

Inhibition of Lysophosphatidic Acid Receptor-2 Expression by RNA Interference Decreases Lysophosphatidic Acid-induced Urokinase Plasminogen Activator Activation, Cell Invasion, and Migration in Ovarian Cancer SKOV-3 Cells

Gui-li Wang¹, Ze-qing Wen², Wan-peng Xu³, Zhi-yu Wang⁴, Xue-lian Du¹, Fei Wang¹

¹Department of Obstetrics and Gynecology, Shandong Provincial Hospital, Shandong University, Jinan, People's Republic of China

²Department of Obstetrics and Gynecology, Shandong Provincial Hospital, Shandong University, Jinan, People's Republic of China

³Department of Surgery, Affiliated Hospital of Weifang Medical University, Weifang, People's Republic of China

⁴Ministry of Health, Key Laboratory for Biotech-Drugs, Shandong Medical Biotechnological Center, Jinan, People's Republic of China

Aim To explore the role of lysophosphatidic acid receptor-2 (LPA₂) in regulating lysophosphatidic acid (LPA)-induced urokinase plasminogen activator (uPA) activation, cell invasion, and migration in human ovarian cancer cell line SKOV-3.

Methods SKOV-3 cells were stimulated with LPA. Cell supernatant uPA level and activity were measured using enzyme-linked immunosorbent assay. LPA₂ mRNA expression was inhibited with LPA₂-specific small interfering RNA (siRNA) and examined using semiquantitative reverse transcriptase-polymerase chain reaction. LPA-induced cell invasion and migration in transfected cells were evaluated by a Matrigel invasion chamber and a Transwell chemotaxis chamber, respectively.

Results LPA stimulation significantly enhanced in vitro uPA activity in time- and dose-dependent manner. The levels of LPA-induced uPA protein decreased by 55% in LPA₂ siRNA-transfected cells compared with negatively transfected cells at 24 hours after being treated with 80 μmol/L LPA (0.75 ± 0.03 vs 0.34 ± 0.04, *P* = 0.004). In the LPA₂ specific siRNA-transfected SKOV-3 cells, LPA treatment at 80 μmol/L induced considerably less invasion and migration compared with negative control siRNA-transfected SKOV-3 cells (invasion: 178 ± 17.2 vs 36.2 ± 3.3, *P* = 0.009; migration: 220.4 ± 25.5 vs 57 ± 7.6, *P* = 0.009).

Conclusion LPA₂ has an essential role in LPA-induced uPA activation and tumor cell invasion in ovarian cancer SKOV-3 cells.

> **Correspondence to:**

Wen Ze-qing
Department of Obstetrics and Gynecology
Shandong Provincial Hospital
Shandong University
324# Jingwu Road, Jinan 250021
People's Republic of China
wenzeqing@yahoo.com.cn

> **Received:** January 14, 2008

> **Accepted:** April 1, 2008

> **Croat Med J. 2008;49:175-81**

> doi: 10.3325/cmj.2008.2.175

Lysophosphatidic acid (LPA) is a naturally occurring lysophospholipid, which mediates diverse biological responses such as mitogenesis, differentiation, cell survival, angiogenesis, inflammation, and cell migration (1). Extracellular LPA has been shown to be involved in certain diseases, such as atherosclerosis (2) and cancer (3-5). In fact, LPA has been identified as a growth-promoting factor that supports the proliferation of ovarian cancer cells in malignant ascites from ovarian cancer patients (6,7). Indeed, LPA is present at high levels in the ascites of patients with advanced-stage ovarian cancer, in concentrations of 5-200 $\mu\text{mol/L}$ (3,8).

Most of the biological responses to LPA are mediated through the lipid-specific endothelial differentiation gene (EDG) family G protein-coupled receptors, ie, $\text{LPA}_1/\text{EDG-2}$, $\text{LPA}_2/\text{EDG-4}$, and $\text{LPA}_3/\text{EDG-7}$ (9-11), although recent studies have suggested that LPA responses are potentially mediated through $\text{LPA}_4/\text{GPR23}$ (12) and peroxisome proliferator-activated receptor γ (13). These LPA receptors differ with respect to their distribution in various tissues. LPA_1 is the most widely expressed receptor subtype in normal and tumor tissues, whereas LPA_2 and LPA_3 are frequently overexpressed in human tumor tissues, such as ovarian cancer, gastric cancer, and ductal cancer, which may account for the various biological effects of LPA (9,10,14). Previous studies found that malignant transformation results in aberrant expression of LPA_2 in ovarian and thyroid cancers, suggesting that LPA may play a role in tumor biology and that shifts in LPA receptor expression are related to carcinogenesis (4,7).

Proteolysis of extracellular matrix (ECM) proteins is necessary for the invasion and metastasis of cancer cells. Urokinase plasminogen activator (uPA), a serine protease, can promote degradation of ECM and has been shown to correlate inversely with prognosis in ovarian cancer patients. In vitro studies have

shown that uPA expression is induced by LPA in ovarian cancer cell lines but not in normal ovarian epithelial cell (15,16).

Previous studies showed that overexpression of LPA_2 induced the expressions of active uPA and vascular endothelial growth factor in the ovaries of transgenic mice (17). In breast carcinoma cells, LPA_2 was also shown by RNA interference approach to mediate LPA-stimulated cell migration (18). However, the effect of LPA_2 on these processes in ovarian cancer, especially when LPA_2 is inhibited, has not been investigated. In the present study, we inhibited the endogenous expression of LPA_2 mRNA using specific small interfering RNA (siRNA), to investigate more directly the potential role of LPA_2 in LPA-induced uPA activity, cell invasion, and migration in ovarian cancer SKOV-3 cells.

Material and methods

Reagents

1-oleoyl-LPA was purchased from Avanti Polar Lipids Inc (Alabaster, AL, USA). The kit for quantification of uPA activity was obtained from American Diagnostica (Greenwich, CT, USA) and used according to the manufacturer's guidelines.

Cell culture and LPA stimulation

The established ovarian cancer cell line SKOV-3 was obtained from American Type Culture Collection (Manassas, VA, USA). The cells were maintained under standard conditions (37°C and 5% CO_2) in a plastic cell culture flasks and grown in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum, 1% penicillin, and 1% streptomycin (all from Sigma, St. Louis, MO, USA) until their confluence level reached about 80%. They were then washed twice with prewarmed phosphate-buffered saline and cultured in serum-free medium (Invitrogen, Carlsbad, CA,

USA) overnight. The appropriate amount of LPA was diluted in 1% delipidated bovine serum albumin (Sigma) on ice. After starvation, the serum-free medium was replaced, LPA was added to the culture at a concentration of 80 $\mu\text{mol/L}$, and incubation was carried out at 37°C for various time periods up to 24 hours. In a different experiment, various concentrations of LPA (0-80 $\mu\text{mol/L}$) were added to the culture and incubation was carried out at 37°C for 24 hours. Cell culture supernatant was collected and analyzed using uPA enzyme-linked immunosorbent assay (ELISA).

uPA ELISA

Extracts were diluted 1:5 in assay buffer and 100 μL aliquots of each extract were incubated overnight at 4°C in precoated microtest wells. Wells were washed thoroughly with wash buffer and a second, biotinylated antibody that recognizes a specific epitope on uPA molecule was added for each analysis. Wells were washed again after an incubation of 1 hour and 100 μL of enzyme conjugate was added (streptavidin-conjugated horseradish peroxidase), leading to the formation of the antibody-enzyme detection complex. After 1-hour incubation, wells were washed again. Then, 100 μL of perborate 3, 3', 5, 5'-tetramethylbenzidine substrate was added to each well and reacted with horseradish peroxidase, producing a blue solution. We used 50 μL of 0.5 mol/L sulfuric acid as a stopping solution, which yielded a yellow color in the reaction.

siRNA transfection

The human LPA₂ SMARTpool siRNA and control non-targeting siRNA were obtained from Dharmacon (Lafayette, CO, USA). SKOV-3 cells were seeded in 24-well plates at a density of 1.25×10^5 cells/well in a complete medium without antibiotics the day before transfection. The cells were transfected overnight with control and LPA₂-specific siRNA (20 pmol) with LipofectAMINE™

2000 (1 μL) (Invitrogen) resuspended in Opti-MEM (500 μL) (Invitrogen). After transfection, the cells were incubated with fresh complete medium for 36 hours for recovery, parts of the transfected cells were then used for the isolation of total cellular RNA followed by semiquantitative reverse transcriptase-polymerase chain reaction (RT-PCR) analysis of LPA₂ mRNA level. The remaining cells were starved in serum-free medium for 16 hours before stimulation with LPA. The uPA ELISA, Matrigel invasion assay, and cell migration assay were performed.

Semiquantitative RT-PCR analysis

The LPA₂ and β -actin mRNA levels were evaluated by semiquantitative RT-PCR. Briefly, total cellular RNA was extracted by a single-step method using TRIzol (Invitrogen). For RT-PCR, 10 μg of total RNA was used for a 838-bp fragment of LPA₂ complementary DNA (cDNA) synthesis according to the manufacturer's protocol, using 5'-CAATCTGCTGGTCATAGC-3' (forward) and 5'-AGAGGATGTATAGTGGACAGAC-3' (backward) primer. The primers for β -actin (internal control) were also included as follows: 5'-CCTTCAACACCCCAGCCAT-3' (forward) and 5'-ATGCCAGGGTACATGTGGT-3' (backward) that amplified a 550-bp fragment of β -actin cDNA. The PCR cycling parameters were as follows: 5 minutes at 94°C, followed by 30 cycles of 30 seconds at 94°C, 45 seconds at 60°C, and 45 seconds at 72°C, and a final cycle at 72°C for 7 minutes. The PCR products were separated by agarose gel (1.5%) electrophoresis and stained with ethidium bromide. The fluorescence intensity (optical density) was automatically measured and integrated by gel data acquisition AlphaImager HP software (Alpha Innotech Corp., San Leandro, CA, USA). A ratio of LPA₂ and β -actin represented the relative level of LPA₂ expression.

In vitro migration and invasion assay

Cell migration was assayed using a Transwell chemotaxis chamber (8- μm pore filters). The transfected SKOV-3 cells (5×10^4 /well) were starved for 16 hours. Next, these cells were trypsinized and treated with vehicle or 80 $\mu\text{mol/L}$ LPA and loaded into the top chamber. Fetal bovine serum was then placed into the bottom chamber as a chemoattractant. Cells were incubated at 37°C in a humidified atmosphere and allowed to migrate through the chemotaxis chamber for 24 hours. After incubation, the cells remaining at the upper surface were completely removed using a cotton carrier. The migrated cells on the bottom of chemotaxis chamber were stained with hematoxylin and eosin. The experiments were repeated in triplicate wells and the migrated cells were counted microscopically (200 \times) in five different fields per filter. Cell invasion capability was assessed by using a Matrigel invasion chamber (Becton-Dickenson, Bedford, MA, USA) according to the manufacturer's instructions. The procedures were essentially the same as those for the migration assay using Transwell chemotaxis chambers.

Statistical analysis

Results are reported as mean \pm standard deviation. Statistical analyses were performed using rank-sum test. $P < 0.05$ was considered statistically significant. The Statistical Package for the Social Sciences for Windows, version 13.0 (SPSS, Inc., Chicago, IL, USA) was used to perform the analysis.

Results**LPA induces uPA activation in ovarian cancer SKOV-3 cells**

The treatment of SKOV-3 cells with 80 $\mu\text{mol/L}$ LPA for 0-24 hours showed that LPA significantly increased the levels of uPA activity in a time-dependent manner (Figure 1A). Also, the

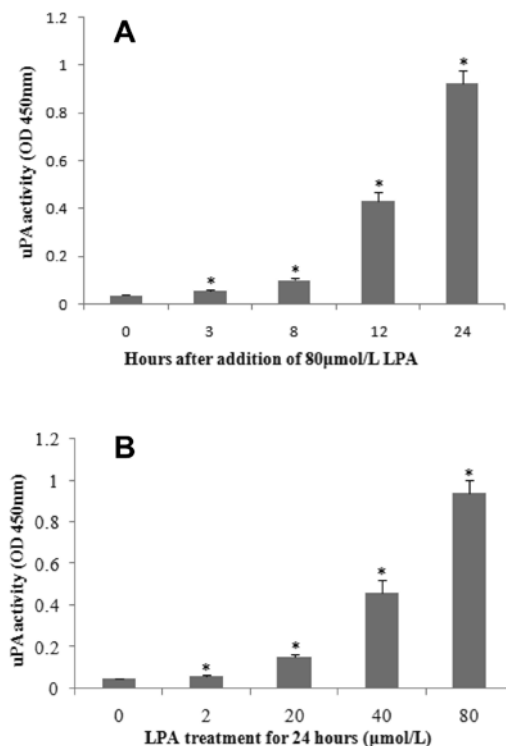


Figure 1. Stimulation of urokinase plasminogen activator (uPA) activity in SKOV-3 ovarian cancer cells by lysophosphatidic acid (LPA). The uPA activity was measured by enzyme-linked immunosorbent assay. (A) SKOV-3 cells were incubated with 80 $\mu\text{mol/L}$ lysophosphatidic acid (LPA) for 0, 3, 8, 12, and 24 hours before the collection of conditioned media. (B) SKOV-3 cells in 24-well plates (5×10^5 per well) were cultured, starved in serum-free medium overnight, and incubated with increasing concentrations of LPA (0, 2, 20, 40, and 80 $\mu\text{mol/L}$) for 24 hours before the collection of conditioned media. Measurements were made in three separate experiments, and data are shown as mean \pm standard deviation. Asterisk indicates $P = 0.004$ vs control. OD – optical density.

treatment with increasing concentrations (0-80 $\mu\text{mol/L}$) of LPA for 24 hours showed that LPA significantly increased the levels of uPA activity in a dose-dependent manner (Figure 1B). These results were consistent with those described previously (15,16). A maximum increase in uPA activity when incubated with LPA occurred at 24 hours and 80 $\mu\text{mol/L}$.

Silencing of LPA₂ reduces LPA-induced uPA activation

SKOV-3 cells were transfected by LPA₂-specific siRNA and control siRNA. The relative LPA₂ mRNA level was determined by semi-quantitative RT-PCR analysis. It was shown

that the transfection with LPA₂-specific siRNA led to >80% decrease in LPA₂ mRNA levels, when compared with non-target control siRNA (NC-siRNA) (Figure 2A). Meanwhile, we found that the silencing of LPA₂ expression resulted in ~55% reduction in LPA-induced uPA activity. In the culture medium without LPA stimulation, however, this change was minor (Figure 2B).

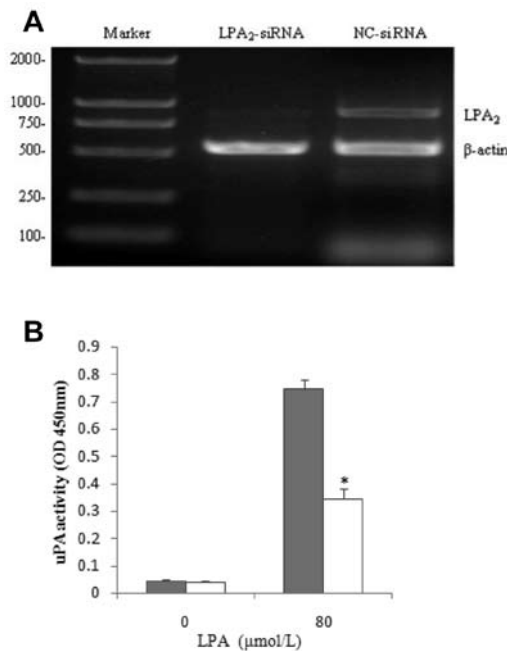


Figure 2. Effect of lysophosphatidic acid receptor-2-specific small interfering RNA (LPA₂-siRNA) transfection on lysophosphatidic acid (LPA)-induced urokinase plasminogen activator activity. (A) Semi-quantitative reverse transcriptase-polymerase chain reaction results showed the average knocking down of LPA₂ mRNA by siRNA. The fragment of LPA₂ and β-actin (internal control) cDNA were 838bp and 550bp, respectively. (B) Enzyme-linked immunosorbent assay showed the decrease of urokinase plasminogen activator (uPA) secreted in the presence of 80 μmol/L LPA in LPA₂ siRNA-transfected SKOV-3 cells compared with non-target control siRNA (NC-siRNA)-transfected SKOV-3 cells. Measurements were made in three separate siRNA transfection experiments, and data were depicted as the mean ± standard deviation. Gray bars – NC-siRNA; open bars – LPA₂-siRNA; OD – optical density; asterisk indicates $P=0.004$, LPA₂-siRNA vs NC-siRNA transfection.

Down-regulation of LPA₂ decreases LPA-induced cell invasion

In the culture medium without LPA stimulation, inhibiting LPA₂ expression showed no significant differences in the invasion activ-

ity of the cells in the NC-siRNA- and LPA₂-specific siRNA-transfected SKOV-3 cells (25.0 ± 3.2 vs 25.0 ± 3.7). In the presence of 80 μmol/L LPA, however, the increase of invasion was down-regulated from 7.3-fold in NC-siRNA-transfected SKOV-3 cells (178.0 ± 17.2 vs 25.0 ± 3.2) to 1.7-fold in LPA₂ siRNA-transfected SKOV-3 cells (36.2 ± 3.3 vs 25.0 ± 3.7), approximately a 4.3-fold decrease in comparison with a negative control (178.0 ± 17.2 vs 36.2 ± 3.3 , $P=0.009$) (Figure 3A).

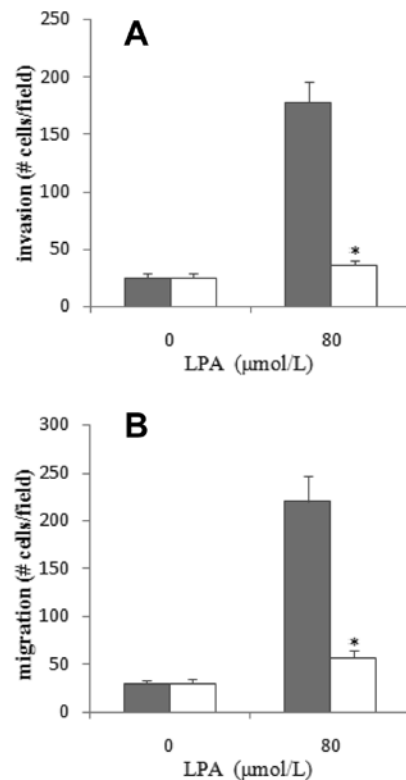


Figure 3. Lysophosphatidic acid receptor-2-specific small interfering RNA (LPA₂-siRNA) transfection reduces LPA-induced increase of SKOV-3 invasion and migration. (A) Matrigel invasion assay of nontarget control and LPA₂-siRNA treated with vehicle (0) or LPA (80 μmol/L). The transfected SKOV-3 ovarian cancer cells were starved for 16 hours and then trypsinized and treated with vehicle or 80 μmol/L LPA and loaded into the top chamber. The cells were incubated at 37°C for 24 hours. The number of cells invaded through the Matrigel was counted as described in Material and Methods section. (B) The migration activity of the transfected cells was examined using Transwell chemotaxis assay. The procedures were essentially the same as those for the invasion assay, except that there was no Matrigel. Data were depicted as the mean ± standard deviation. Asterisk indicates $P=0.009$, LPA₂-siRNA vs non-target control siRNA (NC-siRNA) transfection. Gray bars – NC-siRNA; open bars – LPA₂-siRNA.

Knockdown of LPA₂ decreases LPA-induced cell migration

The number of migrated cells in the NC-siRNA- and LPA₂-specific siRNA-transfected SKOV-3 cells showed no significant differences in the absence of LPA (30.0 ± 3.5 vs 30.0 ± 4.3). However, after being stimulated with 80 $\mu\text{mol/L}$ LPA, the number of migrated cells was significantly decreased in LPA₂-specific siRNA-transfected SKOV-3 cells (57.0 ± 7.6) compared with the NC-siRNA-transfected SKOV-3 cells (220.4 ± 25.5 ; $P=0.009$) (Figure 3B).

Discussion

In this report, we investigated the effect of LPA₂ on LPA-induced uPA activation in human ovarian cancer cell line SKOV-3. The data revealed that LPA induced uPA up-regulation in a dose- and time-dependent manner. Further, we inhibited the expression of LPA₂ by siRNA technique and found that LPA treatment at 80 $\mu\text{mol/L}$ induced a ~55% reduction of uPA activity in LPA₂-specific siRNA-transfected SKOV-3 cells, when compared with control siRNA-transfected SKOV-3 cells. This represents an important signaling pathway from the LPA₂ receptor in the mediation of uPA activation by LPA. LPA mediates diverse responses by binding to its specific receptors. In ovarian cancer, up to 90% tumor specimens have been found by Western blot analysis to overexpress LPA₂ receptor (19).

Degradation of the basement membrane is a requisite to the metastasis of tumor cells. uPA, a key protease that degrades the basement membrane, has been shown to contribute to tumor cell invasion. Since the inhibition of LPA₂ expression could decrease the level of uPA, we analyzed the effect of LPA₂ on LPA-induced ovarian cancer invasion. Matrigel invasion assay showed that down-regulation

of LPA₂ expression by siRNA approach in SKOV-3 cells was accompanied by 78% inhibition of invasive capacity in response to LPA. The result demonstrated that LPA₂ played a crucial role in LPA-induced ovarian cancer cell invasion. Another study also showed that LPA₂ receptor is overexpressed only in ovarian cancer cells and not in normal ovarian epithelial cells (20). The finding suggested that, by inhibiting the LPA₂ receptor expression, we could interfere in the ovarian cancer cells without an impact on normal ovarian surface epithelial cells.

The ability of tumor cells to migrate is a prerequisite for tumor metastasis. We observed that down-regulation of LPA₂ expression in SKOV-3 cells resulted in 74% decrease in the migration capacity of the cells in response to LPA, suggesting that the key receptor for this phenomenon was LPA₂. A previous study found that the migration activity of pancreatic cancer cells was increased by LPA, mainly through LPA₁ receptor (5). Shida et al (21) also proved that LPA enhanced the migration of human colon carcinoma DLD1 cells by LPA₁ receptor, and that LPA₁, rather than LPA₂, was highly expressed in these cell lines. In ovarian cancer, however, LPA₁ had a minor role in LPA-induced cell migration, because it was expressed at similar levels in normal ovarian surface epithelial cells and ovarian cancer cell lines (14) and the effect was markedly inhibited by the down-regulation of LPA₂. Our results strongly suggested that LPA₂ is also capable of inducing cell migration in ovarian cancer cell line SKOV-3, although the mechanism remains unknown.

In summary, our study proved that LPA₂ receptor involved in LPA-dependent uPA activation and LPA₂ down-regulation significantly decreased LPA-induced invasion and migration in SKOV-3 ovarian cancer cells. These results suggest the importance of LPA₂ in response to LPA-induced pathological ac-

tions in ovarian cancer cells. Although there is not LPA₂-selective antagonists at present, LPA₂ may be a viable target for biological therapy in ovarian cancer. Thus, further research is needed to develop a novel LPA₂ receptor antagonist and to investigate the role of LPA-LPA₂ axis in tumor growth and metastasis of ovarian cancer using mouse xenograft models.

References

- Contos JJ, Ishii I, Chun J. Lysophosphatidic acid receptors. *Mol Pharmacol*. 2000;58:1188-96. [Medline:11093753](#)
- Siess W, Zangl KJ, Essler M, Bauer M, Brandl R, Corrinth C, et al. Lysophosphatidic acid mediates the rapid activation of platelets and endothelial cells by mildly oxidized low density lipoprotein and accumulates in human atherosclerotic lesions. *Proc Natl Acad Sci U S A*. 1999;96:6931-6. [Medline:10359816](#) [doi:10.1073/pnas.96.12.6931](#)
- Xu Y, Fang XJ, Casey G, Mills GB. Lysophospholipids activate ovarian and breast cancer cells. *Biochem J*. 1995;309:933-40. [Medline:7639713](#)
- Schulte KM, Beyer A, Kohrer K, Oberhauser S, Roher HD. Lysophosphatidic acid, a novel lipid growth factor for human thyroid cells: over-expression of the high-affinity receptor edg4 in differentiated thyroid cancer. *Int J Cancer*. 2001;92:249-56. [Medline:11291053](#) [doi:10.1002/1097-0215\(200102\)9999:9999::AID-IJC1166>3.0.CO;2-D](#)
- Yamada T, Sato K, Komachi M, Malchinkhuu E, Tobo M, Kimura T, et al. Lysophosphatidic acid (LPA) in malignant ascites stimulates motility of human pancreatic cancer cells through LPA1. *J Biol Chem*. 2004;279:6595-605. [Medline:14660630](#) [doi:10.1074/jbc.M308133200](#)
- Xu Y, Gaudette DC, Boynton JD, Frankel A, Fang XJ, Sharma A, et al. Characterization of an ovarian cancer activating factor in ascites from ovarian cancer patients. *Clin Cancer Res*. 1995;1:1223-32. [Medline:9815916](#)
- Fang X, Schummer M, Mao M, Yu S, Tabassam FH, Swaby R, et al. Lysophosphatidic acid is a bioactive mediator in ovarian cancer. *Biochim Biophys Acta*. 2002;1582:257-64. [Medline:12069836](#)
- Westermann AM, Havik E, Postma FR, Beijnen JH, Dalesio O, Moolenaar WH, et al. Malignant effusions contain lysophosphatidic acid (LPA)-like activity. *Ann Oncol*. 1998;9:437-42. [Medline:9636836](#) [doi:10.1023/A:1008217129273](#)
- An S, Bleu T, Hallmark OG, Goetzl EJ. Characterization of a novel subtype of human G protein-coupled receptor for lysophosphatidic acid. *J Biol Chem*. 1998;273:7906-10. [Medline:9525886](#) [doi:10.1074/jbc.273.14.7906](#)
- Bandoh K, Aoki J, Hosono H, Kobayashi S, Kobayashi T, Murakami-Murofushi K, et al. Molecular cloning and characterization of a novel human G-protein-coupled receptor, EDG7, for lysophosphatidic acid. *J Biol Chem*. 1999;274:27776-85. [Medline:10488122](#) [doi:10.1074/jbc.274.39.27776](#)
- Ye X, Ishii I, Kingsbury MA, Chun J. Lysophosphatidic acid as a novel cell survival/apoptotic factor. *Biochim Biophys Acta*. 2002;1585:108-13. [Medline:12531543](#)
- Noguchi K, Ishii S, Shimizu T. Identification of p2y9/GPR23 as a novel G protein-coupled receptor for lysophosphatidic acid, structurally distant from the Edg family. *J Biol Chem*. 2003;278:25600-6. [Medline:12724320](#) [doi:10.1074/jbc.M302648200](#)
- McIntyre TM, Pontsler AV, Silva AR, St Hilaire A, Xu Y, Hinshaw JC, et al. Identification of an intracellular receptor for lysophosphatidic acid (LPA): LPA is a transcellular PPARgamma agonist. *Proc Natl Acad Sci U S A*. 2003;100:131-6. [Medline:12502787](#) [doi:10.1073/pnas.0135855100](#)
- Hu YL, Tee MK, Goetzl EJ, Auersperg N, Mills GB, Ferrara N, et al. Lysophosphatidic acid induction of vascular endothelial growth factor expression in human ovarian cancer cells. *J Natl Cancer Inst*. 2001;93:762-8. [Medline:11353786](#) [doi:10.1093/jnci/93.10.762](#)
- Pustilnik TB, Estrella V, Wiener JR, Mao M, Eder A, Watt MA, et al. Lysophosphatidic acid induces urokinase secretion by ovarian cancer cells. *Clin Cancer Res*. 1999;5:3704-10. [Medline:10589790](#)
- Li H, Ye X, Mahanivong C, Bian D, Chun J, Huang S. Signaling mechanisms responsible for lysophosphatidic acid-induced urokinase plasminogen activator expression in ovarian cancer cells. *J Biol Chem*. 2005;280:10564-71. [Medline:15653692](#) [doi:10.1074/jbc.M412152200](#)
- Huang MC, Lee HY, Yeh CC, Kong Y, Zaloudek CJ, Goetzl EJ. Induction of protein growth factor systems in the ovaries of transgenic mice overexpressing human type 2 lysophosphatidic acid G protein-coupled receptor (LPA2). *Oncogene*. 2004;23:122-9. [Medline:14712217](#) [doi:10.1038/sj.onc.1206986](#)
- Chen M, Towers LN, O'Connor KL. LPA2 (EDG4) mediates Rho-dependent chemotaxis with lower efficacy than LPA1 (EDG2) in breast carcinoma cells. *Am J Physiol Cell Physiol*. 2007;292:C1927-33. [Medline:17496233](#) [doi:10.1152/ajpcell.00400.2006](#)
- Wang P, Wu X, Chen W, Liu J, Wang X. The lysophosphatidic acid (LPA) receptors their expression and significance in epithelial ovarian neoplasms. *Gynecol Oncol*. 2007;104:714-20. [Medline:17204312](#) [doi:10.1016/j.ygyno.2006.10.016](#)
- Hu YL, Albanese C, Pestell RG, Jaffe RB. Dual mechanisms for lysophosphatidic acid stimulation of human ovarian carcinoma cells. *J Natl Cancer Inst*. 2003;95:733-40. [Medline:12759391](#)
- Shida D, Kitayama J, Yamaguchi H, Okaji Y, Tsuno NH, Watanabe T, et al. Lysophosphatidic acid (LPA) enhances the metastatic potential of human colon carcinoma DLD1 cells through LPA1. *Cancer Res*. 2003;63:1706-11. [Medline:12670925](#)