Sphingosine-1-phosphate modulates growth and adhesion of ovarian cancer cells

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Abstract Sphingosine-1-phosphate (S1P) is a bioactive lipid molecule. It stimulates the growth of some cells, but inhibits the growth of others. In this study, we describe the detection of sub-μM to μM concentrations of S1P in the ascitic fluids of patients with ovarian cancer. In ovarian cancer cells cultured in vitro, S1P exhibited a dual effect on growth and/or survival. S1P (10 μM) induced cell death when cells were in suspension but stimulated cell growth when cells were attached. The calcium-dependent induction of cell death by S1P is apparently associated with its inhibitory effect on cell attachment and cell adhesion. S1P (10–30 μM) also induced calcium-dependent cell-cell aggregation.

Key words: Sphingosine-1-phosphate (S1P); Lysophosphatidic acid (LPA); Ovarian cancer; Cell adhesion; Aggregation

1. Introduction

Ovarian malignancies have the worst prognosis of any gynecological malignancy, due to the difficulty in early detection and the lack of effective treatment for the metastasized disease. We have shown that ascitic fluids from patients with ovarian cancer contain biologically active lipid molecules, such as lysophosphatidic acid (LPA), which stimulates the growth of tumor cells [1,2]. We also have shown that LPA levels are elevated in blood from patients with ovarian cancer and may represent a useful marker for the early detection of ovarian cancer [3]. Another bioactive lipid molecule, sphingosine-1-phosphate (S1P), has been shown to stimulate cell proliferation of fibroblasts and other cell lineages but inhibits growth of breast cancer cells [4,5]. Low concentrations of S1P (10–100 nM) inhibit tumor invasion of melanoma cells through their receptors [6] and 5 μM of S1P inhibits cell invasion of MDA-MB-231 breast cancer cells [4]. S1P (5–20 μM) induces cell-cell aggregation in S1P-receptor (Edg1)-transfected HEK293 cells through up-regulation of cadherin molecules [7]. We report here that S1P is present in the ascites of patients with ovarian cancer and it exhibits a dual effect on the growth of ovarian cancer cells in vitro: it inhibited growth and induced cell death if cells were in suspension when S1P was added but stimulated growth of attached cells. S1P inhibited cell attachment to the surface of tissue culture dishes or adhesion to extracellular matrix (ECM) proteins: laminin, collagens I and IV and fibronectin. S1P also induced cell-cell aggregation in suspended cells. Our data suggest that S1P is an important growth and adhesion modulator for ovarian carcinoma.

2. Materials and methods

2.1. Materials

RPMI 1640 was purchased from Fisher Scientific (Springfield, NJ, USA). The medium supplement (insulin-transferrin-selenium) was purchased from GIBCO BRL (Grand Island, NY, USA). Human plasma fibronectin (HFN) and human laminin were from Chemicon International (Temecula, CA, USA). Human collagens type I and IV were from Becton Dickinson Labware (Bedford, MA, USA). Fatty acid free BSA, soybean trypsin inhibitor and MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) were from Sigma (St. Louis, MO, USA). Antibodies against catenins and integrin β_1 were from Transduction Laboratories (Lexington, KY, USA). BCA protein assay kit was from Pierce (Rockford, IL, USA). [^{3}H]acetic anhydride (50 mCi/mmol) was from NEN Life Sciences (Boston, Ma).

2.2. S1P quantitation

Quantitation of S1P from ascitic fluids was performed as described by Yatomi et al. [8,9]. Briefly, S1P was extracted from 0.5 ml of ascites followed by *N*-acylation with [^{3}H]acetic anhydride. The resulting [^{3}H]C_2-Cer-1-P (*N*-[^{3}H]-acetylated S1P) was applied to silica gel 60 TLC plates (EM Science, Gibbstown, NJ, USA) and the plates were developed in butanol/acetic acid/water (3:1:1). Autoradiography was performed with Kodak BioMax MS film using a Kodak BioMax Transcreen-LE Intensifying Screen (Eastman Kodak, Rochester, NY, USA). Radioactive spots corresponding to [^{3}H]C_2-Cer-1-P were scraped and radioactivity was measured via liquid scintillation counting. The S1P in the ascites samples was quantified by extrapolation from S1P standards that were subjected to the same procedures.

2.3. Cell lines and cell culture

Ovarian cancer cell lines, HEY and OCC1, were kind gifts from Dr. Gordon Mills' laboratory (M.D. Anderson). Cells were cultured in RPMI 1640 with 10% FBS. Starvation was conducted for 24–48 h in serum-free RPMI 1640. To detach cells, either trypsin-EDTA (GIBCO, BRL, Grand Island, NY, USA) or 2 mM EDTA in PBS was used. Cells were then washed twice with 0.2% soybean trypsin inhibitor (if trypsin was used) or RPMI 1640, and resuspended in either RPMI 1640 or Medium A [Hams F12:DMEM (1:1 v/v), supplemented with 0.1% fatty acid free BSA, insulin, transferrin and selenium as recommended by the manufacturer GIBCO BRL (Grand Island, NY, USA)].

2.4. Cell growth assays

MTT dye reduction was used to measure proliferation as described previously [10] with some modifications. Briefly, different concentrations of reagents (LPA or S1P) in 100 μl of Medium A were first added to each well of a 96-well plate. Starved ovarian cancer cells

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Abbreviations: ECM, extracellular matrix; HFN, human plasma fibronectin; LPA, lysophosphatidic acid; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; S1P, sphingosine-1-phosphate; SPC, sphingosylphosphoylcholine

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were suspended and added to each well (5×10^3 cells/100 μl). Following a 72-h incubation at 37°C, 10 μl of MTT solution (5 mg MTT/ml in H_2O) was added to each well and incubated at 37°C for 6 h. After removal of the supernatant, the dye was dissolved in 100 μl of ethanol/DMSO (1:1, v/v). The absorbance was measured at 595 nm, with 655 nm as the reference wavelength. The growth effect of LPA and S1P on attached cells was examined by counting the number of living cells in the presence of trypan blue. Briefly, HEY cells were cultured in 24-well plates in RPMI 1640 with 10% FBS and starved in RPMI without FBS for 48 h. While cells were still attached, the medium was changed to Medium A. LPA or S1P was added to the final concentrations as indicated. Cells were counted after 72 h incubation at 37°C with 5% CO_2.

2.5. Cell attachment assays

Starved ovarian cancer cells (HEY or OCC1) were resuspended in RPMI-1640 without serum and 2 ml of cells (1.3×10^5 cells/ml) was added to each well of a 6-well plate. Different reagents were added and the cells were incubated at 37°C. Phase-contrast images were taken 6 h later. The attached cells and the floating cells were counted separately and the percentage of attached cells was calculated by dividing the number of attached cells by the total cell number (both attached and floating living cells).

2.6. Adhesion assays

The adhesion assays were performed as described by Dunn et al. [10] with modifications. Briefly, 96-well plates were coated at 4°C overnight with laminin (in PBS), collagen I or IV (in acetic acid) or HFN (in PBS) each at a concentration of 10 μg/ml. Plates were rinsed and blocked with heat-denatured fatty acid free BSA (0.02%). S1P or LPA (100 μl) at various concentrations was added to each well and the starved cells [100 μl HEY or OCC1 resuspended in RPMI 1640 without serum (2×10^5 cells/ml)] were added to each well and incubated for 2 h at 37°C. Phase contrast photos were taken and non-adherent cells were removed by washing with PBS. Cells were fixed and stained with 0.5% crystal violet in 20% MeOH for 10 min at room temperature. The crystal violet stain was washed with PBS and dissolved in 0.1% SDS and the optical absorbance was measured at 595 nm using a microplate reader (SpeedMax 340, Molecular Devices Corporation, Sunnyvale, CA, USA).

3. Results

3.1. S1P was present in ascites from patients with ovarian cancer

We have previously detected LPA in ascites from patients with ovarian cancer and showed that LPA is growth-stimulating for ovarian tumor cells [1–3]. S1P is a similar bioactive lipid molecule, stimulating growth of different cell types [4]. To determine whether S1P plays a patho-/physiological role in ovarian cancer development, we measured S1P levels in ascites from seven patients with ovarian cancer, using the method described by Yatomi et al. [8,9]. Ascitic fluids from all seven patients contained S1P. The levels of S1P in the ascitic fluids ranged from 0.42–2.17 μM (Mean ± S.D. = 1.073 ± 0.25).

3.2. S1P displayed a dual effect on the growth/survival of ovarian cancer cells

The presence of S1P in ascites promoted us to determine the potential role of S1P in ovarian cancer cells. Interestingly, S1P displayed a dual effect on the growth of ovarian cancer cells. When cells were detached with trypsin-EDTA, mixed with S1P or LPA and replated in tissue culture wells, LPA stimulated, but S1P inhibited cell growth as analyzed using a MTT dye incorporation method. A narrow concentration range of S1P (0.5–2 μM) consistently showed a very weak stimulation (Fig. 1A). However, if cells were allowed to attach before addition of S1P or LPA, both lipids stimulated cell growth as analyzed by cell counting, even though S1P was a weaker

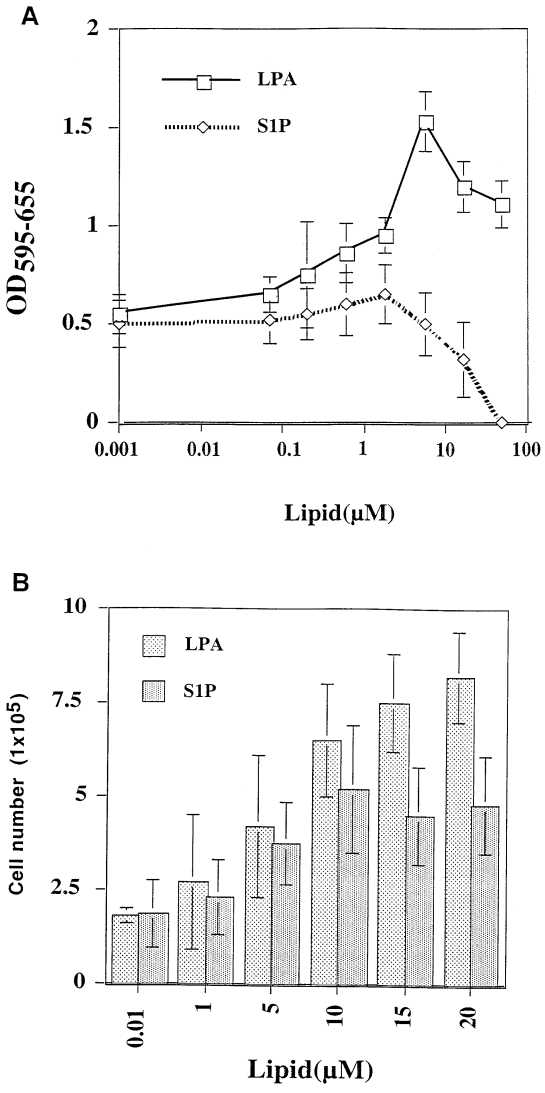

Fig. 1. Effects of LPA and S1P on cell growth and/or survival of ovarian cancer cells (HEY). A: LPA or S1P was titrated from 0.07 μM to 50 μM in 96-well plates as triplets. MTT assays were performed as described in Section 2. B: LPA or S1P was added to the attached cells (HEY) in Medium A to final concentrations as indicated. Cells were counted after 72 h. Data are mean values ± S.D. of three separate experiments.

mitogen than LPA (Fig. 1B). Since S1P (up to 50 μM) was not cytotoxic to attached ovarian cancer cells as assessed by trypan blue exclusion, the inhibitory effect of S1P on suspended cells shown in Fig. 1A may result from the inhibitory effect of S1P on cell attachment. Similar results were observed in another ovarian cancer cell line, OCC1.

3.3. S1P inhibited cell attachment and induced cell death

The dual effects described above suggest that S1P may inhibit cell attachment. We examined the effect of S1P and related lipids on cell attachment to the surface of tissue culture plates. Control cells in RPMI 1640 attached to the surface of tissue culture dishes in a time-dependent manner (1 h, 5%; 3 h, 25%; 6 h, 45% and 20 h, 100%). We found that S1P (20 μM) strongly inhibited cell attachment, concurrent with the induction of cell-cell aggregation (Fig. 2c). The inhibitory effect of S1P was dose-dependent. While 1 μM of S1P was not

effective, 5–20 μM of S1P inhibited 20–100% of cell attachment.

Other related lipid molecules at the same concentration (20 μM) displayed different effects. LPA had some stimulatory effect (Fig. 2B) compared to control cells (Fig. 2A), sphingosine did not have any detectable effect on cell attachment (Fig. 2E) and sphingosylphosphorylcholine (SPC) inhibited cell attachment, but did not induce cell-cell aggregation (Fig. 2D). The stimulatory effect of LPA was dose- and time-dependent. For 5, 10 and 20 μM of LPA, respectively, the percentage of attached cells was: 1 h: 3, 5, 5%; 3 h: 20, 28, 35%; 6 h: 56, 78, 100%. Detached cells with either trypsin-EDTA or EDTA alone showed similar results in all cell attachment and adhesion (see below) experiments.

3.4. S1P inhibited cell adhesion

To examine the effect of S1P in a more physiological manner, we determined the effect of LPA or S1P on cell adhesion to different ECM proteins. As shown in Fig. 3A and B, LPA (20 μM) did not significantly affect cell adhesion to laminin, collagens I and IV or HFN (P values range from 0.29 to 0.65). In contrast, S1P (20 μM) significantly inhibited cellular adhesion to laminin ($P = 0.0092$) and HFN ($P = 0.0033$). The inhibitory effects of S1P on collagen I and collagen IV were less significant ($P = 0.0660$ and 0.0169, respectively) (Fig. 3A and B). These effects were dose-dependent. Two types of dose-dependent curves were observed (Fig. 3C). For collagens I and IV and laminin, a dual effect of S1P was observed. Curve a in Fig. 3C represents a typical dose-dependent effect of S1P on these ECM proteins. S1P from as low as 10 nM to 5 μM was stimulatory on cell adhesion and 10–50 μM of S1P was inhibitory. For HFN, a stronger and monophasic inhibition was observed (Fig. 3C, curve b).

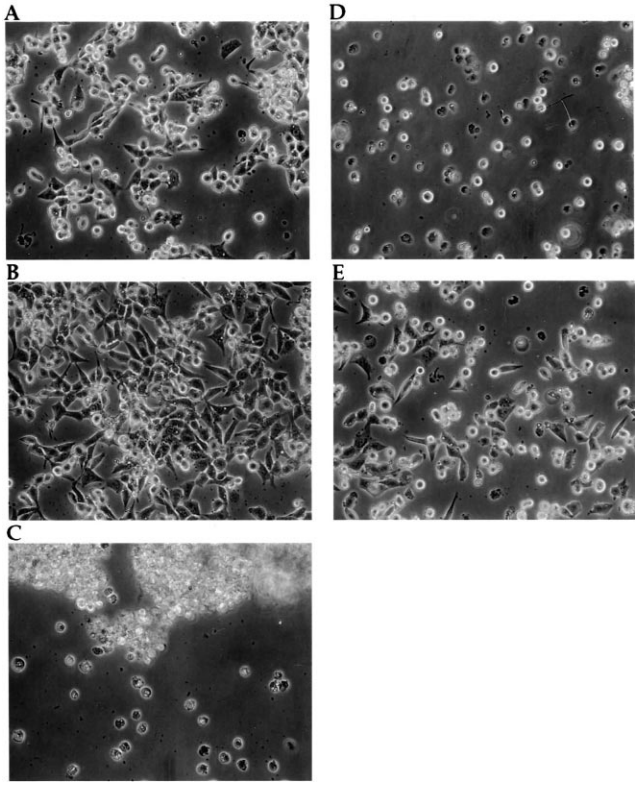

Fig. 2. Effects of different lipids on cell attachment to the plastic surface of a tissue culture plate (6-well plate). One milliliter of starved and resuspended HEY cells (2×10^5 cells/ml) were mixed with PBS (Control, A), LPA (B), S1P (C), SPC (D) or sphingosine (E) at a final concentration of 20 μM, respectively. Cells were incubated in 6-well plates in duplicates and pictures were taken 6 h after plating.

3.5. The cell-death induced by S1P

Apparently, S1P-induced death of resuspended cells (shown in Figs. 1A and 2C) was associated with its inhibitory effect on cell attachment. We tested whether prevention of attachment was the main reason for cell death induced by S1P. We cultured HEY cells in bacterial plates and most of the cells were not attached, but were viable. When these cells were replated in tissue culture dishes after 24 h in suspension, they had the ability to attach and proliferate. In addition, we cultured HEY cells in Medium A in the presence of 3 mM EGTA, which prevented cells from attaching even after 28 h incubation. At this time point, the control, 20 μM LPA-treated and 20 μM S1P-treated cells had a similar number of viable cells, suggesting that S1P-induced cell death is calcium-dependent. However, when these floating cells were replated in RMPI 1640 with 10% FBS after washing with PBS, and allowed to grow for another 72 h, cells treated with 20 μM S1P showed 20–30% growth inhibition.

The effect of the incubation time with S1P on cell survival (in the absence of EGTA) was tested. HEY cells were treated with 20 μM of S1P and incubated in 6-well plates. At each time point (0.5, 2.5, 5, 8 and 24 h), cells from three different wells were washed twice with RPMI 1640 and replated in the medium without S1P. The number of cells surviving at 48 h after initial plating were counted. The survival rates (compared to controls) were: 0.5 h: 40.5%; 2.5 h: 31.4%; 5 h: 10.2%; 8 h: 7.1% and 24 h: 0.86%. The S1P treated cells tended to be round, small and condensed, which are characteristics of apoptotic cells [11].

3.6. S1P induced cell-cell aggregation

Concurrently with the inhibition of cell attachment, S1P (10 μM) induced cell-cell aggregation reproducibly (Fig. 2C). The aggregation occurred as early as 2 h after initial incubation and maximized at approximately 6 h. Addition of 3 mM of EGTA completely prevented the induction of aggregation by S1P (data not shown), suggesting that S1P-induced aggregation is calcium-dependent. Cells in aggregates induced by S1P were not able to attach even after prolonged incubation and actually died within a few hours incubation with S1P as assessed by trypan blue exclusion.

3.7. The mechanisms of S1P-induced aggregation, inhibition of adhesion/attachment and cell death

HEY and OCC1 cells express comparable endogenous amounts of Edg1 as that of HEK293 (unpublished results). Overexpression of Edg1 in HEK293 induces cell-cell aggregation, which is related to upregulation of P- and E-cadherins [7]. We observed that S1P (10–20 μM) induced cell-cell aggregation in suspended HEY or OCC1 cells without Edg1 overexpression. Moreover, the expressions of E-, P- and K- cadherins were not observed in HEY cells (data not shown), although the involvement of other forms of cadherins, such as VE-cadherin or cadherin-5 remains to be tested.

On the other hand, α-, β- and γ-catenins, paxillin and β1

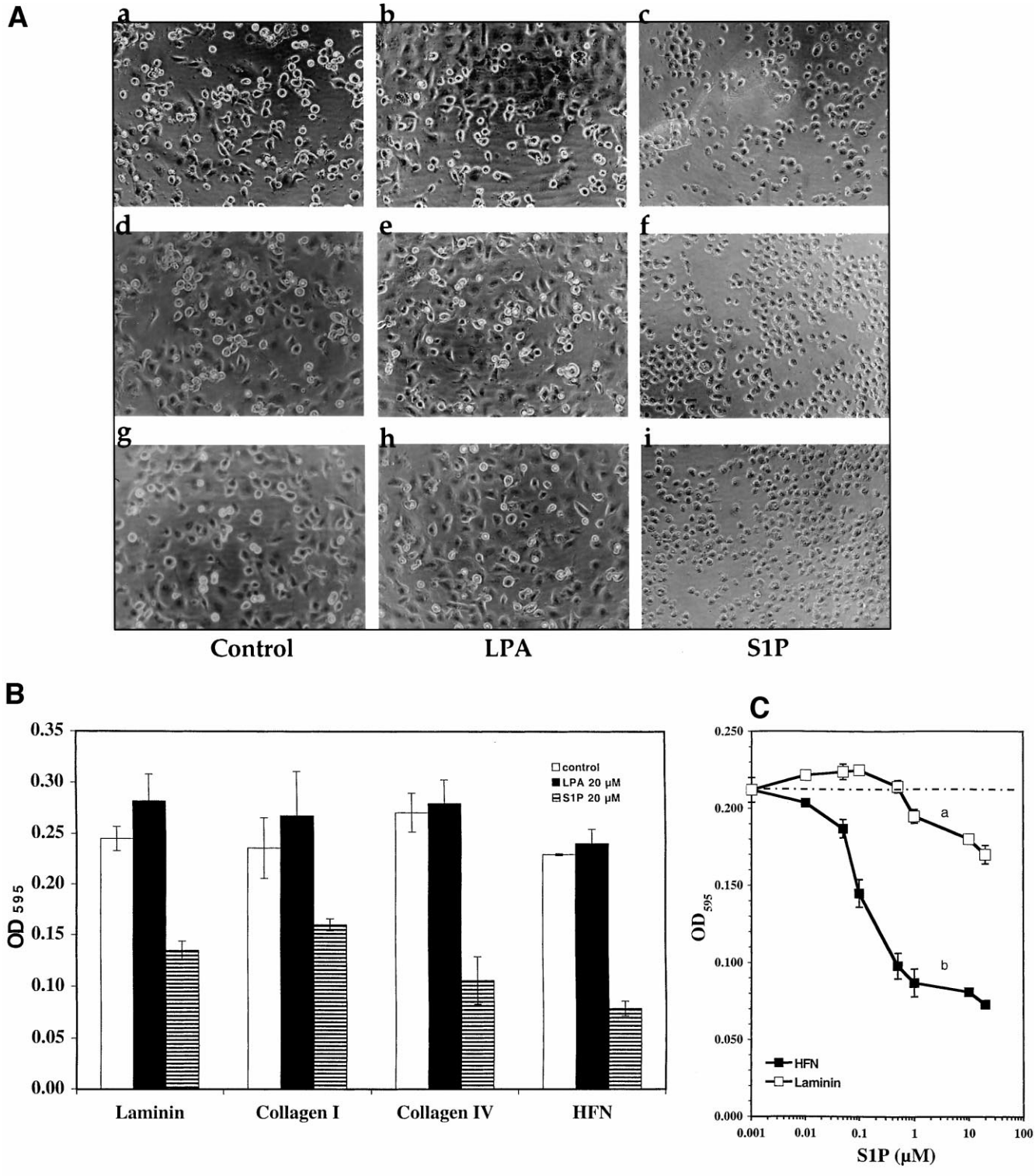

Fig. 3. Effects of LPA or S1P on cell adhesion to ECM proteins. A 96-well plate was coated with 10 μg/ml of laminin, collagen I or IV, or HFN and blocked with denatured fatty acid free BSA (0.02%). Starved HEY cells treated with 20 μM of LPA or S1P (2×10^4 cells/100 μl) were plated in each well. A: Phase contrast photos were taken 3 h later. B: Adherent cells were quantified by crystal violet staining (Section 2). C: Dose-dependent effect of S1P on cell adhesion. Curve a: The dual effect of S1P on cells (HEY) adhesion to laminin. Curve b: The inhibitory effect of S1P on cell adhesion to HFN. The same experimental procedure described in A and B, but using different concentrations of S1P, was performed. Data were analyzed by Student's *t* test using the GraphPad Instat software (San Diego, CA, USA). $P<0.05$ was considered to be significant.

integrin were expressed in HEY cells. While the expression of α- and β-catenins was unaffected by LPA or S1P (15 μM for 6 h), paxillin was weakly upregulated (1.2-fold) by both LPA and S1P and γ-catenin was downregulated (approximately 20%) by S1P (Fig. 4). In addition, the protein expression of integrin β1 was downregulated (approximately 25%) by 15 μM S1P (3.5 h treatment; data not shown). These data suggest the potential role of γ-catenin and integrin β1 in S1P-induced aggregation and/or inhibition of cell attachment or adhesion.

4. Discussion

The dual effects of S1P have been described in several different contexts. Unlike LPA, which has been shown to act mainly extracellularly as a first messenger, S1P acts both as an intracellular second messenger and extracellular first messenger through its receptors [5,6]. S1P has been shown to display opposite growth effects on different cell lines [4]. It inhibits invasion but not adhesion of melanoma cells at low concentrations (10–100 nM) using Transwell assays [11,12]. S1P at 5 μM also inhibits invasion of breast cancer cells in Boyden chambers lined with Matrigel (rich in laminin and collagen IV) [4], but stimulates invasion of T-lymphoma cells (1–15 μM of S1P) using a rat embryo fibroblast monolayer cell invasion assay [13]. Here, we describe for the first time that in the same cells, S1P exhibited dual effects on cell proliferation and/or cell survival, dependent on whether or not cells are attached. We showed that the inhibitory effect of S1P on cell growth or survival was apparently associated with its inhibitory effect on cell attachment or cell adhesion. This association is reflected by both the dose correlations between growth inhibition and adhesion (Fig. 1A and 3C, curve a)

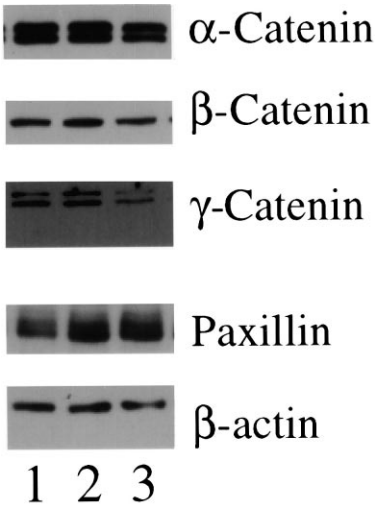

Fig. 4. The effect of LPA or S1P on protein expression of catenins and paxillin. HEY cells were suspended with trypsin-EDTA, washed and seeded in 10-cm tissue culture plates. LPA or S1P was added to a final concentration of 15 μM and incubated for 6 h. Cells were lysed and the protein concentrations were measured using the Pierce BCA protein assay kit. Equal amounts (30 μg) of proteins were loaded on a 8% SDS-PAGE gel. Antibodies against catenins or paxillin were from Transduction Laboratories (Lexington, KY, USA) and anti-β-actin antibody was from Sigma. The ECL detection kit (Amersham) was used for visualization. Lane 1: Control; lane 2: LPA (15 μM); lane 3: S1P (15 μM).

and structural specificity. We have shown that SPC is growth inhibitory for these ovarian cancer cells [2] and also inhibitory for cell attachment (Fig. 2). In contrast, LPA stimulated both growth and attachment. We noted that cells in aggregates died quickly, but aggregation may not be necessary for cell death, since SPC-induced cell death was not associated with aggregation. In addition, the degree of aggregation varied from experiment to experiment, but the inhibition was consistent.

Our results further indicate that the prevention of cell attachment may not be sufficient to induce cell death and additional mechanisms may be involved. We showed that HEY cells in suspension when cultured in bacterial plates or in the presence of EGTA can survive for at least 24 h and cells could reattach and proliferate after removal of EGTA. In contrast, incubation with 20 μM of S1P for as short as 0.5 h resulted in the death of the majority of cells (∼60% cell death). The mechanism of S1P-induced cell death is not clear at present. Our results, however, have provided a few clues, (1) the same concentration of S1P was growth-stimulatory for attached, but inhibitory for suspended cells. These results suggest that the attachment-stimulated cell signalling may be a prerequisite for the stimulatory effect of S1P; (2) a calcium-dependent signalling pathway is required for the S1P-induced cell death in suspended cells, since S1P did not induce cell death until removal of EGTA. Upon removal of both EGTA and S1P, a slower growth rate was observed in cells pretreated with S1P, suggesting that S1P may be internalized during the pre-incubation time.

S1P (5–20 μM) has been shown to induce apoptosis in human hepatoma cells [14] but inhibit apoptosis or anoikis (apoptosis when adherent cells are detached or adherence is inhibited) in human HL60, Jurkat and U937 cells [15–17]. Whether S1P induces apoptosis in ovarian cancer cells remains to be determined.

Sadahira et al. reported that S1P inhibits invasion without affecting cell adhesion in melanoma cells [12]. However, we found that S1P at low concentrations (< 1 μM) had either a stimulatory effect (on laminin, collagens I and IV) or an inhibitory effect (on HFN) in ovarian cancer cells. At higher concentrations (10–20 μM), S1P strongly inhibited cell attachment or cell adhesion to laminin, collagens I and IV and HFN. At these concentrations, S1P was not cytotoxic in attached HEY or OCC1 cells as assessed by trypan blue exclusion. S1P has also been shown to be non-cytotoxic at high concentrations (up to 100 μM) in fibroblasts [4].

Ovarian cancer cells express various adhesion molecules, such as integrins β_1 and β_3, and CD44 [18,19]. Integrins $\alpha\beta_1$ and $\alpha\beta_3$ bind a wide variety of ECM proteins, of which collagens I and IV, HFN and laminin are known to be produced by ovarian tumor cells [18,19]. The different effects of LPA or S1P on adhesion of ovarian cancer cells described here suggest that LPA and S1P may regulate integrin molecules differently.

It is unclear at this point whether effects described here are receptor-mediated. Due to the lack of specific antagonists for S1P receptors at present, this question has not been addressed directly. Nevertheless, we tested the requirement for the presence of S1P in the media to exert its effect. It has been reported that a short incubation time (0.5 h) with S1P, followed by washing and replacement of media, resulted in 50% of maximum DNA synthesis induced by 24 h of treatment [4]. This observation, together with other evidence, has been used to argue that S1P-induced proliferation depends on S1P up-

take and is not receptor-mediated [4]. We have conducted a similar experiment to determine whether the continuous presence of S1P is necessary for its effect. We showed that S1P strongly inhibits cellular proliferation (~60%) after a short incubation time (0.5 h) with cells, supporting the idea that cells may internalize S1P. On the other hand, the further inhibitory effect of S1P on cell survival was dependent on the presence of S1P in the medium. Increased percentages of the cells died when the incubation time with S1P increased. Even at 8 h, the removal of S1P resulted in a 9-fold increase in cell survival compared to 24 h. These data suggest at least two possibilities. First, S1P may play a dual effect: both extracellular and intracellular. S1P was not completely internalized and the presence of S1P in the media is necessary to exert further inhibition. Therefore, part of the effect of S1P may be mediated through its receptor(s). Secondly, S1P induced a secondary factor(s), which was present in the cell media resulting in the further inhibitory effect. Currently, we are unable to distinguish between these two possibilities. We found that both HEY and OCC1 express S1P receptors Edg1 and Edg3 (authors' unpublished data). Some receptor-mediated effect requires μM concentrations of the agonist. For example, S1P (5–20 μM) induces cell-cell aggregation in S1P-receptor (Edg1)-transfected, but not the mock-transfected HEK-293 cells, suggesting the effect is receptor-mediated [7].

Preliminary mechanistic studies suggest that ovarian cancer cells may represent a different model system from that previously described for HEK293 cells. S1P (1–20 μM) has been shown to induce morphological changes in S1P receptor (Edg1) overexpressed HEK293 cells, but not in the parental cells [7]. Overexpression of the S1P receptor (Edg1) in HEK293 cells upregulates P- and E-cadherins, but not cytoplasmic cadherin-associated proteins, such as α-, β- and γ-catenins [7]. In addition, the expression of focal adhesion kinase (FAK) and paxillin in these cells was unaltered by overexpression of Edg1. In contrast, S1P induced similar aggregation effects without overexpression of exogenous Edg1 in HEY ovarian cancer cells. In addition, E-, P-, and N-cadherins were not detected, consistent with the notion that cadherins are down-regulated in metastatic tumor cells [18,20]. Moreover, γ-catenin, paxillin and integrin β1 were regulated by S1P, although the functional role of these regulations in S1P-induced cellular effects remains to be examined. Cell-cell aggregation induced by S1P may involve a different mechanism in HEK293 cells compared to ovarian cancer cells.

Ovarian tumor cells inherently possess strong invasiveness to the peritoneum through direct dissemination, rather than through lymphatic or hematogenous routes [21,22]. This metastatic potency relies on the ability of tumor cells to detach from the primary site and to attach and subsequently implant and proliferate in the mesothelium of the peritoneal cavity. The effect of S1P on growth, attachment, adhesion and aggregation, which are critically important processes in tumor cell invasion, suggest that S1P may play a role in ovarian tumor development and/or metastasis. Importantly, we found that S1P was present in ascitic fluids from patients with ovarian cancer in sub-μM to μM concentrations, suggesting a potential pathological role in ovarian cancer. This is in contrast to the reports that S1P is absent in ascites [9]. However, the sources of these ascitic fluids were not specified [9]. Dependent on the concentrations of S1P in ascites and the attachment status of tumor cells, S1P may play a dual or more complex role in ovarian tumor development and metastasis.

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