http://biology.num.edu.mn
http://mjbs.100zero.org/
Volume 11(1-2), 2013

Mongolian Journal of Biological Sciences

ISSN 1684-3908 (print edition)
MJBS
ISSN 2225-4994 (online edition)

Original Article

Involvement of LPA Receptor 3 in LPA-induced BGC-803 Cell Migration

Erdene Oyungerel, Tserennadmid Tseveensuren, Oyun Gansukh, Li Rui Juan and Alatangaole Damirin

College of Life Sciences, Inner Mongolia University, Huhhot, Inner Mongolia 010021, P.R.China
e-mail: uk_og77@yahoo.com, bigaole@imu.edu.cn

Abstract

Key words:
Lysophosphatidic acid receptor 2, 3 (LPAR2, LPAR3), cell migration, gastric cancer

Article information:
Received: 06 Jan. 2014
Accepted: 31 Mar. 2014
Published: 20 Apr. 2014

Correspondence:
uk_og77@yahoo.com

Lysophosphatidic acid（LPA）is a bioactive phospholipid mediator, which elicits a variety of biological functions mainly through G-protein coupled receptors. Although LPA is shown to stimulate proliferation and motility via LPA receptors, LPAR1 and LPAR3 in several cancer cell lines, but the role of LPA receptors in gastric cancer cells is still being unknown. However, several researches reported that LPAR2 play an important role in the carcinogenesis of gastric cancer, but there is no report to show the LPAR3 involvement in the carcinogenesis. For this reason, we examined LPA receptors (LPAR1, LPAR2 and LPAR3) in BGC-803 cells along with real time PCR method. Real-time PCR analyses were used to evaluate the expression of LPA receptors in BGC-803 cells. Among these receptors, LPAR3 was shown to be highly expressed in BGC-803 cells, a human gastric cancer cell line. Transient transfection with LPAR3 siRNA was observed to reduce LPAR3 mRNA in BGC-803 cells and eliminate the LPA-induced cell migration. The results suggest that the LPAR3 regulates LPA-induced BGC-803 cell migration.

Cite this paper as: Oyungerel, E., Tseveensuren, Ts., Gansukh, O., Juan, L. R, & Damirin, A., 2013. Involvement of LPA receptor 3 in LPA-induced BGC-803 cell migration. *Mong. J. Biol. Sci.*, 11(1-2): 25-30.

Introduction

Lysophosphatidic acid (LPA), a bioactive phospholipid with diverse physiological actions involved in triggering tumor cell invasion and metastasis, as well as malignant cell growth. In recent years, also found that, LPA as an intercellular messenger, could lead to a very wide range of biological effects, and its significant biological effects including the promotion of cell proliferation, promote platelet aggregation, aggregation and smooth muscle cells involved in tumor cell infiltration (Damirin *et al.,* 2007; Komachi *et al.,* 2009). Lysophosphatidic acid (LPA) is a bioactive phospholipid mediator, which elicits a variety of biological functions mainly, through G-protein coupled receptors (Shida *et al.,* 2003; Aoki *et al.,* 2002). Lysophosphatidic acid acts as an extracellular signaling molecule by binding to and activating at least eight known G-protein coupled receptors (GPCRs): LPA1-LPA8 (Noguchi *et al.,* 2009; Komachi *et al.,* 2009). The biological roles of LPA are diverse and include developmental, physiological, and pathophysiological effects (Contos *et al.,* 2000). This diversity is mediated by broad and overlapping expression patterns and multiple downstream signaling pathways activated by cognate LPA receptors (Bandoh *et al.,* 1999; Hama & Aoki, 2010). LPA receptors through different types of LPA showed a lot of biological activity, including the mobilization of Ca^{2+}, changes of cAMP accumulation in actin rearrangement and combined changes in cell

shape, motility, and it can also lead to various types of cell proliferation (An *et al.,* 1998; Yamada *et al.,* 2003). Lysophosphatidic acid and their respective families of G protein-coupled receptors have been implicated in progression of gastric cancer, the second most common cause of cancer related deaths. Binding of either of these lysophospholipids to their cognate receptors on gastric cancer cells transactivates the epidermal growth factor receptor (EGFR) (Ramachandran *et al.,* 2010). The LPA1-3 receptors have been investigated in progression of gastric cancer (Shida *et al.,* 2004; Yang *et al.,* 2013). Here we investigated the roles of specific LPA receptor subtypes in BGC803 cell migration and on receptor-mediated signaling pathway. Furthermore, our data indicated that LPA is an important component for the motility of BGC803 cells through Ki-16425-sensitive LPA receptors, especially LPA_{1-3}.

Materials and Methods

Cell culture and reagents. The human gastric cancer cell line BGC-803 was provided by Institute of Zoology of China (Beijing, China). The cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco Carlsbad, CA, USA) which was supplemented with 10% (v/v) fetal bovine serum (Gibco) at 37°C in a humidified atmosphere containing 5% CO2. 1-Oleoyl-sn-glycero-3-phosphate (LPA), fatty acid-free BSA and PTX were obtained from Sigma (St. Louis, MO, USA). The Ki-16425 and YM-254890 were provided by Fumikazu Okajima (Gunma University, Maebashi, Japan) as gifts.

Cell migration assays. Cell migration was measured using 24-well Transwell plates (Corning, Tewksbury, MA, USA) with 8 μm-pore polycarbonate membranes. The Transwell plates were coated with 1% gelatin and the serum-free DMEM supplemented with LPA and 0.1% fatty acid-free BSA in the lower chamber was used as a lysophospholipid carrier. Cells (2 x 105/ml) suspended in serum-free DMEM containing 0.1% fatty acid-free BSA were added to the upper chamber and incubated for 12 h at 37°C. When the effects of LPA antagonists were examined, the cells were preincubated for 10 min with antagonists before being loaded. Unmigrated cells were removed from the top filter surface with a cotton swab and fixed 100% methanol for 10 min. Migrated cells were observed to attach to the underside of the transwell plates and counted under a light microscope using a 200x objective after staining with 0.2% crystal violet. The experiments were repeated more than three times for each condition and for each experiment , five random fields were counted.

Quantitative RT-PCR. Total RNA was isolated with a total RNA extraction reagent kit (Takara Bio Inc) according to the manufacturer's instructions. After PrimeScriptTM RT reagent Kit with gDNA Eraser (Takara Bio Inc) treatment to remove possible traces of genomic DNA in the RNA preparations, 1 μg total RNA was used in reverse-transcription with SYBR® Green qPCR assay kit (Takara Bio Inc). The primers used in the reaction were: LPAR1 forward, 5'-TCCTGTCCCGCGCCAGGTACAC-3'; LPAR1 reverse, 5'-GGTGGTGAACACGCCCCAGAACT-3'; LPAR2 forward, 5'-ACCGCAGTGTGATGGCCGTG-3'; LPAR2 reverse, TAGGAGCGGCTGAGCAGGGG-3'; LPAR3 forward, 5'-GCCGTGGAGAGGCACATGTC-3', LPAR3 reverse, 5'-TGGCGATGGCCCAGACAAGC-3'; GAPDH forward, 5'-TCAAGTGGGGCGATGCTGGC-3′; GAPDH reverse, 5'-TGGGGGCA TCAGCAGAGGGG-3′. Quantitative RT-PCR was performed using Light Cycler® Real Time PCR Reagent kits (Takara Bio Inc.). The cycling conditions were: 95°C for 30 sec, then 50 cycles at 95°C for 5 sec and 60°C for 20 sec. The mRNA level of the genes of interest of each sample was normalized to that of the GAPDH mRNA and presented as unit values of 2[Cp(GAPDH) - Cp(target gene)]. Quantitative RT-PCR was performed in a LightCycler®480 Instrument II (Roche Diagnostics, Mannheim, Germany).

RNA interference. Cells ($3x10^5$) were incubated in a six-well plate overnight. Transient shRNA transfection was performed with LTX and Plus Reagent (Invitrogen) according to the manufacturer's instructions. Predesigned vectors expressing control shRNA or LPAR2-specific shRNA and LPAR3-specific shRNA were purchased from Inovogen (Inovogen, Beijing, China). The shRNA oligonucleotide sequences of LPAR2 and LPAR3 were 5'-AGTACTTCCTACTGTTGGC-3', 5'-GCTAATGAAGACGGTGATG-3'. The transfected cell clones were designated BGC-803/shLPAR2, BGC-803/shLPAR3 and BGC-803/shRNA control. The LPAR2 and LPAR3 expression were detected by quantitative real-time PCR (RT-PCR)

in these transfected cell clones.

Statistical analysis. The student's t-test and one-way ANOVA using Graphpad Instat 5 software were used for the statistical analyses.

Results

LPA-induced migration of BGC-803 cells was inhibited by Ki-16425. LPA is a natural phospholipid, is able to modulate diverse cellular responses through LPA receptors (LPARs). To detect the role of LPA in cell migration, BGC-803 cells were stimulated with LPA at 1 μM concentration. LPA was observed to significantly increase cell migration. However, Ki-16425 was suppressing the LPA-induced migration of BGC-803 cells. Antagonist for LPARs 1 and 3, Ki-16425 affect the migration of BGC-803 cells. This result suggests that the LPA-induced migration may depend on LPAR1 and LPAR3 (Fig. 1).

Involvement of Gi and Gq proteins in LPA-induced BGC-803 cell migration. To identify which G protein is involved in LPA-induced BGC-803 cell migration, pertussis toxin (PTX), a specific Gi protein inhibitor and YM-254890, a specific Gq protein inhibitor, were used in the cell migration experiment. It was observed that pertussis toxin (PTX) more reduced the LPA-induced cell migration. The results indicate that Gi appears to be involved more than Gq in LPA-induced cell migration (Fig. 2).

LPAR3 is highly expressed in BGC-803 cells. To evaluate the expression of LPARs 1-3 in the BGC-803 cells, RT-PCR analysis was performed. LPAR3 was shown to be highly expressed in the BGC-803 cells. This result suggests that the LPA-induced migration of BGC-803 cells may be dependent on LPAR3 (Fig. 3).

Silencing LPAR2 and LPAR3 expression by shRNAs inhibits the LPA-induced cell migration of BGC-803. As mentioned, LPAR3 was highly expressed in the BGC-803 cells, suggesting that LPAR3 may be important in LPA-induced cell migration. To investigate the roles of LPAR2 and LPAR3 in the LPA-induced cell migration, their expression was silenced by LPAR2 and LPAR3-specific shRNAs in the BGC-803 cells. RT-PCR analysis showed that LPAR3 expression was decreased by 53 percent compared with the control (Fig. 4). However, LPAR2 expression was decreased less than LPAR3 (Fig. 5). Migration experiments showed that silencing LPAR3 expression significantly decreased the LPA-induced migration of BGC-803 cells compared with the control BGC-803 cells (Fig. 6). The results demonstrate that LPA-induced migration of BGC-803 gastric cancer cells requires LPAR3.

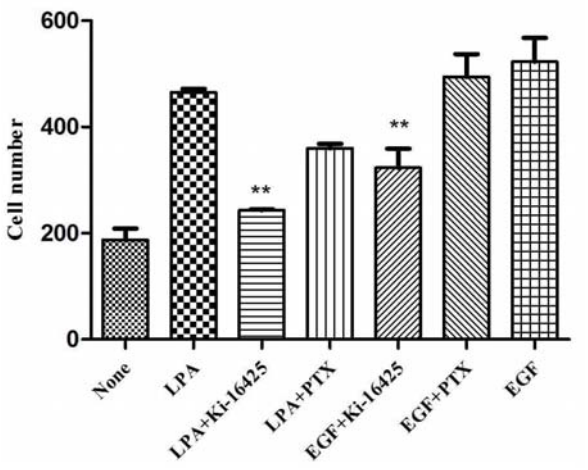

Figure 1. LPA-induced migration of BGC-803 cells was inhibited by Ki-16425 and pertussis toxin (PTX). The LPA-induced migration of BGC-803 cells was assayed after 30 min incubation with 0.1mM Ki-16425 and100ng/mL PTX, as indicated. The values are the average (±SE) of six repeats from three separate experiments *(**P < 0.01)*. LPA, lysophosphatidic acid; PTX, pertussis toxin; EGF, epidermal growth factor.

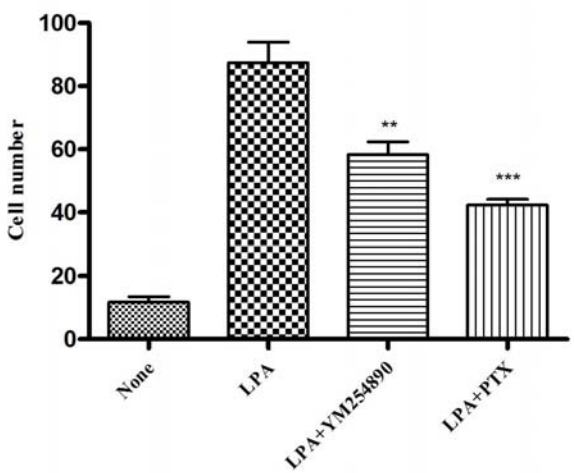

Figure 2. Involvement of Gi and Gq proteins in LPA-induced BGC-803 cell migration. BGC-803 cells were pretreated with 50ng/ml PTX for 16 h and treated with 1μM of YM-254890 for 1 h. The cells were further incubated with 1μM LPA to measure the cell migration. The values are the average (±SE) of six repeats from two separate experiments *(**P < 0.01, ***P < 0.001)*. LPA, lysophosphatidic acid; PTX, pertussis toxin.

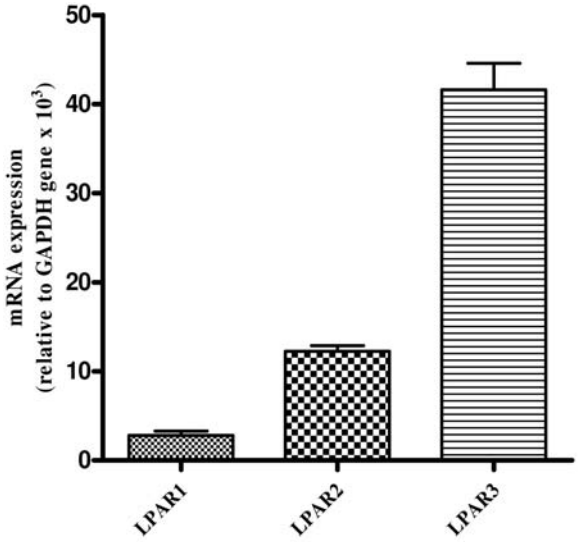

Figure 3. Expression of LPAR1-3 in BGC-803 cells. The total RNA was isolated using a total RNA isolation kit. The mRNA level of LPAR1-3 was reverse transcribed, then assessed by real-time PCR. The values for expression are presented relative to GAPDH mRNA. The values are the average (±SE) of three repeats from a representative experiment. LPAR, lysophosphatidic acid receptor.

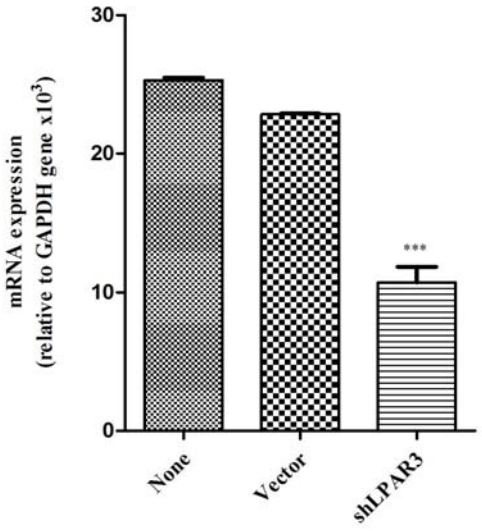

Figure 4. LPAR3 expression was silenced by specific shRNA in the BGC-803 cells. Total RNA was isolated using a total RNA isolation kit. The mRNA level of LPAR3 was reverse transcribed, then assessed by real-time PCR. The values are relative to GAPDH mRNA and are the average (±SE) of three repeats from a representative experiment *(***P < 0.001)*. LPAR3, lysophosphatidic acid receptor 3.

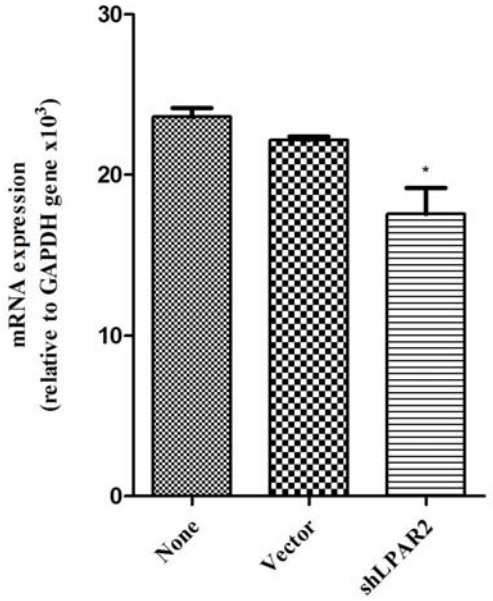

Figure 5. LPAR2 expression was silenced by specific shRNA in the BGC-803 cells. Total RNA was isolated using a total RNA isolation kit. The mRNA level of LPAR2 was reverse transcribed, then assessed by real-time PCR. The values are relative to GAPDH mRNA and are the average (±SE) of three repeats from a representative experiment *(*P < 0.05)*. LPAR2, lysophosphatidic acid receptor 2.

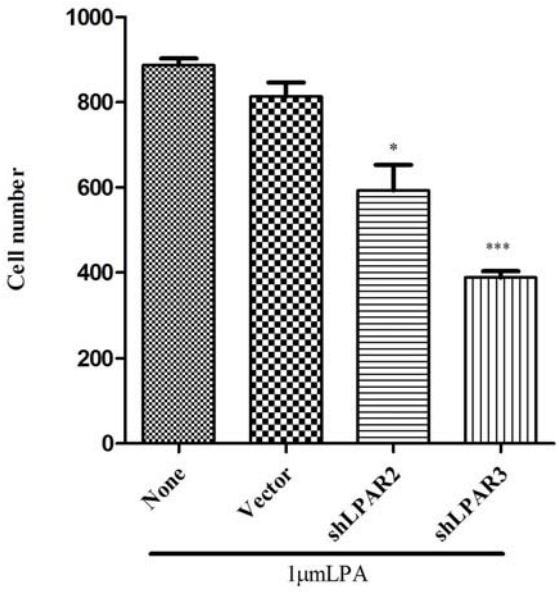

Figure 6. Silencing LPAR2-3 expression with shLPAR2 and shLPAR3 inhibits the LPA-induced cell migration of BGC-803. LPA-induced BGC-803 cell migration was assayed as described in the Materials and methods. The values are the average (±***SE***) of six repeats from two separate experiments *(*P < 0.05, ***P < 0.001)*. LPAR2, lysophosphatidic acid receptor 2, LPAR3, lysophosphatidic acid receptor 3.

Discussion

Associated mechanism between the LPAR and G protein subtypes has not been analyzed in detail for LPA-induced migration in tumor cells. The LPAR1, LPAR2, and LPAR3 are belong to the endothelial differentiation gene family (EDG) of G protein-coupled receptors (Ohta *et al.,* 2003; Fang *et al.,* 2004). Through these receptors, LPA is implicated in numerous cellular processes including cell proliferation and migration (Murray *et al.,* 2008; Zhang *et al.,* 2011; Ramachandran *et al.,* 2010). LPA-activated receptors predominantly stimulate PTX-sensitive Gi/o type heterotrimeric proteins in cells, a property described for all three receptors, and stimulate the small GTP-ases N-Ras, Rac and RhoA (Stahle *et al.,* 2003). The LPA receptors' individual signaling pathways have yet to be fully elucidated. LPAR expression was previously unknown in BGC-803 cells and the present study demonstrated that among the three principle LPARs, LPAR3 is predominantly expressed by BGC-803 cells.

Previous study indicated that the expression of three LPA receptor mRNAs in nine gastric cancer cell lines using Northern blot analysis. This observation suggests that various gastric cancer cells expressed variable levels of LPAR1, LPAR2, and LPAR3 without a consistent pattern (Shida *et al.,* 2004; Yang *et al.,* 2013). LPAR3, in particular, has been shown to be upregulated in a variety of cancer types, including sarcoma, gastric, liver epithelial and pancreatic cancer (Tanabe *et al.,* 2012; Inoue *et al.,* 2013; Kato *et al.,* 2012). The effect of LPA on gastric cancer migration with and without LPAR3 knockdown was then evaluated and the effect of LPA on migration was shown to be blocked in shLPAR3-transfected BGC-803 cells. RT-PCR analysis and migration experiments showed that silencing LPAR3 expression significantly decreased the LPA-induced migration of BGC-803 cells. The present findings suggest that LPAR3 may be a potential target for clinical treatment of gastric cancer. The development of LPAR-specific antagonists might have future therapeutic relevance in the treatment as well as prevention of gastric cancer.

Acknowledgements

This research was supported by the National Natural Science Foundation of China (31160184, 30760054).

References

An, S., Bleu, Th., Zheng, Y. & Goetzl, E. J. 1998. Recombinant human G protein-coupled lysophosphatidic acid receptors mediate intracellular calcium mobilization. *Mol. Pharmacol.,* 54: 881-888.

Aoki, J., Taira, A., Takanezawa, Y., Kishi, Y., Hama, K., Kishimoto, T., Mizuno, K., Saku, K., Taguchi, R. & Arai, H. 2002. Serum lysophosphatidic acid is produced through diverse phospholipase pathways. *J. Biol. Chem.,* 277: 48737-48744.

Bandoh, K., Aoki, J., Hosono, H., Kobayashi, S., Kobayashi, T., Murakami-Murofushi, K., Tsujimoto, M., Arai, Y., & Inoue, K. 1999. Molecular cloning and characterization of a novel human G-protein-coupled receptor, EDG7, for lysophosphatidic acid. *J. Biol. Chem.,* 274: 27776-27785.

Contos, J. A., Ishii, I. & Chun, J. 2000. Lysophosphatidic acid receptors. *Mol. Pharmacol.,* 58: 1188-1196.

Damirin, A., Tomura, H., Komachi, M., Liu, J-P., Mogi, Ch., Tobo, M., Wang, J-O., Kimura, Y., Kuwabara, A., Yamazaki, Y., Ohta, H., Im, D-S., Sato, K., & Okajima, F. 2007. Role of lipoprotein-associated lysophospholipids in migratory activity of coronary artery smooth muscle cells. *Am. J. Physiol. Heart. Circ. Physiol*., 292: H2513-H2522.

Fang, X., Yu, Sh., Bast, R. C., Liu, Sh., Xu, H-J., Hu, Sh-X., LaPushin, R., Claret, F. X., Aggarwal, Bh. B., Lu, Y., & Mills, G. B. 2004. Mechanisms for lysophosphatidic acid-induced cytokine production in ovarian cancer cells. *J. Biol. Chem.,* 279: 9653-9661.

Hama, K. & Aoki. J. 2010. LPA3, a unique G protein-coupled receptor for lysophosphatidic acid. *Prog. Lipid Res*., 49: 335-342.

Inoue, S., Tanabe, E., Shibata, A., Hirane, M., Araki, M., Dong, Y., Fukushima, N. & Tsujiuchi, T. 2013. Ethionine regulates cell motile activity through LPA receptor-3 in liver epithelial WB-F344 cells. *Mol. Cel. Biochem.,* 383(1-2): 173-177.

Kato, K., Yoshikawa, K., Tanabe, E., Kitayoshi, M., Fukui, R., Fukushima, N., Tsujiuchi, T. 2012. Opposite roles of LPA 1 and LPA3 on cell motile and invasive activities of pancreatic cancer cells. *Tumor Biol.,* 33: 1739-1744.

Komachi, M., Tomura, H., Malchinkhuu, E.,

Tobo, M., Moga, Ch., Yamada, T., Kimura, T., Kuwabara, A., Ohta, H., Im, D-S., Kurose, H., Takeyoshi, I., Sato, K. & Okajima, F. 2009. LPA1 receptors mediate stimulation, whereas LPA2 receptors mediate inhibition, of migration of pancreatic cancer cells in response to lysophosphatidic acid and malignant ascites. *Carcinogen.,* 30: 457-465.

Komachi, M., Damirin, A., Malchinkhuu, E., Moga, Ch., Tobo, M., Ohta, H., Sato, K., Tomura, H. & Okajima, F. 2009. Signaling pathways involved in DNA synthesis and migration in response to lysophosphatidic acid and low-density lipoprotein in coronary artery smooth muscle cells. *Vascular Pharmacol,* 50: 178-184.

Moolenaar, W. H. 1995. Lysophosphatidic acid, a multifunctional phospholipid messenger. *J. Biol. Chem.,* 270: 12949-12952.

Moolenaar, W. H. 1995. Lysophosphatidic acid signaling. *Cell Biol.,* 7: 203-210.

Murray, D., Horgan, G., MacMathuna, P. & Doran, P. 2008. NET1-mediated RhoA activation facilitates lysophosphatidic acid-induced cell migration and invasion in gastric cancer. *Brit. J. Cancer,* 99: 1322-1329.

Noguchi, K., Herr, D., Mutoh, Ts. & Chun, J. 2009. Lysophosphatidic acid (LPA) and its receptors. *Pharmacol.,* 9: 15-23.

Ohta, H., Sato, K., Murata, N., Damirin, A., Malchinkhuu, E., Kon, J., Kimura, T., Tobo, M., Yamazaki, Y., Watanabe, T., Yagi, M., Sato, M., Suzuki, R., Murooka, H., Sakai, T., Nishitova, Ts., Im, D-S., Nochi, H., Tamoto, K., Tomura, H. & Okajima, F. 2003. Ki16425, a Subtype-selective antagonist for EDG-family lysophosphatidic acid receptors. *Mol. Pharmacol.,* 64:994-1005.

Ramachandran, S., Shida, D., Nagahashi, M., Fang, X., Milstien, Sh., Takabe, K., Spiegel, S. 2010. Lysophosphatidic acid stimulates gastric cancer cell proliferation via ERK1-dependent upregulation of sphingosine kinase 1 transcription. *Fed. Eur. Biochem. Soc. Lett.,* 584: 4077-4082.

Shida, D., Kitayama, J., Yamaguchi, H., Okaji, Y., Tsuno, N. H., Watanabe, T., Takuwa, Y., & Nagawa, H. 2003. Lysophosphatidic acid (LPA) enhances the metastatic potential of human colon carcinoma DLD1 cells through LPA. *Cancer Res.*, 63: 1706-1711.

Shida, D., Kitayama, J., Yamaguchi, H., Hama, K., Aoki, J., Arai, H., Yamashita, H., Mori, K., Sako, A., Konishi, Ts., Watanabe, T., Sakai, T., Suzuki, R., Ohta, H., Takuwa, Y. & Nagawa, H. 2004. Dual mode regulation of migration by lysophosphatidic acid in human gastric cancer cells. *Exper. Cell Res.,* 301: 168-178.

Stahle, M., Veit, Ch., Bachfischer, U., Schierling, K., Skripczynski, B., Hall, A., Gierschik, P. & Giehl, K. 2003. Mechanisms in LPA-induced tumor cell migration: critical role of phosphorylated ERK. *J. Cell Sci.,* 116: 3835-3846.

Tanabe, E., Kitayoshi, M., Yoshikawa, K., Shibata, A., Honoki, K., Fukushima, N., Tsujiuchi, T. 2012. Loss of lysophosphatidic acid receptor-3 suppresses cell migration activity of human sarcoma cells. *J. Recep. Sign. Transduc.,* 32: 328-334.

Yamada, T., Sato, K., Komachi, M., Malchinkhuu, E., Tobo, M., Kimura, T., Kuwabara, A., Yanagita, Y., Ikeya, T., Tanahashi, Y., Tamoto, K., Tomura, H. & Okajima, F. 2003. Lysophosphatidic acid (LPA) in malignant ascites stimulates motility of human pancreatic cancer cells through LPA1. *J. Biol. Chem.,* 279: 6595-6605.

Zhang, R., Wang, J., Ma, Sh., Huang, Z. & Zhang, G. 2011. Requirement of osteopontin in the migration and protection against taxol-induced apoptosis via the ATX-LPA axis in SGC7901 cells. *BioMedCentral Cell Biol.,* 1-8.

Yang, D., Yang, W., Zhang, Q., Hu, Y., Bao, L. & Damirin, A. 2013. Migration of gastric cancer cells in response to lysophosphatidic acid is mediated by LPA receptor 2. *Oncol. Lett.,* 3: 1048-1052.

*****