Short Communication

No Involvement of Lysophosphatidic Acid Receptor-3 in Cell Migration of Mouse Lung Tumor Cells Stimulated by 12-O-Tetradecanoylphorbol-13-acetate

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Abstract: The tumor promoting agent 12-O-tetradecanoylphorbol-13-acetate (TPA) stimulates cell migration of several tumor cells. Recently, we reported that loss of lysophosphatidic acid (LPA) receptor-3 (LPA3) enhanced cell migration of murine lung tumor LL/2 cells. In the present study, we investigated whether LPA 3 is involved in cell migration of mouse lung tumor cells stimulated by TPA. Exogenous LPA3 gene (Lpar3)-expressing (LL/2-a3) cells and LL/2-AB cells as a vector control generated from LL/2 cells were used. In a cell migration assay, TPA treatment significantly stimulated cell migration of LL/2-AB and LL/2-a3 cells, while the cell migration abilities of LL/2-a3 were markedly lower than those of LL/2-AB cells. Using quantitative real-time reverse transcription (RT)-polymerase chain reaction (PCR) analysis, no effect of TPA treatment on the expression levels of LPA1, LPA2, and LPA3 genes was detected in either type of cells. These results suggest that the LPA3 may not be involved in the enhanced migration ability by TPA in mouse lung tumor cells. (DOI: 10.1293/tox.24.183; J Toxicol Pathol 2011; 24: 183–186)

Key words: LPA3, 12-O-tetradecanoylphorbol-13-acetate, cell migration, lung, mouse

Lysophosphatidic acid (LPA) is a bioactive mediator and interacts with at least six G protein-coupled transmembrane receptors, LPA receptor-1 (LPA1), LPA2, LPA3, LPA4, LPA5, and LPA6. After binding to LPA receptors (LPARs), LPA induces several biological effects, such as cell proliferation, differentiation, morphogenesis and protection from apoptosis. In cancer cells, LPA can also enhance cell growth, migration, invasion and tumorigenicity.

Previously, aberrant expressions of LPAR genes have been detected in human malignancies, including ovary, colon and thyroid tumors. Recently, we have also reported that the distinct expression patterns of LPAR genes due to DNA methylation were found in human and mouse tumor cells. Therefore, this suggests that the biological functions of LPARs may be dependent on the type of cancer cells. In fact, LPA3 enhanced cell migration and invasion of human ovarian cancer cells. By contrast, we have reported that LPA3 inhibited cell migration abilities of murine lung tumor cells.

It is well known that 12-O-tetradecanoylphorbol-13-acetate (TPA) is a tumor promoting agent that inhibits gap junctional intercellular communication in various cells types. TPA also enhances cell migration through activation of protein kinase C (PKC) in some tumor cells. In the present study, to clarify an involvement of LPA signaling pathway in TPA-stimulated cell migration of tumor cells, we investigated cell migration of mouse lung tumor cells treated with TPA and measured the expression levels of LPAR genes in those cells. We used the two cell lines, exogenous LPA3 gene (Lpar3)-expressing (LL/2-a3) cells and LL/2-AB (vector) cells. LL/2-a3 cells showed significantly lower cell migration ability than control LL/2-AB cells.

LL/2-a3 cells were generated from mouse LL/2 lung tumor cells using retroviruses coexpressing green fluorescent protein (GFP) from an internal ribosomal entry site as described previously. LL/2-AB cells were also used as a control clone. All cells were cultured in DMEM containing 10% fetal bovine serum in a 5% CO2 atmosphere at 37 °C. For the cell migration assay, an uncoated Cell Culture Insert (BD Falcon, Franklin Lakes, NJ, USA) with an 8-μm pore size was used. Cells were pretreated with TPA (5 nM).
Total RNA was extracted from each cell using ISO-GEN (Nippon Gene Co., Ltd., Toyama, Japan), and first-strand cDNA was synthesized with Transcriptor First Strand cDNA Synthesis Kit (Roche Diagnostics GmbH, Mannheim, Germany). To assess the expression patterns the Lpar1, Lpar2 and Lpar3 genes in TPA-treated cells, reverse transcription (RT)-polymerase chain reaction (PCR) analysis was performed. The amplified PCR products were separated on 2% agarose gels containing 0.05 μg/ml ethidium bromide. The expression levels of those genes were also measured by quantitative real-time RT-PCR analysis using a SYBR Premix Ex Taq (TaKaRa Bio, Inc., Shiga, Japan) and a SmartCycler II System (TaKaRa). The rat Gapdh gene was used as an internal control gene. The data of the target genes were normalized to Gapdh. Each assay was repeated at least twice for confirmation.

The morphological appearances of the LL/2-AB and LL/2-a3 cells used in this study are shown in Fig. 1. The cell growth rate between the two types of cell indicated the same levels in DMEM containing 10% FBS (data not shown).

To assess cell migration of LL/2-AB and LL/2-a3 cells stimulated by TPA, the cells were treated with TPA for 48 h. While LL/2-a3 cells showed lower cell migration than LL/2-AB cells, the cell migration abilities of LL/2-AB and LL/2-a3 cells were significantly stimulated by TPA treatment (Fig. 2A). Furthermore, to evaluate the effect of LPA on cell migration stimulated by TPA, LPA was added into the lower chamber of the Cell Culture Insert and TPA-treated cells were incubated for 24 h. LPA treatment significantly increased the cell migration of both types of cells treated with TPA (Fig. 2B).

LPAR gene expressions in LL/2-AB and LL/2-a3 cells treated with TPA were measured by semiquantitative RT-PCR and quantitative real-time RT-PCR analyses. No effect of TPA treatment on the expression levels of Lpar1, Lpar2 and Lpar3 was detected in either type of cells (Fig. 3A, B).

LPA3 acts as a positive or negative regulator of cell migration, depending on the cell type. In human ovarian cancer cells, exogenous LPAR1, LPAR2 or LPAR3-expressing cells acquired high malignant potency, and transfection of small interfering RNAs suppressed cell migration and invasion of those cells. By contrast, the exogenous Lpar3-expressing LL/2-a3 cells showed lower cell migration than control LL/2-AB cells.

TPA has several biological effects, including the inhibition of gap junctional intercellular communication and stimulation of cell migration through activation of PKC in various cell types. Thus, to clarify the involvement of LPA3 in cell migration of mouse lung tumor cells treated with TPA, we investigated cell migration of LL/2-a3 and LL/2-AB cells treated with TPA, and measured the expression levels of LPA receptor genes in those cells. The results indicated that TPA stimulated the cell migration abilities of both LL/2-a3 and LL/2-AB cells, but did not affect the expression levels of their LPAR genes, suggesting that LPA3 may not be involved in cell migration of mouse lung tumor cells stimulated by TPA.

A previous report showed that LPARs may form heterodimers with other receptors, resulting in novel signaling and different functional behaviors. Therefore, the involve-
Fig. 2. The cell migration assay. Columns indicate the means of three studies; bars indicate SD. (A) Cell migration of LL/2-AB and LL/2-a3 cells stimulated by TPA. Cells were treated with or without TPA for 48 h. (B) Effects of LPA on TPA-stimulated cell migration of LL/2-AB and LL/2-a3 cells. TPA-treated cells were incubated for 24 h with or without LPA (10 μM). Cells incubated without LPA were used as the control.

Fig. 3. (A) Expression patterns of LPAR gene mRNAs by semiquantitative RT-PCR analysis. (B) Relative expression levels of LPAR gene mRNAs relative to Gapdh mRNA by quantitative real-time RT-PCR analysis. LL/2-AB and LL/2-a3 cells were treated with TPA for 48 h.
ment of other LPARs in cell migration of tumor cells treated with TPA should be further studied.

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References

1. Lin M-E, Herr DR, and Chun J. Lysophosphatidic acid (LPA) receptors: Signaling properties and disease relevance. Prostaglandins Other Lipid Mediat. 91: 130–138. 2010. [Medline] [CrossRef]

2. Aoki J, Inoue A, and Okudaira S. Two pathways for lysophosphatidic acid production. Biochim. Biophys. Acta. 1781: 513–518. 2008. [Medline] [CrossRef]

3. Ishii I, Fukushima N, Ye X, and Chun J. Lysophospholipid receptors: signaling and biology. Annu Rev Biochem. 73: 321–354. 2004. [Medline] [CrossRef]

4. Contos JJA, Ishii I, and Chun J. Lysophosphatidic acid receptors. Mol Pharmacol. 58: 1188–1196. 2000. [Medline] [CrossRef]

5. Fang X, Gaudette D, and Furui T. Lysophospholipid growth factors in the initiation, progression, metastases, and management of ovarian cancer. Ann NY Acad Sci. 905: 188–208. 2000. [Medline] [CrossRef]

6. Fang X, Schummer M, Mao M, Yu S, Tabassam FH, Swaby R, Hasegawa Y, Tanyi JL, LaPushin R, Eder A, Jaffe RB, Erickson J, and Mills GB. Lysophosphatidic acid is a bioactive mediator in ovarian cancer. Biochim Biophys Acta. 1582: 257–264. 2002. [Medline] [CrossRef]

7. Furui T, LaPushin R, Mao M, Khan H, Watt SR, and Watt MAV. Overexpression of Edg-2/vzg-1 induces apoptosis and anoikis in ovarian cancer cells in a lysophosphatidic acid-independent manner. Clin Cancer Res. 5: 4308–4318. 1999. [Medline] [CrossRef]

8. Goetzl EJ, Dolezalova H, Kong Y, Hu YL, Jaffe RB, Kalli KR, and Conover CA. Distinctive expression and functions of the type 4 endothelial differentiation gene-encoded G protein-coupled receptor for lysophosphatidic acid in ovarian cancer. Cancer Res. 59: 5370–5375. 1999. [Medline] [CrossRef]

9. Shida D, Watanabe T, Aoki J, Hama K, Kitayama J, Sonoda H, Kishi Y, Yamaguchi H, Sasaki S, Sako A, Konishi T, Arai H, and Nagawa H. Aberrant expression of lysophosphatidic acid (LPA) receptors in human colorectal cancer. Lab Invest. 84: 1352–1362. 2004. [Medline] [CrossRef]

10. Shida D, Kitayama J, Yamaguchi H, Okaji Y, Tsuno NH, Watanabe T, Takuya Y, and Nagawa H. Lysophosphatidic acid (LPA) enhances the metastatic potential of human colon carcinoma DLD1 cells through LPA1. Cancer Res. 63: 1706–1711. 2003. [Medline] [CrossRef]

11. Schulte KM, Beyer A, Kohrer K, Oberhauser S, and 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. 92: 249–256. 2001. [Medline] [CrossRef]

12. Tsujiuo M, Fujii M, Okabe K, Mori T, Fukushima N, and Tsujiuchi T. Differential expressions and DNA methylation patterns of lysophosphatidic acid receptor genes in human colon cancer cells. Virchows Arch. 457: 669–676. 2010. [Medline] [CrossRef]

13. Okabe K, Hayashi M, Wakabayashi N, Yamawaki Y, Teranishi M, Fukushima N, and Tsujiuchi T. Different expressions and DNA methylation patterns of lysophosphatidic acid receptor genes in mouse tumor cells. Pathobiology. 77: 309–314. 2010. [Medline] [CrossRef]

14. Yu S, Murph MM, Lu Y, Liu S, Hall HS, Liu J, Stephens C, Fang X, and Mills GB. Lysophosphatidic acid receptors determine tumorigenicity and aggressiveness of ovarian cancer cells. J Natl Cancer Inst. 100: 1630–1642. 2008. [Medline] [CrossRef]

15. Hayashi M, Okabe K, Yamawaki Y, Teranishi M, Honoki K, Mori T, Fukushima N, and Tsujiuchi T. Loss of lysophosphatidic acid receptor-3 enhances cell migration in rat lung tumor cells. Biochem Biophys Res Commun. 405: 450–454. 2011. [Medline] [CrossRef]

16. Yotti LP, Chang CC, and Trosko JE. Elimination of metabolic cooperation in Chinese hamster cells by a tumor promoter. Science. 206: 1089–1091. 1979. [Medline] [CrossRef]

17. Enomoto T, Sasaki Y, Shibuya H, Kanno Y, and Yamaki H. Tumor promoters cause a rapid and reversible inhibition of the formation and maintenance of electrical cell coupling in culture. Proc Natl Acad Sci. USA. 78: 5628–5632. 1981. [Medline] [CrossRef]

18. Lin CW, Shen SC, Chien C, Yang LY, Shia LT, and Chen YC. 12-O-Tetradecanoylphorbol-13-acetate-induced invasion/migration of glioblastoma cells through activating PKCa/ERK/NF-kB-dependent MMP-9 expression. J Cell Physiol. 225: 472–481. 2010. [Medline] [CrossRef]

19. Nabeshima K, Komada N, Kishi J, Koita H, Inoue T, Hayakawa T, and Koono M. TPA-enhanced invasion of matrigel associated with augmentation of cell motility but not metalloproteinase activity in a highly metastatic variant (L-10) of human rectal adenocarcinoma cell line RCM-1. Int J Cancer. 55: 974–981. 1993. [Medline] [CrossRef]

20. Zaslavsky A, Singh LS, Tan H, Ding H, Liang Z, and Xu Y. Homo- and hetero-dimerization of LPA/SIP receptors, OGR1 and GPR4. Biochim Biophys Acta. 1761: 1200–1212. 2006. [Medline]