-cell lymphoma, the expression of S1PR1 is elevated, compared with normal splenic myeloid cells, and this upregulation is correlated with the persistent activation of signal transducer and activator of transcription 3 (STAT3) (2,3). S1PR1-STAT3 signaling is also crucial for myeloid cell colonization at future metastatic sites (4). Previous data has suggested a correlation between reduced S1PR1 and reduced survival rates of patients with glioblastoma (5), and the high level of S1PR1 in T-lymphoblastic lymphoma (T-LBL) is a key factor in the inhibition of progression of T-LBL to acute T-lymphoblastic leukemia (6). Studies based on certain S1PR1 inhibitors have also suggested that the degradation of S1PR1 can arrest cell growth in various cancer cell lines (6-8).

Cell growth and apoptosis involve several signaling pathways. The direct executors of apoptosis are intracellular cysteine proteases, termed caspases (9). Caspases are originally translated as inactive zymogens, and subsequently activated by 'mitochondria' or the 'death receptor' pathway. Of note, the release of cytochrome *c* from mitochondria, which triggers caspase activation, appears to be predominantly mediated by the direct or indirect actions of reactive oxygen species (ROS) (10). The response of tumor cells to cell death stimuli is a consequence of cellular redox status, and stimuli, which induce generation of intracellular ROS facilitate the execution of cell death (11). ROS, including hydroxyl, superoxide,

*Correspondence to:* Dr Jian-Min Wang, Department of Hematology, Changhai Hospital, Second Military Medical University, 168 Changhai Road, Shanghai 200433, P.R. China  
E-mail: ellenxxq@qq.com

\*Contributed equally

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nitroxyl radicals, hydrogen peroxide, organic hydroperoxides and hypochlorous acid, can destroy DNA and proteins, and activate cell death (11,12). ROS generation has been shown to be associated with the activation of c-Jun N-terminal kinase (JNK)/stress-activated protein kinase by oxidative inactivation of endogenous JNK inhibitors, including JNK phosphatase receptor-mediated cell death (13-15). JNK and other members of the mitogen-activated protein kinase (MAPK) family, including extracellular signal-regulated kinase (ERK)1/2 and p38 MAPK, are involved in cell proliferation and apoptosis through the activation of specific transcription factors.

With the lowest survival rate among the four major types of leukemia, acute myeloid leukemia (AML) is a significant threat to human health (16). Clinically, AML is characterized as the abnormal differentiation and proliferation of immature blast cells in the bone marrow, and is known to be the most common form of acute leukemia among adults (16). However, the pathogenesis of the disease remains to be elucidated.

Although several studies have suggested that SIPR1 is a notable factor for solid tumors, the role of SIPR1 in AML cell growth remains to be fully elucidated (17). In the present study, the effects of SIPR1 in modulating the apoptosis and proliferation of AML cells were investigated. It was found that SIPR1 signaling mediated anti-apoptotic/pro-proliferative processes in the AML cells through the inhibition of mitochondrion-associated apoptosis and ROS generation, and via the regulation of multiple MAPK signaling cascades.

## Materials and methods

**Cell culture and reagents.** The human promyelocytic leukemia cell lines, HL60, U937 and K562, were purchased from American Type Culture Collection (Manassas, CA, USA) and propagated in RPMI 1640 (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc.) in a 37°C incubator with 5% CO<sub>2</sub>. The 2-7-dichlorofluorescein diacetate (DCFH-DA) and N-acetyl-L-cysteine (NAC) were purchased from Sigma-Aldrich (MO, St. Louis, MA, USA) and dissolved in saline solution at pH 7.0.

**Overexpression and knockdown of SIPR1.** cDNA was synthesized from RNA using a cDNA synthesis kit (Thermo Fisher Scientific, Inc.). Prior to reverse transcription, RNA was treated with DNase (Invitrogen; Thermo Fisher Scientific, Inc.). The coding sequence of SIPR1 was obtained by polymerase chain reaction (PCR), using cDNA generated from the RNA of HL60 as a template, with the following primers: SIPR1, forward 5'-ATGGATGACCTAATCGCCAGACAC-3' and reverse 5'TTAAGAAGAAGAGGTGATATT-3'. The coding sequence was annealed and digested using *AgeI* and *EcoRI*, and then was ligated into pCDNA3 (Invitrogen; Thermo Fisher Scientific, Inc.). The recombinant plasmid was verified by direct sequencing and used to induce the overexpression of SIPR1 in the HL60 cell lines. The siRNA sequence target for SIPR1 was 5'-ATGATCGATCATCTAGCAA-3' (18), and a non-silencing small interfering (si) RNA was used as a control (Life Technologies, Grand Island, NY, USA). For transfection and the analysis of expression levels, a total of 1x10<sup>6</sup> HL60 cells were suspended in 1 ml

OPTI-MEM and subsequently transfected with 100 pM siRNA or 4 µg pCDNA3-SIPR1 using Lipofectamine 2000 (Invitrogen; Thermo Fisher Scientific, Inc.). After 48 h, the expression of SIPR1 was analyzed by quantitative (q)PCR using a SYBR Green PCR kit (Toyobo, Tokyo, Japan) with the 7300 Real-Time PCR system (Applied Biosystems, Darmstadt, Germany). Expression levels were normalized to GAPDH and measured in triplicate. The following primers were used: SIPR1, forward 5'-CCTCGGTGGTGTTCATTC-3' and reverse 5'-GCAGGTAGCTGTGTAGG-3'; GAPDH, forward 5'-CACCCACTCCTCCACCTTTG-3' and reverse 5'-CCACCCTGTTGCTGTAG-3'. The PCR cycling conditions were as follows: 95°C for 10 min, followed by 40 cycles at 95°C for 15 sec and 60°C for 45 sec, and a final extension step of 95°C for 15 sec, 60°C for 1 min, 95°C for 15 sec and 60°C for 15 sec. The gene expression was calculated using the 2<sup>-ΔΔC<sub>q</sub></sup> method (19).

**Western blot analysis.** Whole cell protein extracts were isolated using RIPA buffer containing 50 mM Tris-HCl, (pH 8.0), 150 mM NaCl, 1% Nonidet P-40, 0.1% SDS, 2 mM phenylmethylsulfonyl fluoride, phosphatase and protease inhibitor cocktail (CalbioChem, San Diego, CA, USA) at 4°C for 20 min, followed by centrifugation at 12,000 g for 1 min at 25°C. The supernatants were then subjected to SDS-PAGE (Bio-Rad Laboratories, Inc., Richmond, CA, USA). The protein samples were consequently transferred onto PVDF membrane (0.22 µm; EMD Millipore, Billerica, MA, USA) using a wet transfer device (Bio-Rad Laboratories, Inc.), followed by blotting with primary antibodies specific for B cell lymphoma-2 (Bcl-2)-associated X protein (Bax; cat. no. Sc-493), Bcl2 (cat. no. Sc-492; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA), Caspase 3 (cat. no. Ab32351), phosphorylated (p)-c-Jun (cat. no. Ab32385), Jun (cat. no. Ab32137), MKK1 (cat. no. Ab139343), p-MKK1 (cat. no. Ab3338) from Abcam, and p38 (8690), p-p38 (cat. no. 4631), ERK (cat. no. 9102), p-ERK (cat. no. 5726), JNK (cat. no. 9258), p-JNK (cat. no. 5291), GAPDH (cat. no. 5174) from Cell Signaling Technology, Inc. (Beverly, MA, USA) for 2 h at 25°C, prior to incubation with secondary antibodies for 1 h at 37°C, and were subsequently washed three times with Tris-buffered saline with 20% Tween-20 (Amresco, LLC, Solon, OH, USA). The secondary antibodies used were horseradish peroxidase-conjugated goat anti-rabbit IgG (cat. no. A0216; Beyotime Institute of Biotechnology, Shanghai, China) or horseradish peroxidase-conjugated goat anti-mouse IgG (cat. no. A0208; Beyotime Institute of Biotechnology). The bands on the membrane were developed using an ECL kit (EMD Millipore) and visualized by exposure to a UVP bio-imaging system (UVP, Inc., Upland, CA, USA).

**Cell apoptosis and viability assays.** Cell apoptosis was detected using a Fluorescein isothiocyanate (FITC) Annexin V Apoptosis Detection kit with propidium iodide (PI; BioLegend, Inc., San Diego, CA, USA). Briefly, the cell samples were washed twice with ice-cold Annexin V binding buffer, and then stained with Annexin V-FITC and PI for 15 min at room temperature, followed by the addition of another 400 µl Annexin V binding buffer and analysis using flow cytometry (Accuri C6; BD Biosciences, San Diego, CA,

USA). Early stage apoptotic cells were stained with FITC Annexin V, but not PI, whereas late stage apoptotic cells and necrotic cells stained positively for FITC Annexin V and PI. Cell viability was assessed using a Cell Counting Kit-8 (CCK-8; Dojindo Laboratories, Kumamoto, Japan), according to the manufacture's protocol. Briefly,  $2.5 \times 10^3$  target cells were seeded in triplicate into a 96 well plate, following which 10  $\mu$ l of 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt (WST-8) solution was added to each well and incubated for 4 h in the incubator. The viable cell-mediated formation of WST-8 formazan was measured at an absorbance of 450 nm using a microplate reader.

**Analysis of intracellular ROS levels.** To assess the involvement of S1PR1 in the reduction of ROS, the HL60 cells were transfected with S1PR1 constructs, with or without pre-treatment with 100  $\mu$ M NAC for 2 h. After 48 h, the cell samples were incubated with 5 M of the redox-sensitive dye, DCF-DA, for 30 min at 37°C. The ROS-mediated oxidation of the fluorescent compound, DCF, was measured by excitation at 488 nm and an emission wavelength of 525 nm using a flow cytometer (BD Biosciences).

**Statistical analysis.** Data are presented as the mean  $\pm$  standard deviation. Statistical analysis was performed by two-way analysis of variance followed by Tukey's test. Statistical analyses were performed using GraphPad Prism software, version 5 (GraphPad Software, Inc., La Jolla, CA, USA).  $P < 0.05$  was considered to indicate a statistically significant difference.

## Results

**Modulation of the expression of S1PR1 by transient transfection with plasmids.** To elucidate the effects of S1PR1 in myeloid leukemia cell proliferation and apoptosis, the present study first constructed a mammalian cell expression plasmid for the overexpression of S1PR1 and an siRNA plasmid for knockdown of the expression of S1PR1. As shown in the qPCR assay, the mRNA level of S1PR1 was increased markedly in the HL60 cells transfected with the S1PR1 expression plasmid, whereas transfection with the siRNA plasmid led to a significant reduction in the endogenous expression of S1PR1 (Fig. 1A;  $P < 0.001$ ).

**S1PR1 inhibits the apoptosis of human myeloid leukemia cells and promotes their proliferation.** The present study examined the effects of the expression of S1PR1 on the proliferation of human myeloid leukemia cells using a CCK-8 kit. The U937 cells were transfected with the siRNA plasmid, the S1PR1 expression plasmid, or the corresponding control plasmids. Cell proliferation was measured at 0, 24 and 48 h post-transfection using a CCK-8 assay. As shown in Fig. 1B, knockdown of the expression of S1PR1 by transfection with the siRNA plasmid significantly inhibited U937 cell proliferation, and the overexpression of S1PR1 appeared to result in a marginal enhancement of cell proliferation. To investigate whether S1PR1 contributes to the inhibition of cell apoptosis, the effect of S1PR1 on cell apoptosis was examined by Annexin-PI staining and flow cytometry. As shown in Fig. 1C, knockdown

of the expression of S1PR1 by transfection with the siRNA plasmid markedly induced the apoptosis of leukemia cells, whereas cell apoptosis appeared to be inhibited by the overexpression of S1PR1, suggesting that S1PR1 signaling likely led to the suppression of apoptosis and the promotion of proliferation in the human leukemia cells.

**S1PR1 confers resistance to mitochondrion-associated apoptosis in myeloid leukemia cells.** The apoptotic process in cells is strictly controlled by the expression of anti- and pro-apoptotic proteins. For example, the Bcl-2 family contributes to the regulation of cell apoptosis by functioning as promoters, including Bax, or inhibitors, including Bcl-2 of mitochondrion-associated apoptosis. To further elucidate the molecular mechanism by which S1PR1 suppresses the apoptosis of human myeloid leukemia cells, the present study assessed the protein expression levels of pro-apoptotic Bax and anti-apoptotic Bcl-2, in the context of the overexpression or downregulation of S1PR1. As shown in Fig. 2, the overexpression of S1PR1 resulted in a significant increase in the expression of Bax, thus reducing the ratio of Bax/Bcl-2. This suggested that S1PR1 affected cell apoptosis by regulating the expression of mitochondrion-associated anti- and pro-apoptotic proteins. In addition, the effects of the expression of S1PR1 on the activation of caspase-3 were examined. There was an increase in the level of cleaved caspase-3 (17 kD) in the cells with downregulated levels of S1PR1, whereas the overexpression of S1PR1 led to the inhibition of caspase-3 cleavage (Fig. 2). Taken together, these data demonstrated that S1PR1 interfered with myeloid leukemia cell apoptosis via the suppression of mitochondrion-associated apoptotic processes by regulating the expression of Bax and activation of caspase-3.

**S1PR1 suppresses the generation of ROS.** Several previous studies have shown that ROS is important in stress-induced apoptosis. During cellular stress, ROS can act directly on the apoptotic machinery, by accelerating depolarization and causing dysfunction of mitochondria (20), which in turn, functions as a major source of ROS in the course of apoptosis (21). To investigate whether ROS is involved in the regulation of S1PR1-mediated myeloid leukemia cell apoptosis, the present study compared the levels of ROS in cells with S1PR1 overexpression or knockdown with the control cells. As shown in the results of the flow cytometric analysis (Fig. 3), the downregulation of S1PR1 led to a marked increase in the intracellular levels of ROS. By contrast, the generation of ROS in cells overexpressing S1PR1 was significantly suppressed. To further confirm the involvement of ROS in the S1PR1-mediated regulation of cell apoptosis, NAC, a ROS scavenging agent was used in flow cytometric analysis of S1PR1-mediated regulation of cell apoptosis. As expected, cell apoptosis induced by the downregulation of S1PR1 was partially reversed in cells treated with NAC, further indicating the direct implication of ROS in the S1PR1-mediated resistance of cell apoptosis. These results suggested that S1PR1 confers resistance to cell apoptosis likely by suppressing the generation or accelerating the elimination of ROS.

**S1PR1 inhibits JNK-c-Jun signaling.** Apart from the direct action of ROS on mitochondrial apoptotic machinery, ROS



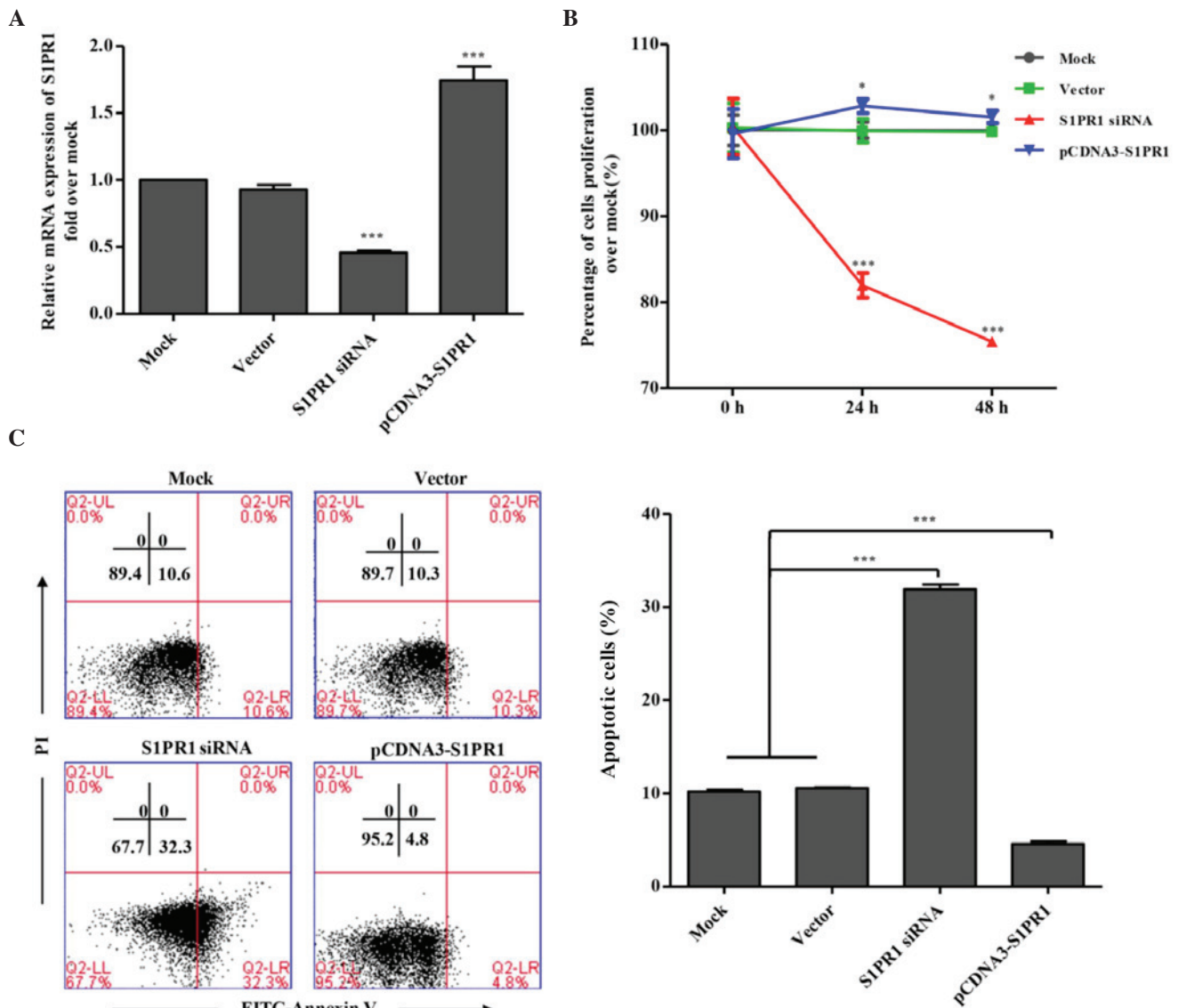


Figure 1. Effects of the expression of SIPR1 on the proliferation and apoptosis of AML cells. (A) Modulation of the expression of SIPR1 by transient transfection with plasmids. HL60 cells were transfected with the indicated plasmids. At 48 h post-transfection, total RNA was isolated from the cell samples and the mRNA levels of SIPR1 were analyzed using quantitative polymerase chain reaction analysis. Data are presented as the mean  $\pm$  standard deviation ( $n=3$ ). (B) Effects of the expression of SIPR1 on the proliferation of human AML cells. U937 cells were transfected with the indicated plasmids. Cell proliferation was measured at the indicated times post-transfection using a CCK-8 assay. Data are presented as the mean  $\pm$  standard deviation ( $n=3$ ). (C) Effects of the expression of SIPR1 on apoptosis of human AML cells. U937 cells were transfected with SIPR1 siRNA or overexpression plasmids, or control plasmids. After 48 h, cell apoptosis was analyzed with Annexin-PI staining using flow cytometry. A representative assay is shown, with data presented as the mean  $\pm$  standard deviation ( $n=3$ ). \* $P<0.05$  and \*\*\* $P<0.001$ , compared with mock and vector. SIPR1, sphingosine-1-phosphate receptor 1; AML, acute myeloid leukemia; siRNA, small interfering RNA; FITC, fluorescein isothiocyanate; PI, propidium iodide.

can also promote apoptosis by triggering the pro-apoptotic signaling pathway mediated by JNK and p38 MAPK. As ROS is involved in the regulation of cell apoptosis by SIPR1, it was hypothesized that the regulation may also involve ROS-induced JNK or/and P38 MAPK signaling. To confirm this hypothesis, the activation of JNK and P38 were examined by western blot analysis using antibodies against corresponding phosphorylated proteins. The phosphorylation of p38 was not affected by either the overexpression or knockdown of SIPR1. However, JNK activation was not changed in SIPR1-overexpressing cells, and the knockdown of SIPR1 appeared to induce a significant increase in JNK phosphorylation, compared with the controls (Fig. 4A), suggesting that the downregulated expression of SIPR1 contributed to the activation of JNK. Similar results

were obtained from the investigation of the level of c-Jun phosphorylation downstream of the JNK signaling. These findings indicated that SIPR1 specifically interfered with JNK signaling and likely inhibited cell apoptosis.

*SIPR1 positively modulates MKK1-ERK signaling, likely resulting in the promotion of cell proliferation.* In parallel with JNK and P38, ERK is a member of the MAPK superfamily. However, JNK and p38 MAPK are preferentially activated by proinflammatory cytokines and oxidative stress, resulting in cell apoptosis, whereas ERK1/2 are activated by mitogens and growth factors, leading to cell growth and survival. Thus, the present study evaluated the activation of ERK. The overexpression of SIPR1 increased the phos-

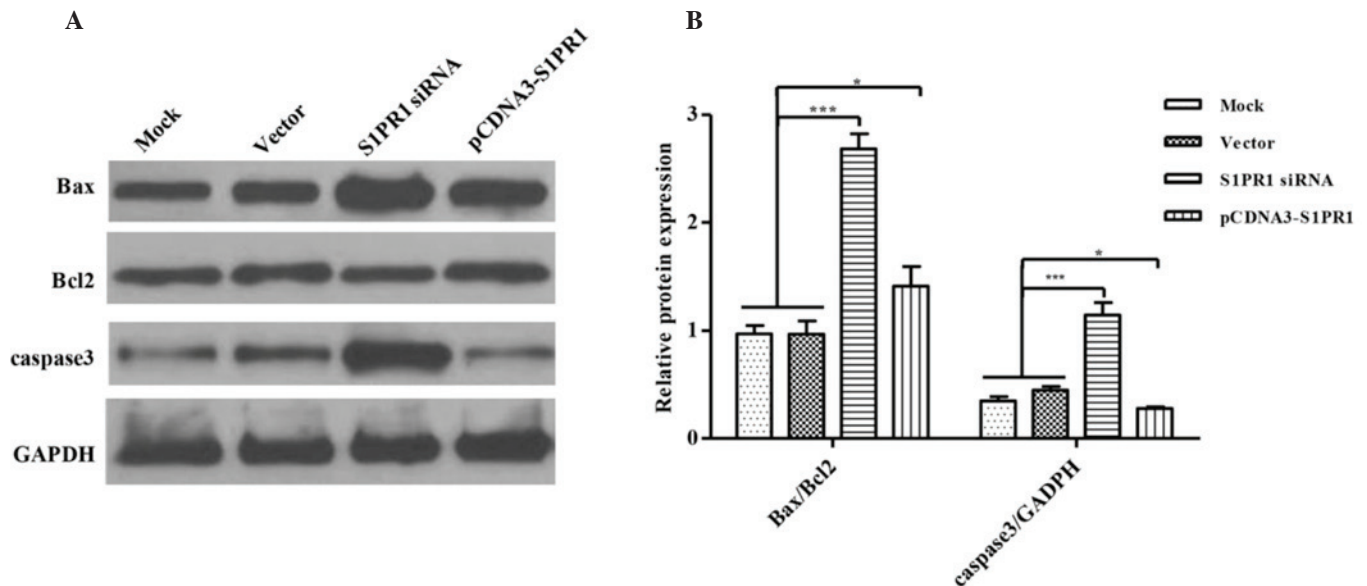


Figure 2. S1PR1 confers resistance to mitochondrion-associated apoptosis on acute myeloid leukemia cells. Cells were transfected with the indicated plasmids and lysed for western blot analysis 24 h post-transfection using antibodies against the indicated proteins. GAPDH was detected as a control for sample loading. (A) A representative assay is shown. (B) Intensities of the Bax and Bcl2 bands were analyzed and the ratios of Bax/Bcl2 are shown. The protein expression level of caspase 3 was also analyzed. Data are presented as the mean  $\pm$  standard deviation. \* $P$ <0.05, \*\*\* $P$ <0.001. S1PR1, sphingosine-1-phosphate receptor 1; Bcl2, B cell lymphoma-2; Bax, Bcl2-associated X protein; siRNA, small interfering RNA.

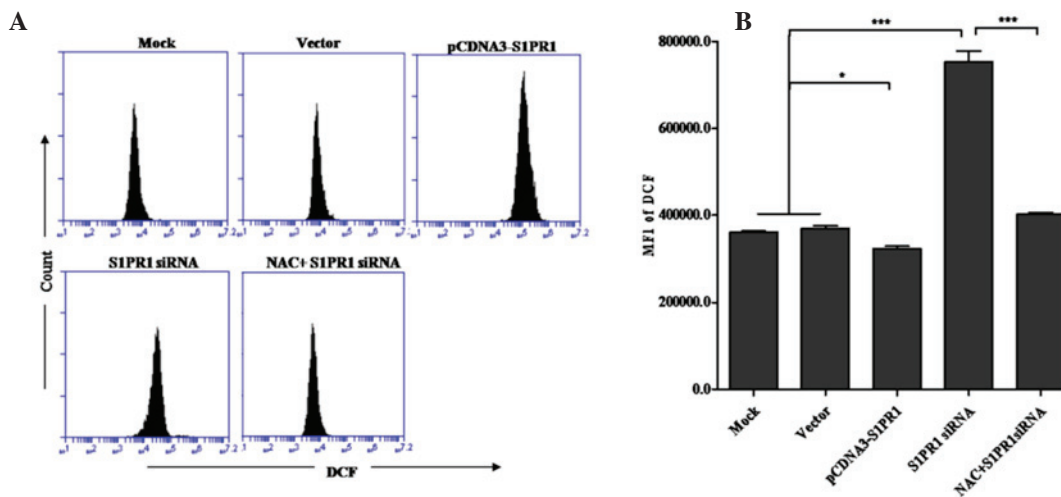


Figure 3. Effects of the expression of S1PR1 on the generation of ROS in AML cells. The cells were transfected with the indicated plasmids, and the levels of intracellular ROS were analyzed using flow cytometry. (A) A representative assay is shown. (B) Mean fluorescence intensity (MFI) of DCF values from three independent experiments are presented as the mean  $\pm$  standard deviation. \* $P$ <0.05, \*\*\* $P$ <0.001. S1PR1, sphingosine-1-phosphate receptor 1; AML, acute myeloid leukemia; siRNA, small interfering RNA; DCF 2,7-dichlorofluorescein.

phorylation of ERK, whereas kinase activation appeared to be marginally attenuated in the S1PR1-knockdown cells (Fig. 4B). These results indicated that S1PR1 had a positive effect in the activation of ERK. In addition, the effects of the expression of S1PR1 on the phosphorylation of MKK1, the upstream kinase of ERK, was also examined and a similar result was obtained, suggesting that the enhancement of ERK activation was due to the positive regulation of MKK1 signaling by S1PR1. Considering the roles of ERK signaling in cell growth and survival, it was hypothesized that the promotion of cell proliferation observed results, at least in part, from the positive modulation of MKK1-ERK signaling by S1PR1. Taken together, S1PR1 likely suppresses JNK

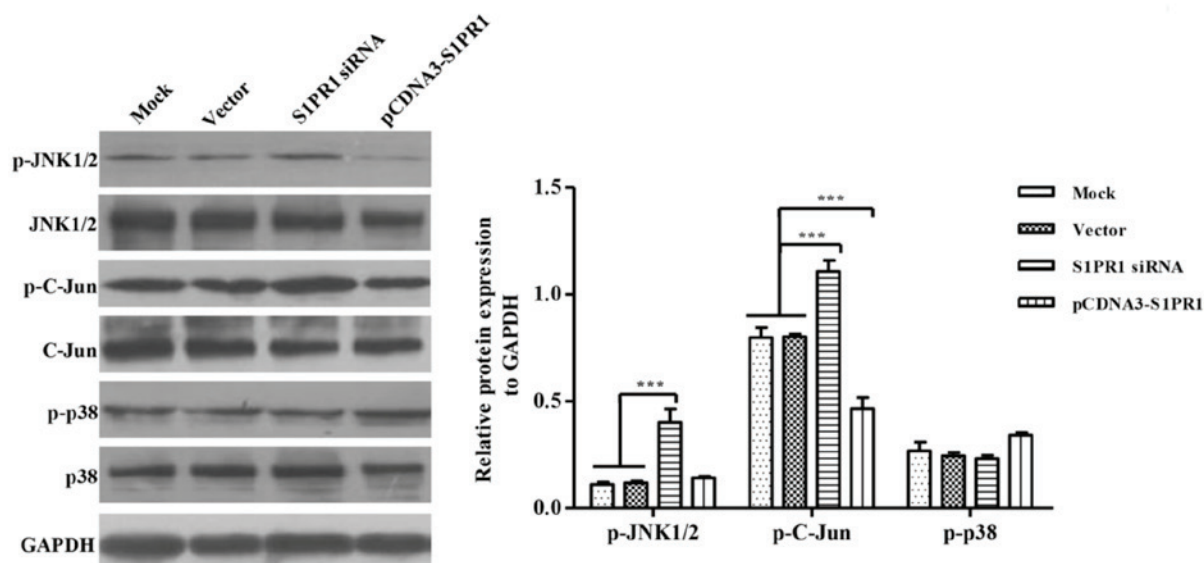
signaling, leading to the promotion of myeloid leukemia cell apoptosis, and enhances cell proliferation by activating ERK signaling.

In conclusion, S1PR1 not only suppressed JNK signaling and promoted myeloid leukemia cell (mitochondrion-associated) apoptosis, but also facilitated cell proliferation, likely by suppressing the generation or accelerating the elimination of ROS, and by activating ERK signaling.

## Discussion

AML is a heterogeneous clonal malignancy. The disease is characterized as multiple genetic alterations in hematopoietic

A



B

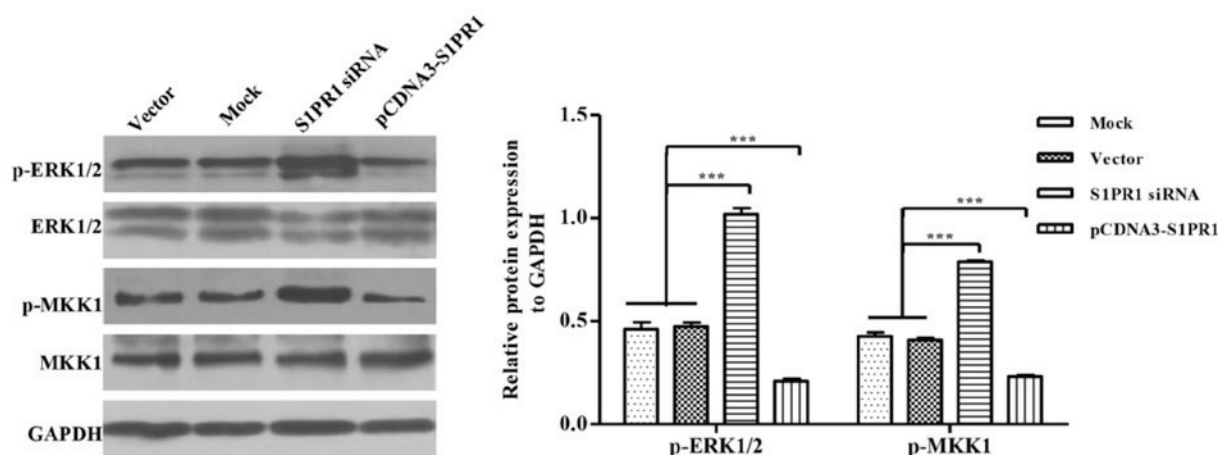


Figure 4. Western blot analysis. (A) S1PR1 specifically promoted the activation of JNK-c-Jun signaling. The cells were transfected with the indicated plasmids and lysed for western blot analysis at 24 h post-transfection using antibodies against the indicated proteins. GAPDH was detected as a control for sample loading. (B) S1PR1 stimulated MKK1-ERK signaling. Cells were transfected with the indicated plasmids and lysed for western blot analysis at 24 h post-transfection using antibodies against the indicated MKK1-ERK1/2 signaling proteins. GAPDH was detected as the control of sample loading. \*\*\* $P < 0.001$ . S1PR1, sphingosine-1-phosphate receptor 1; JNK, c-Jun N-terminal kinase; MKK1, mitogen-activated protein kinase kinase 1; ERK, extracellular signal-regulated kinase; p-, phosphorylated; siRNA, small interfering RNA.

stem cells, including the translocation of a t(12;18) (p13;q12) involving Ets Variant 6, which results in the overexpression of SET binding protein 1 and promotes the proliferation of leukemic cells (22). In previous years, several genetic markers in AML have been identified, leading to increased understanding and providing targets for treatment of the disease (23-26). However, the relevant aberrations that contribute to the pathogenesis of this disease remain to be fully elucidated. In the present study, the importance of S1PR1 in regulating the apoptosis and proliferation of AML cells were identified. S1PR1 inhibited AML cell apoptosis through interfering with mitochondrion-mediated apoptosis processes, ROS generation and JNK MAPK signaling. S1PR1 also promoted the proliferation of AML cells, which was at least partially due to the activation of ERK signaling by S1PR1.

ROS are diffusible chemical mediators. They can act directly on the apoptotic machinery, by accelerating

depolarization and causing dysfunction of mitochondria (20), which in turn, function as a major source of ROS in the course of apoptosis (21). The results of the present study suggested that S1PR1 prevented mitochondrion-associated apoptosis and ROS generation, and confirmed the importance of S1PR1 as an anti-apoptotic factor, with elucidation of the corresponding mechanism. However, the upstream reason for the suppression of apoptosis remains to be elucidated, in terms of the reciprocal causation between mitochondria injury and ROS generation.

Impaired apoptosis can promote cancer cell survival. In the evolutionarily conserved 'stress' pathway-mitochondria signal transduction of apoptosis, the Bcl-2 family are core cell death machinery, and consist of pro-apoptotic proteins, including Bcl-2-associated death promoter and Bax, and anti-apoptotic proteins, including Bcl-2 and Bcl-extra large, which interact to maintain a balance (27). When anti-apoptotic Bcl-2 family members are overexpressed, the ratio of pro- and anti-apoptotic Bcl-2 family members is disturbed, which in turn potentially



drives malignant progression and confers chemoresistance to cancer cells (23). According to the observations in the present study, S1PR1 inhibited mitochondrion-associated apoptosis by reducing the levels of Bax and preventing the cleavage of caspase-3 (Fig. 4). Although the inhibition of ROS generation by S1PR1 may explain the anti-apoptotic mechanism of the protein, there may be other factors involved in the S1PR1 knockdown-induced apoptosis of AML cells in addition to ROS.

MAPK signaling pathways are crucial in cell proliferation and apoptosis. Until now, three MAPK families have been clearly characterized: ERK1/2, JNK/MAPK and p38 kinase. The distinct MAPK members mediate different physiological activities; the ERKs function in proliferation and differentiation, whereas the JNKs and p38 MAPKs are predominantly involved in the stress response and apoptosis through responding to various stresses (25). Several studies have suggested that stimulated ERK1 activity results in enhanced cell proliferation (24,26). Of note, S1PR1 can promote the MKK1/ERK signaling pathway, which was observed in the AML cells examined in the present study. Therefore, the enhancement of ERK activation may be a reason for the S1PR1-mediated promotion of AML cell proliferation observed in the present study. However, the mechanism by which S1PR1 enhances MKK/ERK signaling remains to be elucidated.

According to the results of the present study, the reduction of ROS is likely to be involved in S1PR1-induced cell proliferation. Although the S1PR1-induced reduction of ROS levels may be one reason for the inhibition of JNK signaling by S1PR1, the possibility that S1PR1 counteracts JNK activation in a ROS-independent manner cannot be excluded. Further investigations are required to determine whether S1PR1 suppresses JNK signaling by other mechanisms.

In conclusion, the present study identified the anti-apoptotic/pro-proliferative roles of S1PR1 in AML cells, and elucidated the corresponding mechanism. These findings may provide insights into the molecular mechanism underlying the progression and pathogenesis of AML, and benefit the development of therapeutical approaches for the disease.

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