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ORIGINAL ARTICLE

Effect of S1P5 on proliferation and migration of human esophageal cancer cells

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Abstract

AIM: To investigate the sphingosine 1-phosphate (S1P) receptor expression profile in human esophageal cancer cells and the effects of S1P5 on proliferation and migration of human esophageal cancer cells.

METHODS: S1P receptor expression profile in human esophageal squamous cell carcinoma cell line Eca109 was detected by semi-quantitative reverse transcription polymerase chain reaction. Eca109 cells were stably transfected with S1P5-EGFP or control-EGFP constructs. The relation between the responses of cell proliferation and migration to S1P and S1P5 expression was evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide and migration assay, respectively.

RESULTS: Both normal human esophageal mucosal epithelium and Eca109 cells expressed S1P1, S1P2, S1P3 and S1P5, respectively. Esophageal mucosal epithelium expressed S1P5 at a higher level than Eca109 cell line. S1P5 over-expressing Eca109 cells displayed spindle cell morphology with elongated and extended filopodia-like projections. The proliferation response of S1P5-transfected Eca109 cells was lower than that of control vector-transfected cells with or without S1P stimulation ($P < 0.05$ or 0.01). S1P significantly inhibited the migration of S1P5-transfected Eca109 cells ($P < 0.001$). However, without S1P in transwell lower chamber, the number of migrated S1P5-transfected Eca109 cells was greater than that of control vector-transfected Eca109 cells ($P < 0.001$).

CONCLUSION: S1P binding to S1P5 inhibits the proliferation and migration of S1P5-transfected Eca109 cells. Esophageal cancer cells may down-regulate the expression of S1P5 to escape the inhibitory effect.

Key words: Sphingosine 1-phosphate; Esophageal cancer; Sphingosine 1-phosphate 5; Proliferation; Migration

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INTRODUCTION

Esophageal cancer is one of the most common malignancies worldwide, occurring mostly in developing countries with marked regional variations in incidence. Its mortality is very similar to its incidence due to relatively late diagnosis and inefficient treatment. About 300 000 people would die of esophageal cancer each year in the world[1]. The incidence and mortality of esophageal cancer are unusually high in China, especially in Henan, Shanxi, Hebei and Sichuan Provinces[2]. Even though a small number of esophageal cancer patients can survive more than 5 years after initial surgical treatment, over 60% of patients still die of metastasis and local recurrence[3,4]. The ability to reverse the outcome of esophageal cancer is limited due to a poor understanding of its biology.

Sphingosine 1-phosphate (S1P) is a pleiotropic phospholipid mediator that mediates diverse cellular responses, such as cell proliferation, differentiation, migration, adhesion, and morphogenesis. S1P with a low nanomolar affinity binds to its 5-related G-protein coupled receptors (GPCR), and is designated as S1P1-5 (originally termed EDG-1, 3, 5, 6, and 8, respectively). S1P1, S1P2, and S1P3 receptors are ubiquitously expressed, whereas S1P4 is only expressed in lymphoid tissues and platelets, and S1P5 is predominantly expressed in the central nervous system[5-11].

Previous reports have highlighted the importance of S1P and its receptors for the growth, metastasis and infiltration of tumors, such as gastric cancer[5], thyroid cancer[12], ovarian cancer[13-16], and glioma[17]. In general, S1P stimulates the migration of S1P3 dominantly expressing cell lines and inhibits the migration of S1P2 expressing cell lines. S1P1, S1P2 and S1P3 contribute positively to S1P-stimulated glioma cell proliferation, and S1P5 blocks glioma cell proliferation. S1P1 and S1P3 enhance glioma cell migration and invasion, while S1P2 inhibits cell migration but unexpectedly enhances glioma cell invasiveness by stimulating cell adhesion[17].

The possible role and importance of S1P and its receptors in the growth and metastasis of human esophageal cancer have not been investigated. In this study, we focused on esophageal squamous cell carcinoma (ESCC), characterized S1P receptor expression patterns and investigated the role of S1P receptors in proliferation and migration of ESCC cells. ESCC cell line Eca109 expressed 4 S1P receptors (S1P1, S1P2, S1P3 and S1P5). Compared with normal esophageal mucosa epithelium, Eca109 cells expressed a very low level of S1P5. Furthermore, by overexpressing human S1P5 in Eca109 cells, the effects of S1P5 on esophageal cancer cells were partly clarified.

MATERIALS AND METHODS

Materials

S1P was obtained from Cayman Chemical (Ann Arbor, MI, USA). Dispase Ⅱ, fibronectin and fatty acid-free bovine serum albumin (BSA) were purchased from Sigma (St Louis, MO, USA). SV total RNA isolation system and pGEM-T easy vector system were bought from Promega (Madison, WI, USA). PrimeScript™ reverse transcription polymerase chain reaction (RT-PCR) kit and PrimerSTAR HS DNA polymerase with GC buffer were purchased from TakaRa Biotech (Dalian, China). Lipofectamine 2000 and G418 were obtained from Invitrogen (Carlsbad, CA, USA).

Preparation of normal human esophageal mucosal epithelium

Normal human esophageal mucosa samples were obtained from surgically resected esophagus of patients with esophageal carcinoma, and then confirmed by an experienced pathologist to contain neither macroscopic tumor nor histologically detectable metaplastic cells or cancer cells. Samples were collected with the signed informed consent from patients. Tunica mucosa was separated from the tissues with forceps, and cut into 1 cm × 1 cm sections which were then transferred to a 100 mm culture dish containing 10 mL PBS with 0.25% dispase Ⅱ and incubated for 18-24 h at 4℃. Epithelial layers were separated from the full-thickness mucosa with forceps.

Cell culture

Human ESCC cell line Eca109 was obtained from Shanghai Institute of Cell Biology, Chinese Academy of Sciences. Eca109 cells were cultured in RPMI 1640 medium supplemented with 10% (v/v) fetal bovine serum (FBS) and 100 U/mL of penicillin and streptomycin at 37℃ in a humidified incubator containing 5% CO_2.

Preparation of total RNA and semi-quantitative reverse transcription polymerase chain reaction

Total RNA was isolated with the SV total RNA isolation system following its manufacturer's instructions. RNA quality and integrity were checked by absorbance spectrometry and agarose gel electrophoresis. Semi-quantitative RT-PCR was performed to confirm differential expression of S1P receptors in Eca109 cell line. The sequences of primers used are as follows: 5'-TATCAGC GCGGACAAGGAGAACAG-3' (sense) and 5'-ATAG GCAGGCCACCCAGGATGAG-3' (antisense) for S1P1 receptor (429-bp product), 5'-TCGGCCTTCATCGTC ATCCTCT-3' (sense) and 5'-CCTCCCGGGCAAACCA CTG-3' (antisense) for S1P2 receptor (220-bp product), 5'-CTGCCTGCACAATCTCCCTGACTG-3' (sense) and 5'-GGCCCGCCGCATCTCCT-3' (antisense) for S1P3 receptor (394-bp product), 5'-GAGAGCGGGGCCACC AAGAC-3' (sense) and 5'-GGTTGACCGCCGAGTTGA GGAC-3' (antisense) for S1P4 receptor (454-bp product), 5'-GGAGTAGTTCCCGAAGGACC-3' (sense) and 5'-T CTAGAATCCACGGGGTCTG-3' (antisense) for S1P5 receptor (236-bp product), 5'-GATGACATCAAGAAGG TGGTGAA-3' (sense) and 5'-GTCTTACTCCTTGGAG GCCATGT-3' (antisense) for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (246-bp product). Thirty-two cycles of amplification were performed using a thermal

cycler, Mastercycle® gradient (Eppendorf, Hamburg, Germany) at 94℃ for 30 s, at 60℃ for 30 s, and at 72℃ for 1 min. The PCR products were electrophoresed on a 1.6% agarose gel and visualized with ethidium bromide staining.

Generation of stably-transfected Eca109 cell line

Human S1P5 open reading frame was cloned from normal esophageal mucosal epithelium cDNA *via* PCR using the PrimerSTAR HS DNA polymerase with GC buffer. The sequences of primers used are 5'-CATTG AAGCTTCCACC*ATG*GAGTCGGGGCTGCTG-3' (sense) and 5'-CATTGTCTAGAGTCTGCAGCCG GTTCTGATA-3' (antisense). The sense primer was designed to add a *Hin*dⅢ site upstream of a consensus Kozak sequence and the S1P5 initiating Met (italics). The antisense primer was designed to remove the S1P5 stop codon and to add a *Xba* I site. PCR conditions, established to amplify the S1P5 receptor sequence, were 94℃ for 1 min, followed by 35 cycles at 94℃ for 30 s, at 60℃ for 30 s, at 72℃ for 2 min, and a final extension at 72℃ for 8 min. The PCR products were electrophoresed on 0.8% agarose gel and the corresponding band was cut and purified. The purified PCR products were ligated into the pGEM-T easy vector and the sequence was confirmed by sequence analysis. The *S1P5* gene was spliced out with *Hin*dⅢ/*Xba* I and ligated into the Myc-His-EGFP-N1 frame, which was purified from similarly digested S1P1-Myc-His-EGFP-N1 as previously described[18]. The initiating methionine of the GFP open reading frame in pEGFP-N1 was mutated to alanine. The resulting plasmid was designated as S1P5-EGFP. A *Hin*dⅢ/*Xba* I -digested DNA oligonucleotide containing both sense (5'-CATTGAAGCTTCCACCATGGAATTCTGCAGT CGACGGTACTCTAGACCG-3') and antisense (5'-CG GTCTAGAGTACCGTCGACTGCAGAATTCCATG GTGGAAGCTTCAATG-3') sequences was cloned into similarly digested Myc-His-EGFP-N1 frame as a control vector. The DNA oligonucleotide contained initiating Met and multiple cloning sites. The plasmid was designated as control-EGFP.

Cells were transfected using Lipofectamine 2000, according to its manufacturer's instructions. Briefly, 5 × 10^5 Eca109 cells in 2 mL of growth medium without antibiotics were seeded into each well of 6-well plates and grown overnight, then transfected using Lipofectamine 2000. The cells were split at a 1:20 dilution into a fresh growth medium 24 h after transfection. G418 was added at a concentration of 0.8 mg/mL the following day. Resistant colonies were picked to a 96-well plate 14 d after selection and several clones were analyzed. The cells were subsequently maintained in a medium containing G418 at 0.2 mg/mL.

Proliferation assay

Cells (5 × 10^3/well in 100 μL/well) were seeded into a 96-well plate and cultured in RPMI 1640 medium containing 10% FBS for 8 h at 37℃ in an atmosphere containing 5% CO_2 to allow attachment. The medium was then aspirated and a fresh medium containing 0.1% fatty acid-free BSA and varying concentrations of S1P was added into three replicate wells every 48 h. After incubation at 37℃ in an atmosphere containing 5% CO_2 for 72 h, the number of living cells was calculated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. Briefly, a MTT solution was added into each well and the cells were incubated for an additional 4 h. The number of living cells was then counted by measuring the absorbance at 490 nm.

Migration assay

Migration assay was performed on a 6.5 mm-diameter transwell chamber with an 8 μm pore size (Corning, NY, USA). The underside of transwell membranes was precoated with fibronectin (10 μg/mL) and allowed to dry. Cells at 70%-80% confluence were serum starved overnight, detached from culture plates by 0.02% EDTA, washed and resuspended in RPMI 1640 medium with 0.1% fatty acid-free BSA at 1 × 10^6 cells/mL. For migration assay, cells (1 × 10^5 cells/well) were added into the upper chamber of transwell chambers separated by inserts with 8 μm pores in the presence or absence of S1P. Following an 8-h incubation, nonmigrating cells on the upper surface of membranes were removed with cotton swabs, fixed and stained with 0.1% crystal violet in phosphate-buffered saline (PBS) at the ambient temperature, and then destained with PBS. Migrating cells were counted in 5 randomly selected high-power fields per membrane under an Olympus IX71 inverted microscope.

Statistical analysis

Data are expressed as mean ± SD and shown as error bars in the figures. Statistical comparisons were made by Student's *t*-test. One-way ANOVA with a *post hoc* Bonferroni testing was employed in case of multiple comparisons. $P < 0.05$ was considered statistically significant.

RESULTS

Expression of S1P receptors in normal human esophageal mucosal epithelium and Eca109 cell line

Semi-quantitative RT-PCR of mRNA was performed to observe which S1P receptors are expressed in Eca109 cell line and normal human esophageal mucosal epithelium. For comparison, data were also normalized to the expression of the reference gene GAPDH, which gave similar results. Four S1P receptors were found in normal human esophageal mucosal epithelium with the following rank order of mRNA abundance: S1P1>S1P5>S1P3>S1P2. In contrast to Eca109 cell line, the mRNA abundance in S1P receptors was S1P3>S1P1>S1P5>S1P2. S1P4 expression was absent or minimal in Eca109 cell line and normal human esophageal mucosal epithelium (Figure 1). Eca109 cell line expressed S1P3 at a higher level than normal esophageal mucosal epithelium. In contrast, normal

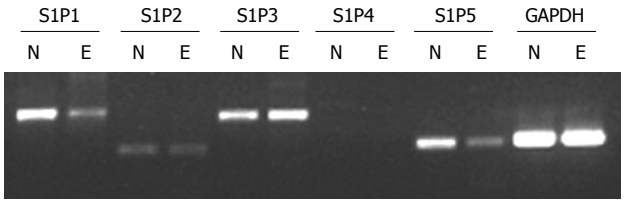

Figure 1 Expression of sphingosine 1-phosphate (S1P) receptors in normal human esophageal mucosal epithelium and Eca109 cell line. N: Normal human esophageal mucosal epithelium; E: Eca109 cell line.

esophageal mucosal epithelium expressed S1P1 and S1P5 at a higher level than Eca109 cell line. On the basis of these results, S1P5 was chosen for further study.

Generation of S1P5 overexpressing Eca109 cell line

Eca109 cells transfected with the S1P5-EGFP or Control-EGFP constructs were cultured in a medium containing 10% FBS. Their localization was visualized by fluorescence microscopy. As shown in Figure 2, control-EGFP was localized at the cytosol of Eca109 cells. In contrast, the S1P5-EGFP was localized at the plasma membrane. Interestingly, control-EGFP-transfected cells displayed the characteristic Eca109 cell morphology. In contrast, S1P5-EGFP-transfected cells displayed spindle cell morphology with elongated and extended filopodia-like projections in a medium containing 10% FBS or 0.1% fatty acid-free BSA.

Effect of S1P on proliferation of control-EGFP or S1P5-EGFP expressing Eca109 cells

Since S1P regulates the proliferation of many cell types, including cancer cells, it is of great interest to investigate whether S1P5 or S1P binding to S1P5 has an effect on the proliferation of control vector and S1P5 transfected Eca109 cells. To assess induction of proliferation, stably transfected cells in 96-well plates containing 10% FBS or 0.1% fatty acid-free BSA were counted after treatment with a vehicle (DMSO) and 10 or 100 nmol/L S1P for 72 h. The number of cells from both control vector and S1P5 transfectants did not increase in response to S1P ($P > 0.05$), suggesting that S1P does not behave as a mitogen at S1P5 (Figure 3).

Interestingly, the proliferation response of S1P5-transfected Eca109 cells was lower than that of control vector-transfected cells in a medium containing 10% FBS (8% lower, $P < 0.05$) or 0.1% fatty acid-free BSA with or without S1P (20%-29% lower, $P < 0.05$ or 0.01), suggesting that S1P5 has an intrinsic activity and inhibits cell proliferation.

Effect of S1P on migration of control-EGFP or S1P5-EGFP expressing Eca109 cells

Migration of Eca109 cells was quantified by transwell-based assay. Transwell membranes were precoated with fibronectin. At a concentration of 10 or 100 nmol/L S1P, the migration of S1P5-EGFP-transfected Eca109 cells was significantly lower than that of control-EGFP-transfected Eca109 cells (48% *vs* 84% at 10 nmol/L S1P, 39% *vs* 94% at 100 nmol/L S1P, $P < 0.001$) (Figure 4A and B). Interestingly, without S1P in the lower chamber, the migrated number of S1P5-EGFP transfected Eca109 cells (145 ± 10) was more than that of control-EGFP-transfected Eca109 cells (51 ± 4, $P < 0.001$) (Figure 4A and C). Both cells failed to migrate on gelatin-coated or non-coated transwell membranes (data not shown). Control-EGFP and S1P5-EGFP transfected Eca109 cells exhibited spontaneous migration without S1P, and the addition of S1P to the lower chamber potently inhibited cell migration.

DISCUSSION

In the present study, normal esophageal mucosal epithelium expressed S1P5 at a high level, and Eca109 cells expressed S1P5 at a low level. S1P5 was predominantly expressed in the white matter tracts and oligodendrocytes, and was particularly abundant in the anterior commissure, corpus callosum, and optic tract. S1P5 mRNA was detected in spleen, peripheral blood leukocytes, placenta, lung, aorta, and fetal spleen. A low level of signals has been detected in many tissues[19,20]. In this study, normal human esophageal mucosal epithelium expressed S1P5 at a high level, suggesting that S1P5 is also a ubiquitous S1P receptor and plays an important role in different tissues and cells.

S1P is believed to be generated intracellularly by all cell types during sphingolipid degradation. Plasma S1P is mainly hematopoietic in origin with erythrocytes as a major contributor to the sources of extracellular S1P, whereas lymph S1P is from a distinct radiation-resistant source[21,22]. The concentration of S1P in plasma and serum is 0.1-0.6 μmol/L and 0.4-1.1 μmol/L, respectively, while the S1P level in tissues is 0.5-75 pmol/mg[23]. Although S1P was originally thought to act as an intracellular second messenger, it has been widely accepted as an intercellular agonist. S1P binding to its receptors activates different intracellular signaling pathways depending on which intracellular Gα protein they couple to. The S1P1 receptor primarily couples through $G\alpha_{i/o}$, whereas S1P2 and S1P3 can couple through $G\alpha_{i/o}$, $G\alpha_{q}$ and $G\alpha_{12/13}$, S1P4 and S1P5 can couple through $G\alpha_{i/o}$ and $G\alpha_{12/13}$[6,7,24-26]. Signaling through $G\alpha_{i/o}$ has been associated with the activation of small guanosine triphosphatase (GTPase) Ras and extracellular signal-regulated kinase (ERK) to promote proliferation, and induction of Pi3K and small GTPase Rac to promote migration. Signaling through $G\alpha_{12/13}$ can promote the activation of small GTPase Rho and Rho-associated kinase (ROCK) to inhibit migration[6]. Furthermore, S1P5 is also a constitutively active receptor with the ability to intrinsically stimulate various effector pathways *albeit* at a different extent in the absence of stimulating ligand[27].

In the present study, normal esophageal mucosal epithelium expressed S1P5 at a higher level than Eca109 cell line. Therefore, Eca109 cells were transfected with the expression constructs of S1P5 or control vector in order to delineate the effects of S1P5 on the growth, migration

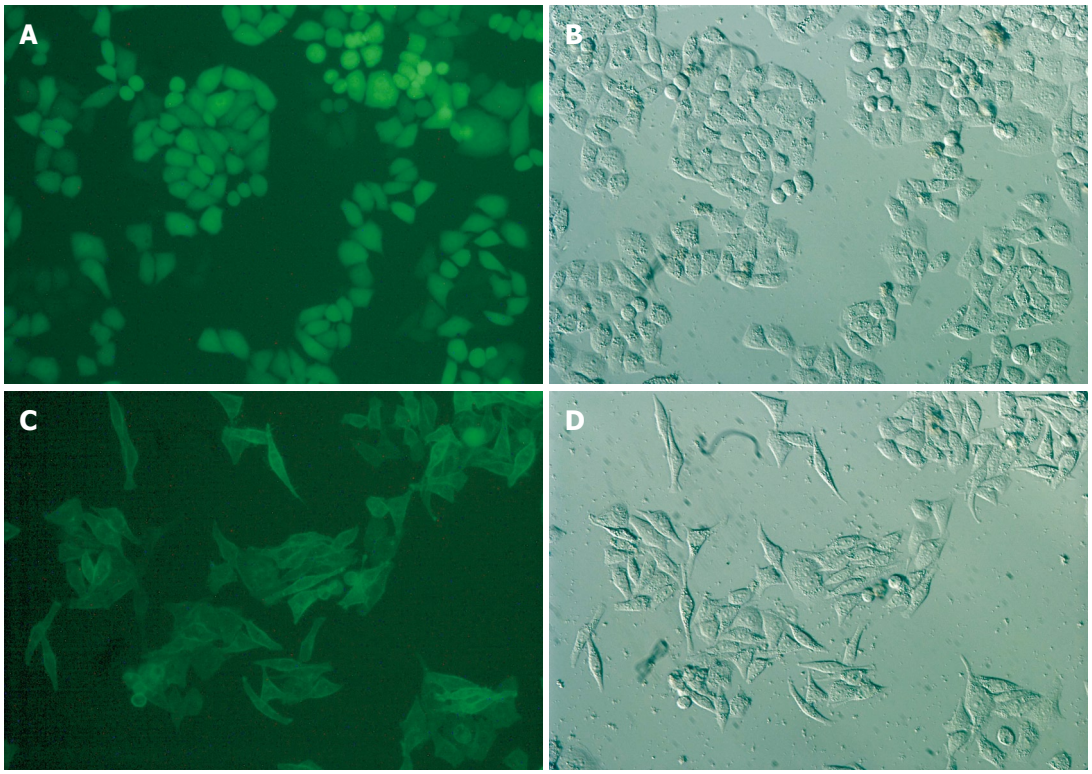

Figure 2 S1P5 receptor overexpression in Eca109 cells causes cell spindle change with elongated and extended filopodia-like projections in a medium containing 10% FBS or 0.1% fatty acid-free BSA (× 200). A, B: Eca109/control-EGFP; C, D: Eca109/S1P5-EGFP.

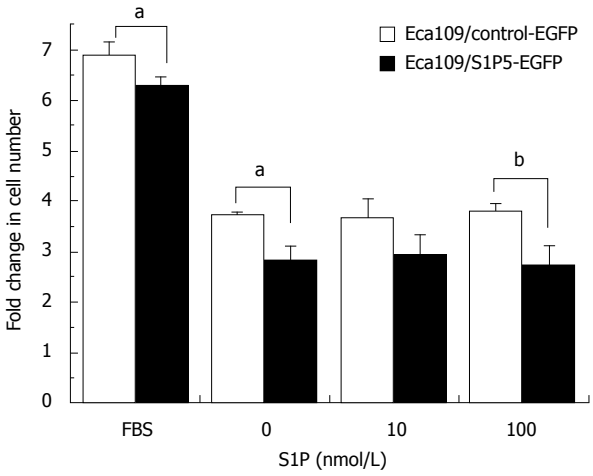

Figure 3 MTT assay showing proliferation of control-EGFP or S1P5-EGFP-transfected Eca109 cells. $^{a}P < 0.05$, $^{b}P < 0.01$ *vs* control group.

and morphogenesis of esophageal cancer cells. Eca109 cells that overexpress S1P5 were analyzed by phase contrast microscopy. Increased expression of S1P5 induced cell spindle change with elongated and extended filopodia-like projections in a medium containing 10% FBS or 0.1% fatty acid-free BSA. This morphological change is characterized by motile cells. However, transfection of several mammalian cell lines (CHO-K1, HEK293, NIH3T3, RH7777) with the S1P5 receptor induced cell rounding change, which is more pronounced in the presence of S1P-containing serum[27]. Differences in morphological alteration may correlate well with cell type or malignant degree, because CHO-K1, HEK293, NIH3T3 and RH7777 are the normally-derived mammalian cell lines.

In this study, the expression of S1P5 receptor in Eca109 cells reduced the proliferation of Eca109 cells whether or not the medium contained S1P, indicating that the S1P5 receptor is constitutively active in the absence of stimulating ligand. Antiproliferative effect is an intrinsic ability of the S1P5 receptor that is insensitive to ligand stimulation. Analogously, S1P5-transfected CHO cells exhibit an intrinsic activity and inhibit serum-stimulated cell proliferation[28]. S1P5 can negatively and constitutively regulate cellular proliferation by reducing ERK1/2 activity *via* protein-tyrosine phosphatase. $G\alpha_{12/13}$ is a likely candidate G protein for the inhibitory effects of S1P5 on ERK activity. It has been shown that mutation- activated $G\alpha_{12/13}$, but not $G\alpha_{q}$, $G\alpha_{s}$, or $G\alpha_{i2}$, can inhibit EGF stimulation of ERK activity in COS-7 cells in a Ras- and Raf-independent manner[29]. Down-regulation of S1P5 in esophageal cancer cells may weaken its antiproliferative effect.

In this study, overexpression of S1P5 receptor constitutively increased the migration of esophageal cancer cells to fibronectin in the absence of S1P, which was consistent with morphological alteration. However, addition of 10 and 100 nmol/L S1P significantly inhibited the constitutive migration of S1P5-transfected Eca109 cells. It has been shown that small GTPases of the Rho family, including Rac, Cdc42, and RhoA, are well-known regulators of cell migration[5,30]. Cell migration induced by S1P receptors (S1P1 and S1P3) involves $G\alpha_{i/o}$-mediated activation of phosphatidylinositol 3-kinase (Pi3K) and

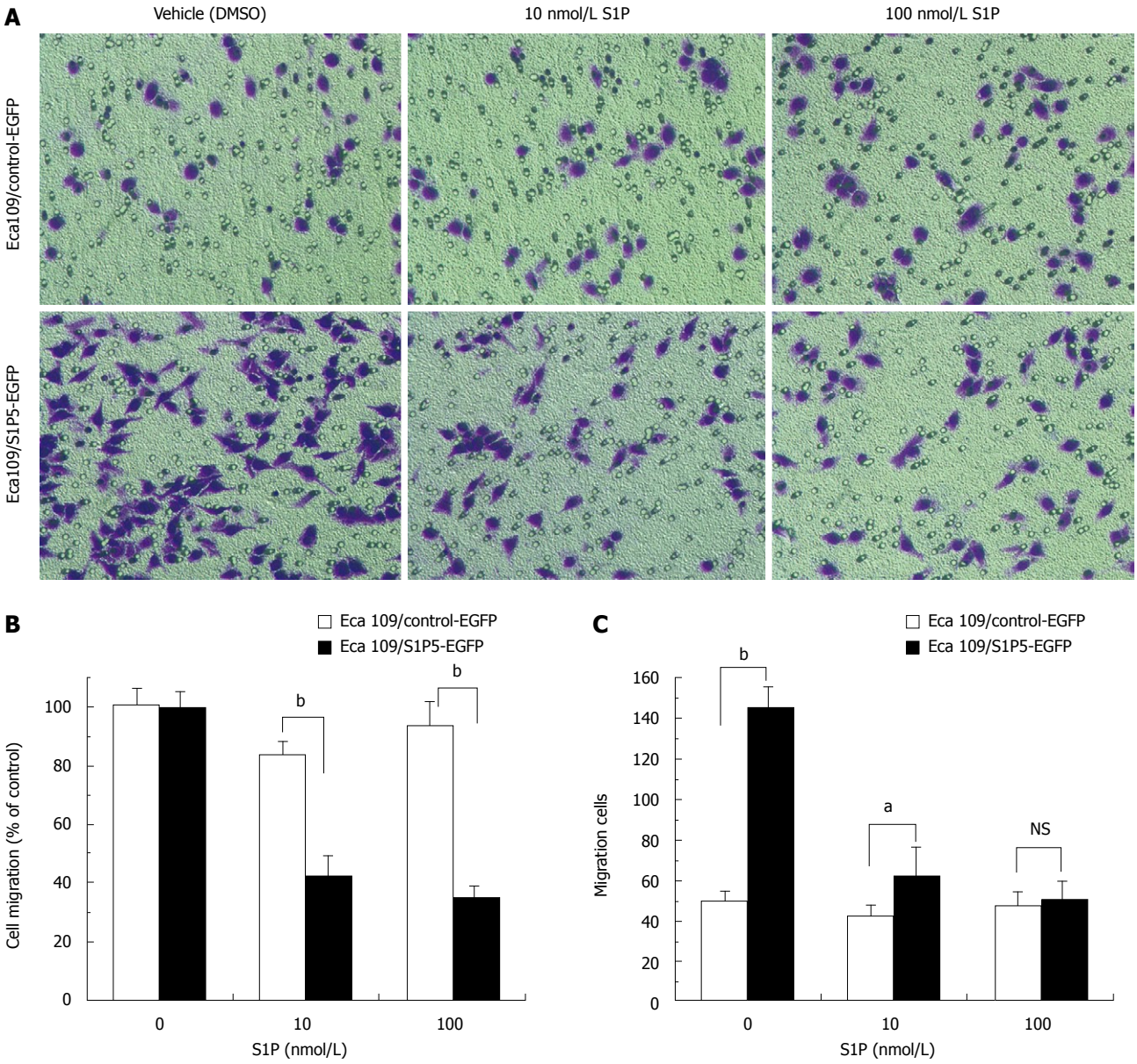

Figure 4 Effect of S1P on migration of S1P5-EGFP-transfected Eca109 cells and control-EGFP Eca109 cells. A: Eca109 cells are allowed to migrate for 8 h, and stained with crystal violet (× 200); B: Results are expressed as percentage in cell migration of S1P-treated cells compared to vehicle (DMSO)-treated cells; C: Results are expressed as migration cell number. $^{a}P < 0.05$, $^{b}P < 0.001$ indicate a statistically significant effect between S1P5-EGFP and control-EGFP transfected Eca109 cells treated by indicated concentrations of S1P or vehicle. NS: Not significant.

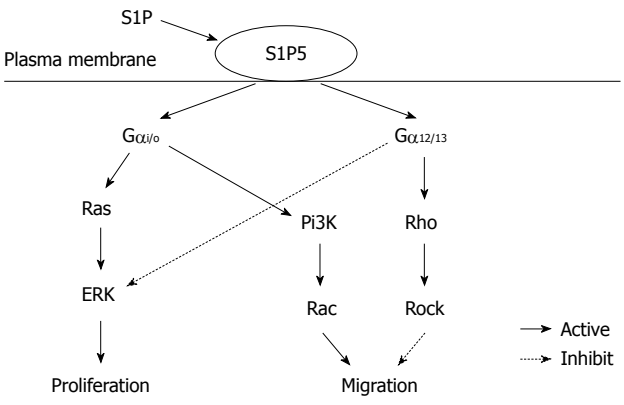

Figure 5 Putative signaling pathway of S1P5.

Rac. S1P receptors (S1P2 and S1P5) mediate inhibition of cell migration by $G\alpha_{12/13}$-evoked stimulation of Rho[12]. It was reported that fibronectin binding to $\alpha_v\beta_3$ integrin can decrease Rho activity and stimulate cell motility and spreading, suggesting that enhanced Rho activity may reduce integrin-driven cell migration[7,31]. Our data support that S1P5 may constitutively induce cell migration due to $G\alpha_{i/o}$-activated Rac and inhibit cell migration due to $G\alpha_{12/13}$-stimulated Rho. In a microenvironment containing S1P interstitial fluid, esophageal cancer cells may escape the inhibitory effect of S1P-S1P5 on their migration by down-regulating the expression of S1P5. The putative signaling pathway of S1P5 is shown in Figure 5.

In summary, S1P5 is a constitutively active receptor

with the ability to intrinsically inhibit cell proliferation and induce cell migration. Cell migration can be inhibited by stimulating extracellular S1P. In normal esophageal mucosa cells, a high expression level of S1P5 may inhibit cell proliferation and migration while esophageal cancer cells may down-regulate the expression of S1P5 to promote cell proliferation and escape the inhibitory effect of S1P-S1P5 on cell migration. Our study is the first to show that the deficiency in inhibitory effect of S1P-S1P5 is of importance in the growth and metastasis of esophageal cancer cells. Further study is needed on the role of S1P and S1P5 receptor in esophageal cancer.

COMMENTS

Background

Esophageal cancer is one of the most common malignancies worldwide. Its mortality is very high due to relatively late diagnosis and inefficient treatment. The ability to reverse the outcome of esophageal cancer is limited due to a poor understanding of its biology. Progression of esophageal cancer may be associated with sphingosine 1-phosphate (S1P) and its receptors S1P1-5, which play an important role in other cancers.

Research frontiers

S1P is a pleiotropic phospholipid mediator. S1P binding to its 5 receptors S1P1-5 mediates diverse cellular responses such as cell proliferation, differentiation, migration, adhesion, and morphogenesis. Recent studies have shown that S1P and its receptors are related with cancer growth and metastasis. In this study, esophageal squamous cell carcinoma (ESCC), S1P receptor expression pattern, and the role of S1P receptors in proliferation and migration of ESCC cells were studied.

Innovations and breakthroughs

Recent reports have highlighted the importance of S1P and its receptors for the growth, metastasis and infiltration of tumors, such as gastric cancer, thyroid cancer, ovarian cancer, and glioma. This study showed that deficiency in inhibitory effect of S1P-S1P5 may be of importance in the growth and metastasis of esophageal cancer.

Applications

The results of this study indicate that deficiency in inhibitory effect of S1P-S1P5 may be of importance in the growth and metastasis of esophageal cancer. S1P5 or its associated signaling molecules may serve as a future strategy in biotherapy for esophageal cancer.

Terminology

S1P is a bioactive lipid that regulates central cellular processes such as cell proliferation, differentiation, migration, adhesion, and morphogenesis. S1P is present in plasma and serum at high nanomolar concentrations. S1P binding to related G-protein coupled receptors is designated as S1P1-5. S1P1, S1P2, and S1P3 receptors which are ubiquitously expressed, whereas S1P4 is restricted to lymphoid tissues and platelets and S1P5 is predominantly expressed in the central nervous system. Binding of S1P to its receptors activates different signaling pathways *via* the heterotrimeric G proteins.

Peer review

The paper is well written and presents an interesting aspect of the pathophysiology of esophageal mucosa which is related to the evolution of esophageal cancer.

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