Short Communication

Enhancement of Drug Resistance by Lysophosphatidic Acid Receptor-3 in Mouse Mammary Tumor FM3A Cells

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Abstract: Lysophosphatidic acid (LPA) acts as a simple phospholipid that interacts with G protein-coupled transmembrane LPA receptors. Recently, it has been reported that each LPA receptor plays different biological roles in acquisition of the malignant property of tumor cells. In this study, to assess the involvement of LPA receptor-3 (LPA3) in cell survival after treatment with anticancer drugs, we generated Lpar3-expressing FM3A-a3A9 cells from mouse mammary tumor FM3A cells and examined the cell survival rate after treatment with anticancer drugs compared with Lpar3-unexpressing cells. Cells were treated with 0.005 to 10 μM of cisplatin (CDDP) or doxorubicin (DOX) for 3 days. For the CDDP and DOX treatments, the cell survival rate of FM3A-a3A9 cells was significantly higher than that of Lpar3-unexpressing cells. The expression level of the Mdr1a gene in FM3A-a3A9 cells was higher than that of Lpar3-unexpressing cells, whereas no significant difference in multidrug resistance 1b (Mdr1b) and glutathione S-transferase mu1 (Gstm1) expressions was found. These results suggest that LPA3 may enhance the cell survival rate after treatment with anticancer drugs in mouse mammary tumor cells, correlating with increased expression of the Mdr1 gene. (DOI: 10.1293/tox.25.225; J Toxicol Pathol 2012; 25: 225–228)

Key words: LPA, LPA receptor-3, drug resistance, FM3A, mammary, mouse

Lysophosphatidic acid (LPA) is a simple phospholipid that interacts with at least G protein-coupled transmembrane LPA receptors, LPA1 to LPA6. LPA has several cellular effects through the binding of LPA receptors, such as cell proliferation, migration, differentiation, morphogenesis and protection from apoptosis. There are various expression patterns of LPA receptors in not only normal tissues but also tumor cells, suggesting that their biological functions may be essentially different. In our recent studies using endogenous LPA3 unexpressed cells, exogenous LPA3 stimulated cell migration and tumorigenicity in rat liver tumor cells, whereas cell migration ability was inhibited by exogenous LPA3 in lung tumor cells of the rat and mouse. In cancer cells, multidrug resistance (MDR) refers to a phenomenon of simultaneous resistance to structurally and functionally unrelated anticancer drugs. It is considered that one of the most important mechanisms underlying the acquisition of multidrug resistance is the activation of efflux transporter proteins, such as P-glycoprotein, which is encoded by MDR genes. Moreover, the induction of detoxification enzyme is also involved in multidrug resistance in cancer cells. It is well known that glutathione S-transferases (GSTs) represent a major family of detoxification enzymes. Recently, we reported that exogenous LPA3 increased the cell survival rate after treatment with cisplatin (CDDP) and doxorubicin (DOX) in rat liver tumor cells.

Although aberrant expression levels of LPA3 were detected in breast cancer cells, the biological functions of LPA2 and LPA3 in the pathogenesis of breast cancer are still unclear. Multidrug resistance and poor clinical outcome are closely linked in several cancers, including breast cancer. In the present study, to better understand the involvement of LPA3 in the acquisition of multidrug resistance, we generated Lpar3-expressing cells from mouse mammary tumor FM3A cells, and investigated cell survival rate after CDDP and DOX treatment. Furthermore, the expression levels of multidrug resistance 1a (Mdr1a), Mdr1b and glutathione S-transferase mu1 (Gstm1) genes were also measured. FM3A cells expressed Lpar2, but not Lpar1 and Lpar3 due to DNA methylation.

Cells were maintained in Dulbecco’s modified Eagle’s medium (DMEC) (Wako Pure Chemical Industries, Ltd.,...
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Osaka, Japan) containing 10% fetal bovine serum (FBS) in 5% CO₂ atmosphere at 37°C. To generate Lpar3-expressing (FM3A-a3A9) cells from FM3A cells, we used retroviruses co-expressing green fluorescent protein (GFP) from an internal ribosomal entry site as described previously. As control cells, FM3A-AB (vector) cells were also used.

Cells were plated at 2000 cells/well in a 96-well plate and cultured with 100 μl of DMEM containing 10% FBS. To measure cell growth rate for 3 days, solution from a Cell Counting Kit-8 (CCK-8) (Dojin Chemistry, Kumamoto, Japan) was added to each well at 0, 1 or 3 days and further incubated for 1 h. The absorbance of the culture medium at 450 nm was then determined. To assess the effects of LPA on cell growth, cells were also cultured in serum-free DMEM with or without 1 or 10 μM LPA (Avanti Polar Lipids, Inc., Alabaster, AL, USA) for 3 days, which were added every 24 h. Cell viability was also measured with CCK-8 (Dojin Chemistry).

To evaluate the expression levels of Mdr1a, Mdr1b and Gstm1 genes, quantitative real-time RT-PCR (RT, reverse transcription; PCR, polymerase chain reaction) analysis using SYBR Premix Ex Taq (TaKaRa Bio, Inc., Shiga, Japan) and a Smart Cycler II System (TaKaRa) was performed. The primer pairs used in this study were as follows: for mouse Mdr1a, F 5’-AAATCCAGCGACAGCAGC-3’, R 5’- TGCCAAATGGTGAAGCCCTG-3’; for mouse Mdr1b, F 5’-TCATGAAACTGCCCCACCAA-3’, R 5’-GGGCAATGGCGATTCTCTGT-3’; and for mouse Gstm1, F 5’-AAGTTCAAGCTGGCCCTGGA-3’, R 5’-GATGGCATTGCTCTGGGTGA-3’. The data of the target genes were normalized to Gapdh.

Since the Lpar3 gene expression in FM3A cells was undetectable, we generated Lpar3-expressing FM3A-a3A9 cells (Fig. 1A). The Lpar3 expression levels in each

![Fig. 1](image-url)

(A) Morphology of FM3A-AB (control vector), and the Lpar3-expressing FM3A-a3A9 cells in serum-containing medium. (B) Expression patterns of the Lpar3 gene in mouse mammary tumor cells by semiquantitative RT-PCR analysis. (C) Cell proliferation rate of mouse mammary tumor cells. Cells were cultured in serum-containing medium. Data are expressed as percentage of cell numbers on day 0. (D) The effects of LPA on cell growth in FM3A-AB and FM3A-a3A9 cells. Cells were cultured with or without LPA (1 or 10 μM), and cell proliferation was measured using a CCK-8. Columns indicate the means of three studies; bars indicate SD.
cell were confirmed by semi-quantitative RT-PCR analysis (Fig. 1B). The cell growth rate of each clone was the same in DMEM containing 10% FBS (Fig. 1C). To examine the effects of LPA on cell proliferation of each clone, cells were cultured in serum-free DMEM with or without LPA treatment. LPA significantly increased cell proliferation ability at 1 and 10 μM in FM3A-a3A9 cells, while the proliferation rate of FM3A-AB cells was stimulated at 10 μM of LPA (Fig. 1D). The stimulation of growth rate of FM3A-AB cells may be due to other expressed LPA receptors.

Next, to assess whether Lpar3-expressing cells may show multidrug resistance, two chemotherapeutic drugs with different modes of action, CDDP and DOX, were used. Cells were treated with CDDP or DOX for 3 days. The cell viability of FM3A-a3A9 cells was significantly higher at a dose of 0.1, 0.5 and 1 μM of CDDP than that of FM3A-AB cells (LC50=0.24 μM and 0.09 μM, respectively). For DOX treatment, FM3A-a3A9 cells showed significantly higher cell viability at a dose of 0.1, 0.5, 1 and 10 μM than FM3A-AB cells (LC50=0.23 μM and 0.18 μM, respectively) (Fig. 2A). Using quantitative real-time RT-PCR analysis, the expression levels of Mdr1a, Mdr1b and Gstm1 genes in FM3A-a3A9 and FM3A-AB cells were measured. No significant differences were found in the Mdr1b and Gstm1 genes between FM3A-a3A9 and FM3A-AB cells. By contrast, the expression of the Mdr1a gene in FM3A-a3A9 cells was approximately 2.0-fold higher than that in FM3A-AB cells (P<0.01) (Fig. 2B). Mdr1a and Mdr1b proteins are multidrug transporters and reduce the intracellular drug content to sublethal levels in cells. Overexpression of these transporters has been detected in several cancer cells and correlated with poor responses to chemotherapeutic agents8,9. Therefore, this suggests that LPA3 may enhance the cell survival rate after treatment with CDDP and DOX through induction of the Mdr1a gene in FM3A cells. However, the mechanism underlying the induction of Mdr1a expression by exogenous LPA3 remains to be clarified.

In cancer cells, the distinct expression levels of LPA3 have been reported. In our recent report, a variety of LPA3 expression patterns were found in human colon cancer cells, correlating with the DNA methylation status of the LPAR3 gene16. Therefore, it seems that the biological role of LPA3 may not be equivalent, depending on the types of cells. So far, functional analyses for LPA3 in cancer cells have been performed by the induction of exogenous LPA3 or shRNAs. In ovarian cancer cells, LPA3 increased these cell abilities by lentivirus constructs, and inhibited cell migration and invasion by shRNAs17. Moreover, LPAR3-expressing cells increased primary tumor size, ascites volume and metastatic potency to distant organs and reduced the survival rate of mice in a mouse xenograft model17. In rat liver cells, LPA3 induced by 12-O-tetradecanoylphorbol-13-acetate, which is a tumor promoting agent, stimulated cell migration and invasion by shRNAs18. In our recent report, the cell survival of Lpar3-expressing liver tumor cells treated with anticancer drugs was elevated, correlating with the expression levels of Mdr1a, Mdr1b and Gspt6. Therefore, this evidence suggests that LPA3 may contribute to the acquisition of malignant potency in cancer cells. By contrast, exogenous LPA3 inhibited cell motility of Lpar3-expressing lung cancer cells in the murine7.

In conclusion, LPA3 is involved in the enhancement of cell survival rate after treatment with CDDP and DOX in mouse mammary tumor cells, correlating with the increased Mdr1a expression. Taken together with our previous report6,
LPA₃ may be a target molecule for a novel chemotherapeutic approach in cancer cells.

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