Iranian Journal of Basic Medical Sciences

ijbms.mums.ac.ir

IJBMS

Sphingosine 1-phosphate interacts with Survivin pathway to enhance tumorigenesis in cancer cells

Maryam Tabasinezhad [1, 2, 3], Hamid Ghaedi [4], Parisa Qanbari [1, 2], Mahsa Mohseni [1, 2], Mehdi Sabzichi [1, 2], Nasser Samadi [1, 2*]

[1] Research Center for Pharmaceutical Nanotechnology, Tabriz University of Medical Sciences, Tabriz, Iran
[2] Department of Medical Biotechnology, Faculty of Advanced Medical Sciences, Tabriz University of Medical Sciences, Tabriz, Iran
[3] Student Research Center Committee, Tabriz university of Medical Sciences, Tabriz, Iran
[4] Medical Genetics Department, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

ARTICLE INFO

Article type:
Original article

Article history:
Received: Dec 10, 2014
Accepted: Mar 18, 2015

Keywords:
Invasion
Migration
Proliferation
Survivin
S1P

ABSTRACT

Objective(s): Degradation of sphingosine 1-phosphate (S1P), as a bioactive lipid, or deregulation of its production involves in tumor progression, metastasis and chemoresistance. Since the tumor progression effects of S1P and its mechanism in chronic lymphoblastic leukemia and non-small cell lung cancer is not fully understood, we investigated the role and one of the mechanisms of S1P in tumor progression of SKW3 and H1299 cells.

Materials and Methods: The effects of S1P on proliferation, invasion and migration was studied using MTT assay, soft-agar colony forming assay and trans-well migration assay, respectively. In order to find out the mechanisms of S1P action, the role of S1P on expression of *Survivin* gene was assessed by real-time RT-PCR.

Results: Our results demonstrated that although invasion was shown only in H1299 cells, low concentration of S1P, especially at 1 µM, mediated proliferation and migration in both cell lines. In addition, these effects of S1P in tumor progression are S1P receptor-dependent, and Survivin plays a key role in S1P tumorigenesis.

Conclusion: Our results confirmed the involvement of S1P and its receptors in tumor progression of SKW3 and H1299. We also investigated another mechanism of S1P involved in cell survival, tumor progression, and Survivin signaling. In conclusion, data demonstrated the importance of this molecule as a target for designing new anticancer drugs such as anti-S1P monoclonal antibody for inhibiting major downstream signaling, which plays significant role in tumorigenesis.

► *Please cite this article as:*
Tabasinezhad M, Ghaedi H, Qanbari P, Mohseni M, Sabzichi M, Samadi N. Sphingosine 1-phosphate interacts with Survivin pathway to enhance tumorigenesis in cancer cells. Iran J Basic Med Sci 2015; 18:813-821.

Introduction

Sphingolipids are ubiquitous components of eukaryotic cell membranes, which can be metabolized to ceramide, sphingosine, and their phosphorylated forms such as ceramide 1-phosphate (C1P) and sphingosine 1-phosphate (S1P) (1). In the recent years, researches on lipids have shifted from their constitutive roles in the cell membrane to the signaling aspects of these molecules.

Sphingolipids have been implicated in a plethora of biological processes including the regulation of cell growth, cell survival, differentiation, and programmed cell death (2). S1P is one of the most important bioactive sphingolipid metabolites. It plays various roles in physiological processes, promotes cellular proliferation, stimulates cell survival and protects cells against apoptosis through an intracellular receptor-independent mechanism (3, 4). S1P functions not only as an intracellular signaling molecule, but also acts extracellularly through G-protein coupled receptors (GPCRs) that are called S1P1-S1P5 (5). Activation of S1P receptors (S1PRs) induces some signaling pathways, which lead to regulation of cytoskeletal organization, cell proliferation, migration, invasion, angiogenesis and inhibition of apoptosis (6). Pertussis toxin (PTX) by coupling to GPCRs inhibits signal transduction of S1P (7). Intracellular S1P levels are regulated with S1P lyase (SPL), S1P kinase (SphK) and S1P phosphatase (SPP1-SPP2); therefore, alteration in any of these enzymes can change S1P levels and results in cell survival or death (8). Balance between synthesis and degradation of S1P is firmly regulated by SPL, SphK and SPP1-SPP2 (9). Several lines of evidence have demonstrated that deregulation of normal S1P levels in blood or tissues may contribute to pathophysiologic states including cardiovascular

*Corresponding author: Nasser Samadi. Department of Medical Biotechnology, Faculty of Advanced Medical Sciences and Research Center for Pharmaceutical Nanotechnology, Tabriz University of Medical Sciences, Tabriz, Iran. Tel: +98-411-3355789; Fax: +98-411-3355789; email: drnsamadi@yahoo.com

diseases, chronic inflammation, cancer drug resistance and metastasis (10-12). Moreover, various studies have shown that S1P implicated in tumorigenesis such as cell migration, invasion, and metastasis and cancer recurrence in the breast, lung and colon cancer cells (6).

Survivin is an anti-apoptotic protein that in human is encoded by *BIRC5* gene, which can inhibit apoptosis via Bcl-2 family independent pathway, and has an important role in cell survival (13, 14); moreover, it is over expressed in most cancer tissue such as Oral Squamous Cell Carcinoma and Peritumoral tissue (15). Studies showed that anti-Survivin inhibits proliferation and migration in breast cancer cell line (16); in addition, diagnostic role of Survivin in urinary bladder cancer is also reported (17).

We hypothesized that S1P may involve in Survivin pathway. To test our hypothesis, we assessed the effect of S1P on malignant behavior of cancer cell lines (SKW3 and H1299). We studied cellular proliferation, migration and invasion in the presence of S1P and monitored the Survivin levels to find any possible relations. The effect of S1P on these cell lines had not been specified up to now, and we selected them to identifying the role of S1P in different adherent and non-adherent cells.

Materials and Methods

SKW3 and H1299 cell lines were purchased from Pasteur Institute Cell Culture Collection (Tehran, Iran). S1P, bovine serum albumin (BSA), RPMI1640, fibronectin, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazoliumbromide (MTT) and agar obtained from Sigma-Aldrich (St Louis, MO, USA). Transwells® (polycarbonate, 6.5 mm D, 8 µm pore size) were obtained from Corning (Corning, NY, USA). PTX and Fetal Bovin Serum (FBS) purchased from Invitrogen (Auckland, New Zealand). Primers were provided from MWG Biotech (Ebersberg, Germany). RNA isolation kit (RNX-Plus) was obtained from CinnaGen Co. (Tehran, Iran), and REVERTA-L RT reagents kit was purchased from Central Research Institute of Epidemiology of Russia (Moscow, Russia). Power SYBR® Green PCR Master Mix (5 ml) was obtained from Applied Biosystems (Warrington, UK).

Cell culture

SKW3 and H1299 cells were maintained in RPMI supplemented with 10% FBS, 100 U/ml penicillin and 100 µg/ml streptomycin on culture plates at 37 ˚C in a humidified incubator supplemented with 5% CO_2. Cell counting for the two cell line are different. Since SKW3 cells are in suspension, they were first centrifuged, and then supernatant was diluted and counted by use of Neoubar lam and inverted microscope. But, H1299 cells (attachment cells) were trypsinised before counting to detach form the bottom of flaks, and then counted as mentioned for SKW3 cells.

Cell proliferation assessment by MTT assay

Number of the cells optimized for each cell line, which seeded in each well (in 96-well plate) was 6000 and 50000 for H1299 and SKW3 cells, respectively. The cells were treated with different concentration of S1P 0.001-1 µM for 24 and 48 hr. The media in each well was replaced with 200 µl fresh media containing 50 µl of MTT and were incubated for 4 hr at 37 ˚C. After incubation period, media/MTT mixture was removed and 200 µl of DMSO plus 25 µl of Sorenson's glycine buffer (0.1 M glycine, 0.1 M NaCl, pH 10.5) were added to each well. The absorbance of each well was measured at 570 nm after shaking for 10 min, employing a microplate reader (Biotek, ELx 800, and USA). MTT solution with DMSO (without cells and medium) was used as blank control. For confirming the effect of S1P, cells pre-incubated with 200 µM of PTX before treating by S1P and then all the processes repeated.

Boyden Chamber assay for migration

Two million cells were seeded in a 25 cm^2 flask with growth medium for at least 48 hr. Starvation medium (RPMI 1640 containing 0.1% BSA, ≥96% fatty acid free) was added for the last 48 hr before the migration experiment. Cells were centrifuged down, media were removed by aspiration, and cells were re-suspended in starvation media and counted. Cells (300,000) were seeded into each of the fibronectin coated Transwell® filters and incubated for 45 min. Filters were then transferred into bottom chambers that contained 1.5 ml of starvation medium. Chambers were incubated at 37 ˚C, and cells were allowed to migrate through the pores in the filter for 3 or 6 hr. The cells that migrated into bottom chamber were stained with trypan blue and then counted.

Soft agar colony forming assay for invasion

Base agar

One percent agar ((DNA grade) was melted using microwave, and then it was cooled to 40 ˚C in a water-bath. Then 2X RPMI + 20% FBS warmed to 40 ˚C in water-bath and allowed at least 30 min for temperature to equilibrate. Afterwards, equal volumes of the two solutions mixed to give 0.5% Agar + 1X RPMI + 10% FCS. Finally, 1.5 ml of the solution added per well and allowed setting.

Top agar and plating

Agar (0.7%) was melted in microwave and cooled to 40 ˚C in a water-bath. Also 2X RPMI + 20% FCS were warmed at the same temperature. We required 5,000 cells for each well. For plating, 3 ml of 2X RPMI and 3 ml of 0.7% agar were added to tubes containing cells that treated with different concentration of S1P. They were mixed gently and 1.5 ml content of each tube was added to each well plate, and one ml of media (2XRPMI + 0.2% charcoal-

treated FBS) was added to each well and replaced with the fresh media every 4 to 5 days. Plates were incubated at 37 ˚C in humidified incubator for 21 days. At last, plates were stained with 0.5 ml of 0.005% crystal violet, incubated about one hr, and subsequently colonies counted by naked eyes. It is very important to have a positive control line (eg. ras transformed). In addition, all S1P treatments were performed in duplicate.

RNA isolation and cDNA synthesis

Cells were harvested 24 hr after treatment with different concentrations of S1P and were lysed using RNX-PLUS™ (RN7713C) CinnaGen Co., according to manufacturer's protocol. RNA pellet was dissolved in Diethylpyrocarbonate (DEPC) treated water, quantified by optical density measurement (A260/A280 ratio) with NanoDrop 1000 Spectrophotometer (Wilmington, DE, USA), quality checked by agarose gel electrophoresis and then kept at −70 ˚C until use in reverse transcription reactions. cDNA synthesis was performed using REVERTAA-L (RT reagents kit) and samples were stored at -20 ˚C.

Real-time PCR

The iQ5 Optical System (Bio-Rad Laboratories, Inc., CA-USA) was utilized for performing all amplification reactions in a total volume of 25 µl. Each well contained 1 µl of cDNA, 5.75 µM of each primer and 12.5 µl of 2X Power SYBR Green PCR Master Mix. Primer for Glyceraldehyde-3-Phosphate Dehydrogenase (*GAPDH*) was designed according to published Gene Bank sequences using Beacon Designer 5.01, and Survivin primers (F: 5'GACCACCGCATCTCTACATTC-3' R: 5'TGC-TTTT-TATGTTCCTCTATGGG-3') was ordered according to Cho *et al* (18). Thermal cycle conditions were: 1 cycle of primary denaturation at 94 ˚C for 10 min; 40 cycles of amplification at 95 ˚C for 15 sec, 62 ˚C for 30 sec for Survivin and 56.9 ˚C for GAPDH and 72 ˚C for 25 sec; 1 cycle for final extension at 72 ˚C. Interpretation of the results was performed using the Pfaffle method and the cycle threshold (CT) values were normalized with respect to *GAPDH* expression. All reactions were performed in triplicate and negative control was included in each experiment.

Statistical analysis

Results were presented as means from three independent experiments. Statistical analysis was performed using SPSS software through ANOVA or student t-tests. A $P\leq0.05$ was considered as statistically significant, and accordingly the levels of significance were defined as: * ($P\leq0.05$), ** ($P\leq0.01$) and *** ($P\leq0.001$).

Results

Proliferative effects of S1P on SKW3 and H1299 cells

The effects of S1P in induction of proliferation of SKW3 and H1299 cells were evaluated via MTT assay. For this examination, 6000 H1299 cells and 50000 SKW3 cells were seeded in each well of 96-well plate, treated with increasing concentrations of S1P (0.01-10 µM) for 24 hr and 48 hr in starvation media for MTT assay to determine the optimal concentration of S1P that shows maximum proliferative effect. MTT results in both cell lines showed remarkably increase in cell proliferation at a range of S1P concentration between 0.01 and 1 µM S1P after 24 hr incubation. However, there was no significant increase in the number of cells after 48 hr incubation. By increasing S1P concentration to above 1 µM, cell proliferation decreased compared to 1 µM concentration. In addition, the increase in the number of viable cells was similar in 24 hr and 48 hr incubation (Figure 1A and C). These data showed that S1P has little effect on SKW3 and H1299 cells after first 24 hr. In 1 µM S1P, the cell proliferation increased approximately 5.5 fold in SKW3 cells and 2.2 fold in H1299 cells compared to control cells ($P\leq0.05$). Considering these results, the rest of experiments performed in 24 hr and with 0.1-5 µM of S1P.

S1P induced H1299 and SKW3 cell proliferation in PTX-sensitive manner

To investigate the signaling mechanisms by which S1P induces proliferation in H1299 and SKW3 cells, we applied PTX for blockade of S1P receptors and inhibition of cell proliferation. Treatment of cells with PTX inhibited S1P-induced cell proliferation in 24 hr significantly (Figure 1 B and D), indicating that the role of S1P (1 µM) in induction of cell proliferation is S1P receptor dependent ($P\leq 0.01$).

S1P induced migration of H1299 and SKW3 cells

To evaluate migration effects of S1P on cancer cells, at first 300,000 cells of both SKW3 and H1299 cells were seeded in starvation media in each of the fibronectin coated Transwell® filters. One ml of starvation media containing different concentrations of S1P was added to the lower chamber and incubated for 3 to 6 hr. Our results showed that S1P exerts a significant effect on migration of both SKW3 and H1299 cells. SKW3 and H1299 cells showed a similar migratory response to S1P in 0.1 to 1 µM S1P after 3 to 6 hr. Maximum migration occurred in 1 µM S1P in both cell lines. Migration effects of S1P (1 µM) in SKW3 was 1.3 fold more than its effect in H1299 cells ($P\leq0.01$) (Figure 2A and C).

S1P induced migration of SKW3 and H1299 by PTX-sensitive GPCRs

To evaluate the role of S1P in induction of migration in SKW3 and H1299 cells, we examined the effects of S1P on PTX pre-treated cells. For this purpose, 200 ng/ml of PTX added to each well containing the cells and incubated for 2 hr, then the well containing 1 ml of starvation media with different concentrations of S1P incubated for 3 to 6 hr in 37 ˚C. Our results showed that the migration of PTX-treated cells from both SKW3

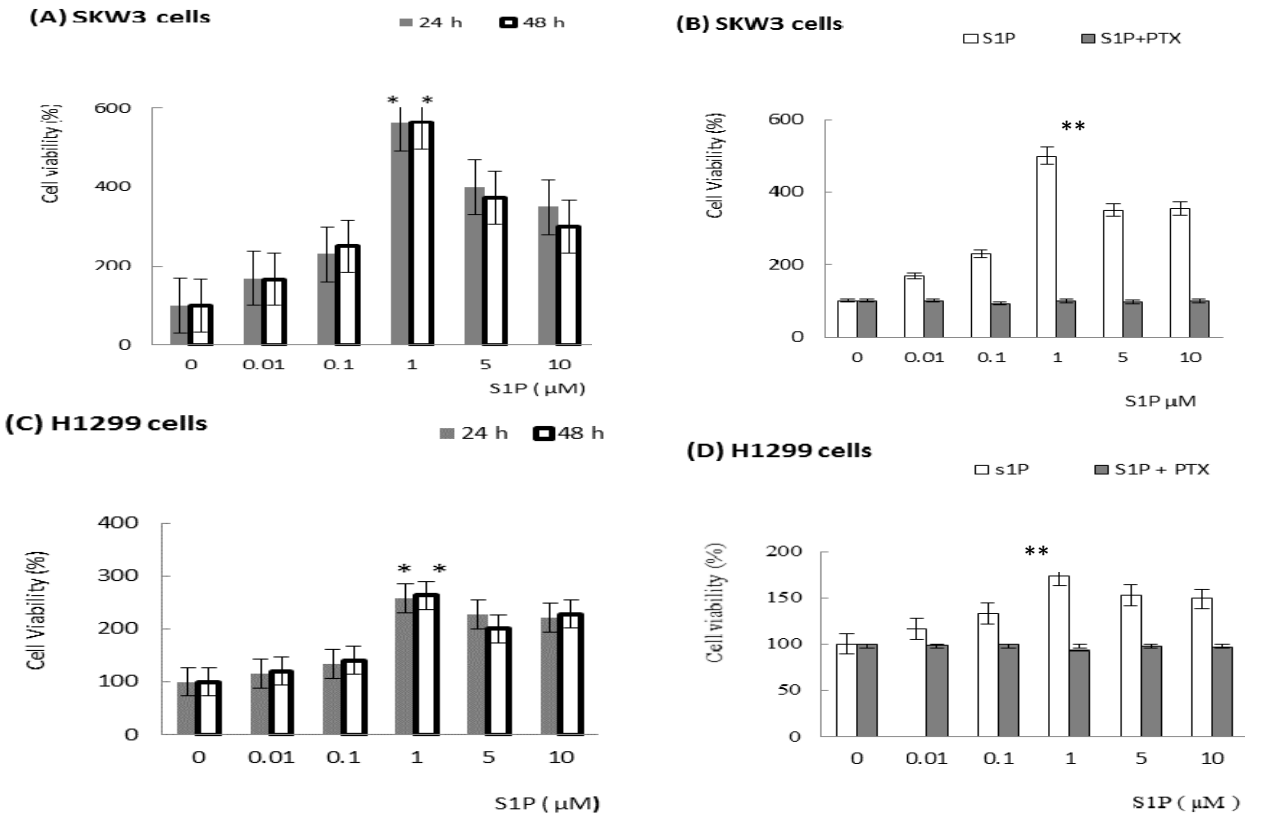

Figure 1. MTT assay for examination of proliferative activity of S1P. SKW3 and H1299 cells treated with 0.01-10 µM S1P for 24 and 48 hr (A and B). Pre-incubated of SKW3 and H1299 cells with PTX (200 ng/ml) for 2 hr prior to treatment with 0.01-10 µM S1P for 24 hr (B and D). *(P≤0.05), ** (P≤0.01)

and H1299 cell lines were decreased remarkably in comparison to control cells (PTX pre-incubated without S1P) and S1P treated cells. These data strongly suggest the involvement of PTX-sensitive GPCRs in induction of migration in H1299 (P≤0.05) and SKW3 (P≤0.01) cells by S1P. The experiments were performed in triplicate (Figure 2B and D).

S1P induced cell invasion just in H1299 cells

To evaluate the effects of S1P on cancer cell invasion, we first optimized the number of seeded cells. For this purpose, different cell number of SKW3 and H1299 without any treatment were seeded in top agar in 6-well plates and incubated in 37 ˚C. After 21 days, no colony was observed in the plates that were seeded with SKW3 cells; however, a huge number of colonies were observed in the wells seeded with H1299 cells. So, we examined the effects of different concentrations of S1P on colony formation of H1299 cells in soft agar (5000 cells in each plate). S1P significantly induced colony formation of these cells with a concentration range of 0.01 to 5 µM. The highest number of colonies was achieved with 1 µM S1P (P≤0.05) (Figure 3A).

S1P induced invasion of cells, mediated by PTX-sensitive G-proteins

We also examined the effects of PTX on S1P induced invasion in H1299 cells. When H1299 cells were pre-incubated with 200 ng/ml of PTX prior to the invasion assay, the number of colonies toward S1P was significantly reduced in comparison to the number of the cells that were not treated with PTX. Our results strongly suggest the involvement of PTX-sensitive GPCRs in colony formation and invasion of H1299 cells (P≤ 0.05) (Figure 3B).

S1P induced overexpression of Survivin gene in H1299 and SKW3 cells

Real-time RT-PCR experiment was performed for the SKW3 and H1299 cells that were treated with variable concentrations of S1P (0, 0.1 and 1 µM) after 24 hr. We found that 1 µM of S1P remarkably increased mRNA levels of Survivin about 5.5 fold in both of cell lines in comparison to control (P≤0.001) (Figure 4A and C). *GAPDH* gene was considered as a house-keeping gene in our experiments, an internal control in gene expression assesses. Analysis of *GAPDH* expression by quantitative Real-time PCR technique demonstrated that cells under treatment with different concentrations of S1P had roughly the same amount of *GAPDH* expression, which is critical before continuing to investigate the expression of target genes (*Survivin*) in tumor progression, as it shows the lack of systematic errors.

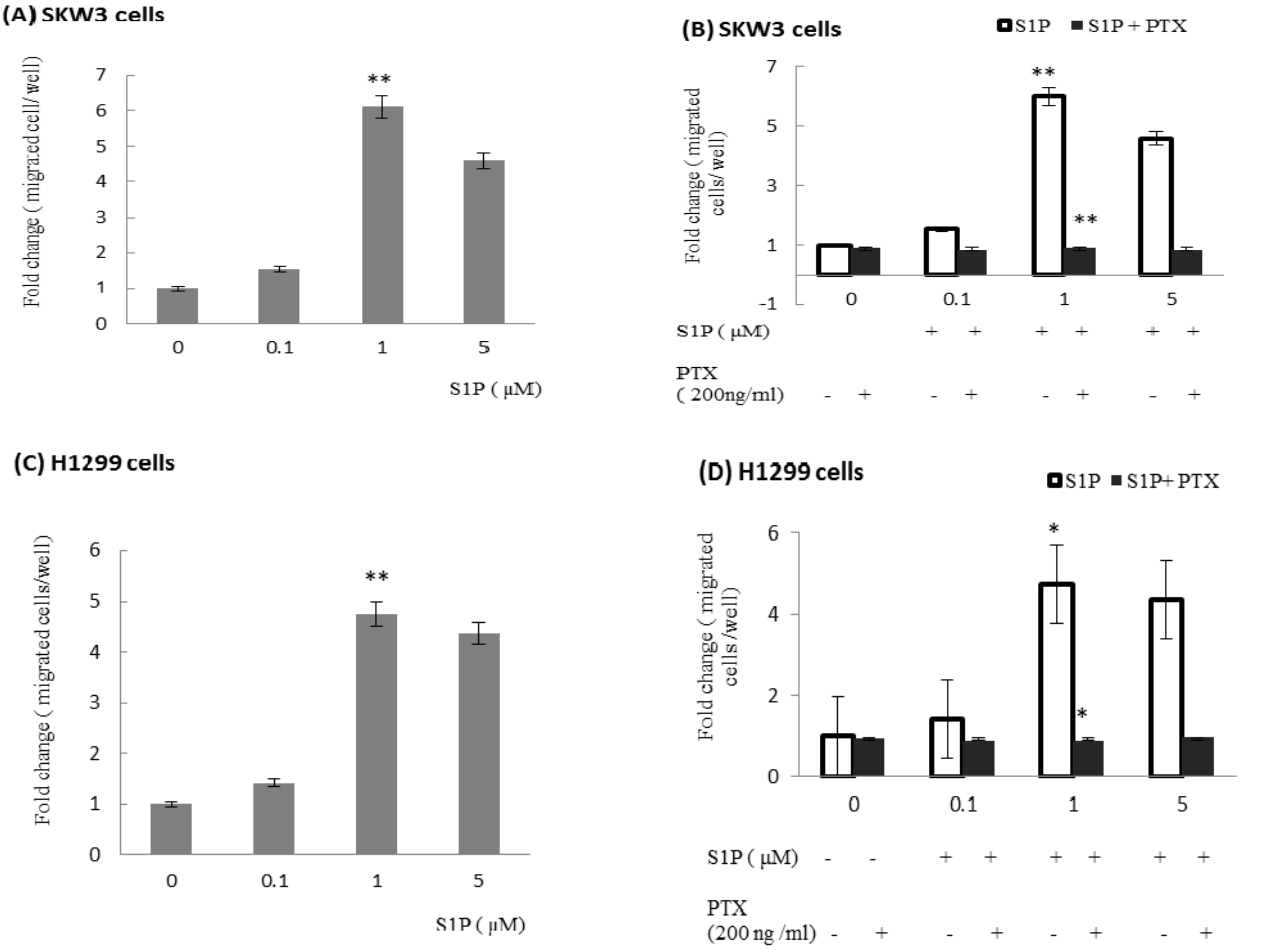

Figure 2. Migration effect of S1P. A) Migration assay showed that different concentrations of S1P increased migration of SKW3 and H1299 cells with a maximum increase at 1 µM (A and C). Pre-incubation of SKW3 and H1299 cells with PTX (200 ng/ml) for 2 hr almost abolished the effects of S1P in induction of tumor cell migration (B and D). *($P \leq 0.05$), ** ($P \leq 0.01$)

S1P induces Survivin expression through GPCRs pathway

In this experiment, we applied PTX to show the effects of S1P and find the role of S1P receptors in overexpression of Survivin. SKW3 and H1299 cells pre-incubated with 200 ng/ml of PTX for 2 hr in 37 ˚C and treated with 1 µM S1P for 24 hr, and Real-time RT-PCR was used to investigate the rate of Survivin expression (we applied 1 µM S1P to assess the PTX effect because the most significant

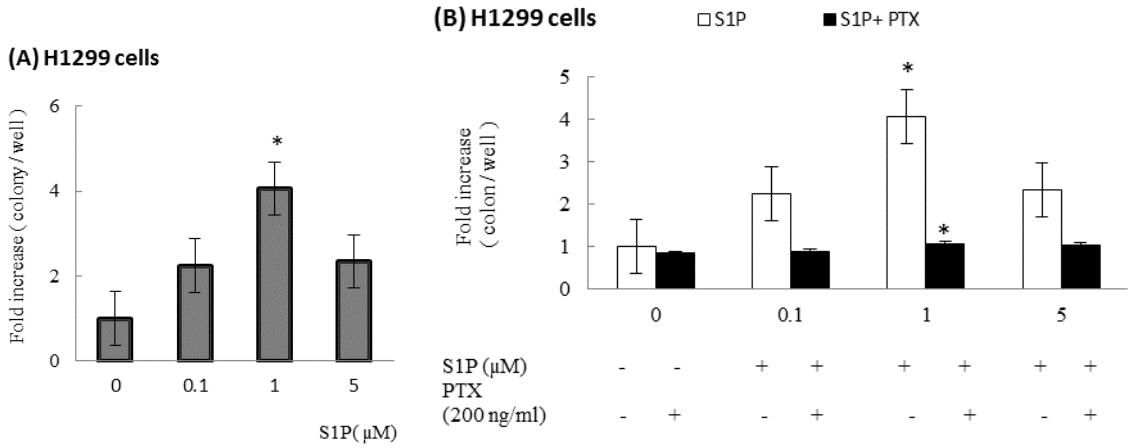

Figure 3. Invasion role of S1P. A) S1P induced colonies forming of H1299 cells in agar after 21 days that significant increase of colonies forming was in 1 µM. B) H1299 cells that pre-incubated with PTX (200 ng/ml for 2hr) and treated with S1P, could not increase the number of colonies in agar due to blockage of GPCRs via PTX in contrast to S1P treated cell * ($P \leq 0.05$)

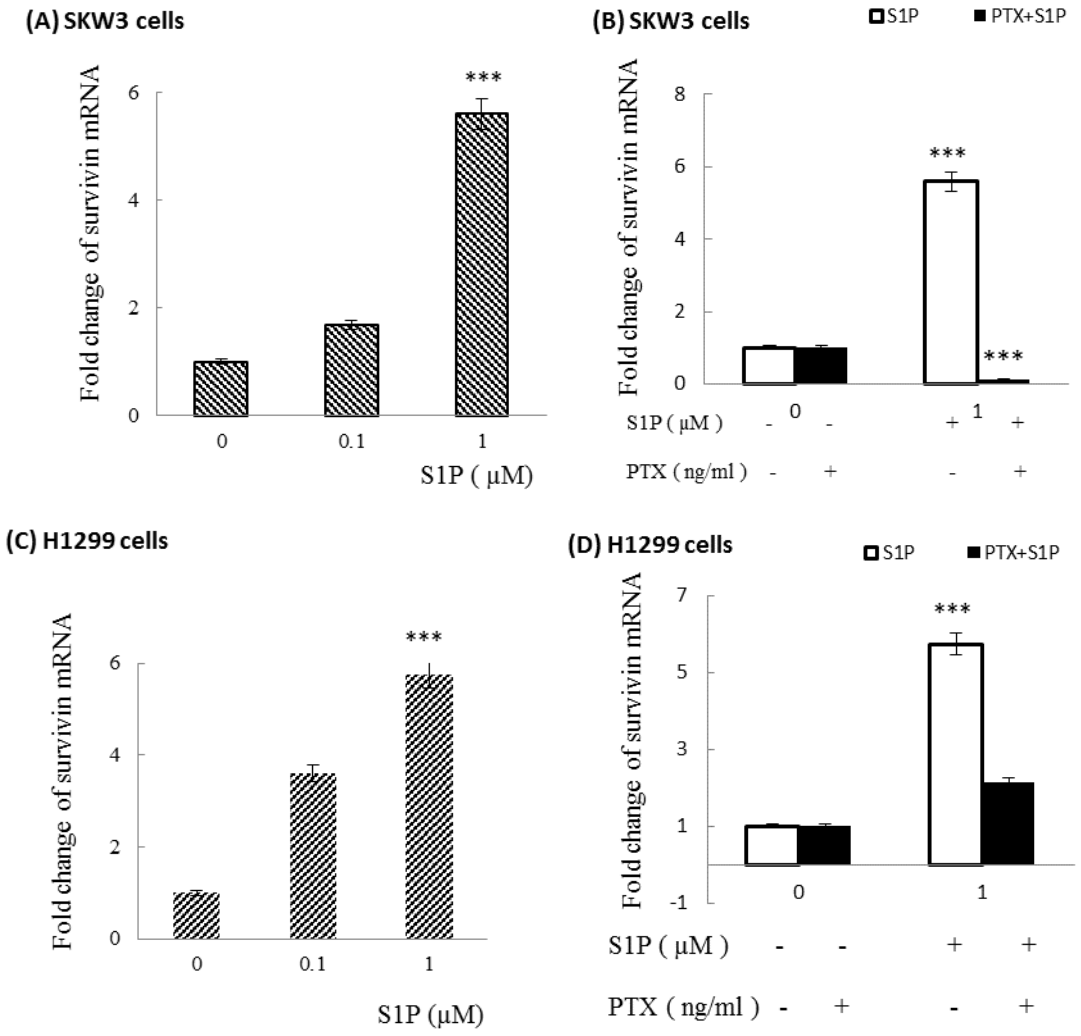

Figure 4. Overexpression of Survivin by S1P. SKW3 and H1299 cells treated with 0.1 and 1 µM S1P showed a significant increase in the expression of *Survivin* gene after 24 hr, especially at 1 µM (A and C). S1P (1 µM) treated SKW3 and H1299 cells that pre-incubated with PTX (200 ng/ml for 2hr) significantly decreased expression of *Survivin* gene after 24 hr. These results showed the involvement of S1P in cell survival in a PTX-sensitive manner (B and D). *** ($P \leq 0.001$)

overexpression of Survivin was observed in 1 µM). Our data demonstrated that S1P has no significant effect on PTX pre-incubated cells, and expression of Survivin was decreased remarkably in both of cell lines ($P \leq 0.001$). However, in S1P-treated cells, PTX caused more significant decrease in Survivin expression in SKW3 cells (Figure 4B and D).

Discussion

S1P through binding to its receptors activates different intracellular signaling pathways depending on which intracellular Gα protein they couple to. The S1P1 receptor primarily couples through Gαi/o, whereas S1P2 and S1P3 receptors can couple through Gαi/o, Gαq and Gα12/13, S1P4 and S1P5 receptors can couple through Gαi/o and Gα12/13 (19-23). Signaling through Gα12/13 can promote the activation of small GTPase Rho and Rho-associated kinase (ROCK) to inhibit migration (19).

S1P is sensitive to PTX by restrain of Gi/o-coupled receptors (24). Therefore, PTX is a PAN-S1P receptor inhibitor. Our data showed that S1P increases proliferation up to 1 µM in both SKW3 and H1299 cell lines in a dose-dependent manner, although PTX pretreated cells did not show cell proliferation in contrast to our control cells. S1P activates S1PRs, which coupled to Gi/o. Therefore, S1P by activating G-protein signaling pathway motivates cell proliferation. PTX by inhibition of Gi/o prevents the S1PR signaling pathway. In conclusion, combination of S1P via PTX attributes to neutralize S1P effect on cell proliferation. Higher effect of S1P in proliferation of SKW3 in comparison to H1299 cells suggests that S1PRs are probably more overexpressed in SKW3. This could be followed by future studies. Yoshida *et al* in 2009 showed that proliferation effect of S1P through S1PR in glioma cells correlated with patient survival (25). Our results are in agreement with these findings.

S1P regulation of cellular migration occurs through the Rho family of GTPases (Rac1, Rho, and CDC42) signaling (26). In the present study, we demonstrated that cell migration increased in the studied cell lines results in S1P signaling. As a result, enhanced migration in SKW3 and H1299 cells may occur by S1PR activation, which is mediated migration via GPCR–Gi coupling. Furthermore, decrease of SKW3 and H1299 cells migration observed after treatment of cells with S1P and PTX. In addition, SKW3 cells migration was 1.3 fold more than H1299, which suggests higher ability of SKW3 cells for migration.

Yamashita *et al* showed that S1PRs expression profile is correlated with the effects of S1P on migration in gastric cancer cells (27). Kluk *et al* showed a similar correlation between S1PRs and migration in classical hodgkin's lymphoma (28).

Although cell motility responses as discussed above are an important aspect of cancer invasion and metastasis, it is only a part of the entire process. In addition, we examined the effects of S1P on SKW3 and H1299 invasion. According to the mentioned process, S1P increased H1299 cells invasion and leaded to more colonies formation in agar resulting in the activation of S1PRs that enhance cell adhesion and invasion. However, treatment of H1299 cells with S1P and PTX contributed to block of S1PRs and decreased colony number of H1299 cells. In addition, SKW3 cells do not have invasive function and are unable to attach together in presence or absence of S1P, and subsequently S1P may not exert adhesion effect on the cell line.

It is inferred from our results that although the SKW3 cells showed high level of migration in the presence of S1P, but they did not have adhesion performance, concluding that SKW3 cells show moderate invasion function in presence of S1P. Furthermore, H1299 cells showed an increase in migration and adhesion of the cells in presence of S1P, so the lipid makes these cells more invasive. Park *et al* in 2007 showed that increasing the invasion of OVCAR3 ovarian cancer cells in the presence of S1P is mediated through S1PR receptor, which is in consistent with our data (29). Gandy KAO and Van Brocklyn J also showed that S1P/S1PR signaling stimulates cell migration and invasion of cancer cells (30, 31).

In addition, S1P has a crucial role in cell survival via different mechanisms. A number of studies showed that S1P have implicated in induction of anti-apoptotic mitogen-activated protein kinase (MAPK) and impediment of pro-apoptotic MAPK pathway in U937 leukemia cells (32, 33). In addition, pro-survival effects of S1P are mediated through prevention of cytochrome C and SMAC/DIABLO release from mitochondria and subsequent inhibition of caspase-3 activation in the human acute leukemia Jurkat, U937, and HL-60 cell lines (33). As well, S1P receptors activate MAPK, PI3K (Phosphoinositide 3-kinase) and AKt (serine/threonine-specific protein kinase) by coupling to Gαi/o and G12/13 and subsequently induce cell survival (34).

PI3K/ Akt pathway is upstream signaling that activates Survivin, a key anti-apoptotic protein, and then Survivin blocks apoptotic pathway by inhibition of SMAC/DIABLO (35-37).

With regard to involvement of S1P in cell survival through S1PRs by activating PI3K/AKt pathway as well as activation of Survivin through PI3K/AKt pathway (38), we demonstrated that one of the mechanisms of S1P in cell survival, tumor proliferation and even chemoresistance is overexpression of Survivin.

Therefore, to evaluate the role of Survivin in S1P-induced tumor progression, the expression of *Survivin* gene was examined in S1P treated-SKW3 and H1299 cells. Our data revealed that 1 μM S1P induces remarkable cell survival and anti-apoptotic signal via overexpression of *BIRC5* gene in SKW3 and H1299 cells. Furthermore, *BIRC5* overexpression as a result of S1P was higher in SKW3. Based on our results, we concluded that S1P mediates activation of Survivin pathway in the SKW3 cells, and hence leads to cell survival. Expression of *BIRC5* gene induced by S1P was decreased remarkably in SKW3 and H1299 cells when they are incubated with PTX. Since PTX blocks the S1PRs, it can be concluded that S1P-induced *BIRC5* gene expression is S1P receptor-dependent. However, more researches are needed to investigate expressions of genes upstream of Survivin pathway in cells treated by S1P.

Conclusion

In this study, we showed involvement of S1P and its receptors in tumor progression of SKW3 and H1299 cell lines. We also demonstrated that increase in gene expression of *Survivin* as another survival mechanism of S1P in cell survival and tumor progression. Consequently, data demonstrate the importance of S1P as a target to design novel anticancer drugs in order to inhibit major downstream signaling, which plays significant role in tumorigenesis.

Acknowledgment

This work was supported by Research Center for Pharmaceutical Nanotechnology, Tabriz, Iran. The results described in this paper were part of student thesis.

References

1. Chalfant CE, Spiegel S. Sphingosine 1-phosphate and ceramide 1-phosphate: expanding roles in cell signaling. J Cell Sci 2005; 118:4605-4612.

2. Hannun YA, Obeid LM. Principles of bioactive lipid signaling: lessons from sphingolipids. Nat Rev Mol Cell Biol 2008; 9:139-150.
3. Goparaju SK, Jolly PS, Watterson KR, Bektas M, Alvarez S, Sarkar S, *et al*. The S1P2 receptor negatively regulates platelet-derived growth factor-induced motility and proliferation. Mol Cell Biol 2005; 25:4237-4249.
4. Hla T, Brinkmann V. Sphingosine 1-phosphate (S1P) Physiology and the effects of S1P receptor modulation. Neurology 2011; 76:S3-S8.
5. Jung B and Hla T. Sphingosin 1-phosphate (S1P) receptors. In: Chun J, Hla T, Spiegel s, Moolenaar W, editors. Lysophospholipid Receptors: Signaling and Biochemistry. 1st ed. John Wiley & Sons Inc; 2013.p.41-60.
6. Tabasinezhad M, Samadi N, Ghanbari P, Mohseni M, Saei AA, Sharifi S, *et al*. Sphingosin 1-phosphate contributes in tumor progression. J Cancer Res Ther 2013; 9:556-563.
7. Windh RT, Lee MJ, Hla T, An S, Barr AJ, Manning DR. Differential coupling of the sphingosine 1-phosphate receptors Edg-1, Edg-3, and H218/Edg-5 to the Gi, Gq, and G12 families of heterotrimeric G proteins. J Bio Chem 1999; 274:27351-27358.
8. Gangoiti P, Camacho L, Arana L, Ouro A, Granado MH, Brizuela L, *et al*. Control of metabolism and signaling of simple bioactive sphingolipids: Implications in disease. Prog Lipid Res 2010; 49:316-334.
9. Alvarez S.E, Milstien S, Spiegel S. Autocrine and paracrine roles of sphingosine-1-phosphate. Trends Endocrinol Metab 2007; 18:300-307.
10. Maceyka M, Harikumar KB, Milstien S, Spiegel S. Sphingosine-1-phosphate signaling and its role in disease. Trends Cell Biol 2012; 22:50-60.
11. Oskeritzian CA, Milstien S, Spiegel S. Sphingosine-1-phosphate in allergic responses, asthma and anaphylaxis. Pharmacol Ther 2007; 115:390-399.
12. Salas A, Ponnusamy S, Senkal CE, Meyers-Needham M, Selvam SP, Saddoughi SA, *et al*. Sphingosine kinase-1 and sphingosine 1-phosphate receptor 2 mediate Bcr-Abl1 stability and drug resistance by modulation of protein phosphatase 2A. Blood 2011; 117:5941-5952.
13. Ambrosini G, Adida C, Altieri DC. A novel anti-apoptosis gene, survivin, expressed in cancer and lymphoma. Nat Med 1997; 3:917-921.
14. Tamm I, Wang Y, Sausville E, Scudiero DA, Vigna N, Oltersdorf T, *et al*. IAP-family protein survivin inhibits caspase activity and apoptosis induced by Fas (CD95), Bax, caspases, and anticancer drugs. Cancer Res 1998; 58:5315-5320.
15. Li S, Chai L, Cai Z, Jin L, Chen Y, Wu H, *et al*. Expression of Survivin and Caspase 3 in Oral Squamous Cell Carcinoma and Peritumoral Tissue. Asian Pac J Cancer Prev 2012; 13:5027-5031.
16. Zhang1 M, Sun YF, Luo S. Ani-survivin DNAzymes inhibit cell proliferation and migration in Breast Cancer Cell Line MCF-7. Asian Pac J Cancer Prev 2012; 13: 6233-37.
17. Srivastava AK, Singh PK, Srivastava K, Singh D, Dalela D, Rath SK, *et al*. Diagnostic role of survivin in urinary bladder cancer. Asian Pac J Cancer Prev 2013; 14: 81-85.
18. Cho GS, Ahn TS, Jeong D, Kim JJ, Kim CJ, Cho HD, *et al*. Expression of the survivin-2B splice variant related to the progression of colorectal carcinoma. J Korean Surg Soc 2011; 80:404-411.
19. Brinkmann V. Sphingosine 1-phosphate receptors in health and disease: mechanistic insights from gene deletion studies and reverse pharmacology. Pharmacol Ther 2007; 115:84-105.
20. Novgorodov AS, El-Alwani M, Bielawski J, Obeid LM, Gudz TI. Activation of sphingosine-1-phosphate receptor S1P5 inhibits oligodendrocyte progenitor migration. FASEB J 2007; 21:1503-1514.
21. Rosen H, Sanna MG, Cahalan SM, Gonzalez-Cabrera PJ. Tipping the gatekeeper: S1P regulation of endothelial barrier function. Trends Immunol 2007; 28:102-107.
22. Kluk MJ, Hla T. Signaling of sphingosine-1-phosphate via the S1P/EDG-family of G-protein-coupled receptors. Biochim Biophys Acta 2002; 1582:72-80.
23. Takuwa Y. Subtype-specific differential regulation of Rho family G proteins and cell migration by the Edg family sphingosine-1-phosphate receptors. Biochim Biophys Acta 2002; 1582:112-120.
24. Okajima F, Tomura H, Sho K, Nochi H, Tamoto K, Kondo Y. Involvement of pertussis toxin-sensitive GTP-binding proteins in sphingosine 1-phosphate-induced activation of phospholipase Cî—¸ Ca< sup> 2+</sup> system in HL60 leukemia cells. FEBS lett 1996; 379:260-264.
25. Yoshida Y, Nakada M, Sugimoto N, Harada T, Hayashi Y, Kita D, et al. Sphingosine-1-phosphate receptor type 1 regulates glioma cell proliferation and correlates with patient survival. Int J Cancer 2010; 126:2341-2352.
26. Huang YL, Huang WP, Lee H. Roles of sphingosine 1-phosphate on tumorigenesis. World J Biol Chem 2011; 2:25-34.
27. Yamashita H, Kitayama J, Shida D, Yamaguchi H, Mori K, Osada M, *et al*, Sphingosine 1-phosphate receptor expression profile in human gastric cancer cells: differential regulation on the migration and proliferation. J Surg Res 2006; 130:80-87.
28. Kluk MJ, Ryan KP, Wang B, Zhang G, Rodig SJ, Sanchez T. Sphingosine-1-phosphate receptor 1 in classical Hodgkin lymphoma: assessment of expression and role in cell migration. Lab Invest 2013; 93:462-471.
29. Park KS, Kim MK, Lee HY, Kim SD, Lee SY, Kim JM, *et al*. S1P stimulates chemotactic migration and invasion in OVCAR3 ovarian cancer cells. Biochem Biophys Res Commun 2007; 356:239-44.
30. Gandy KAO, Adada M, Canals D, Carroll B, Roddy P, Hannun YA, *et al*. Epidermal growth factor-induced cellular invasion requires sphingosine-1-phosphate/sphingosine-1-phosphate 2 receptor-mediated ezrin activation. FASEB J 2013; 27:3155-3166.
31. Van Brocklyn JR. Regulation of cancer cell migration and invasion by sphingosine-1-phosphate. World J Biol Chem 2010; 1:307.
32. Maceyka M, Payne SG, Milstien S, Spiegel S. Sphingosine kinase, sphingosine-1-phosphate, and apoptosis. Biochim Biophys Acta 2002; 1585:193-201.
33. Cuvillier O, Levade T. Sphingosine 1-phosphate antagonizes apoptosis of human leukemia cells by

inhibiting release of cytochrome c and Smac/DIABLO from mitochondria. Blood 2001; 98:2828-2836.
34. Pyne NJ, Pyne S. Sphingosine 1-phosphate and cancer. Nat Rev Cancer 2010; 10:489-503.
35. McNeish I, Lopes R, Bell S, McKay T, Fernandez M, Lockley M, *et al*. Survivin interacts with Smac/DIABLO in ovarian carcinoma cells but is redundant in Smac-mediated apoptosis. Exp Cell Res 2005; 302:69-82.
36. Ling X, Bernacki RJ, Brattain MG, Li F. Induction of survivin expression by taxol (paclitaxel) is an early event, which is independent of taxol-mediated G2/M arrest. J Biol Chem 2004; 279:15196-15203.
37. Zhao P, Meng Q, Liu L-Z, You Y-P, Liu N, Jiang B-H. Regulation of survivin by PI3K/Akt/p70S6K1 pathway. Biochem Biophys Res Commun 2010; 395:219-224.
38. Shi M, Zhang H, Li M, Xue J, Fu Y, Yan L, *et al*. Normal endometrial stromal cells regulate survival and apoptosis signaling through PI3K/AKt/Survivin pathway in endometrial adenocarcinoma cells< i> in vitro</i>. Gynecol Oncol 2011; 123:387-392.