Lysophosphatidic Acid and Autotaxin Stimulate Cell Motility of Neoplastic and Non-neoplastic Cells Through LPA$_1$

Kotaro Hama$^1$, Junken Aoki$^{1*}$, Masahiro Fukaya$^2$, Yasuhiro Kishi$^1$, Teruyuki Sakai$^3$, Rika Suzuki$^3$, Hideo Ohta$^3$, Takao Yamori$^4$, Masahiko Watanabe$^2$, Jerold Chun$^5$ and Hiroyuki Arai$^1$

$^1$ Graduate School of Pharmaceutical Sciences, The University of Tokyo, 7-3-1, Hongo, Bunkyo-ku, Tokyo, 113-0033, Japan

$^2$ Department of Anatomy, Hokkaido University School of Medicine, Sapporo 060-8638, Japan

$^3$ Pharmaceutical Research Laboratory, Kirin Brewery Co., Ltd., 3 Miyahara, Takasaki, Gunma 370-1295, Japan

$^4$ Division of Molecular Pharmacology, Cancer Chemotherapy Center, Japanese Foundation for Cancer Research, Toshima-ku, Tokyo 170-8455, Japan

$^5$ Department of Molecular Biology, The Scripps Research Institute, 10550 North Torrey Pines Rd, ICND-118, La Jolla, CA 92037

* Corresponding author

E-mail: jaoki@mol.f.u-tokyo.ac.jp

TEL: 81-3-5842-4723  FAX:81-3-3818-3173

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Abbreviations: LPA, lysophosphatidic acid; ATX, autotaxin; lysoPLD, lysophospholipase D; LPC, lysophosphatidylcholine; PTX, pertussis toxin; MSF, mouse skin fibroblast; PDGF, platelet-derived growth factor; GAPDH, glyceraldehyde-phosphate dehydrogenase
ABSTRACT

Autotaxin (ATX) is a tumor cell motility-stimulating factor, originally isolated from melanoma cell supernatant that has been implicated in regulation of invasive and metastatic properties of cancer cells. Recently, we showed that ATX is identical to lysophospholipase D that converts lysophosphatidylcholine to a potent bioactive phospholipid mediator, lysophosphatidic acid (LPA), raising the possibility that autocrine or paracrine production of LPA by ATX contributes to tumor cell motility. Here we demonstrate that LPA and ATX mediate cell motility-stimulating activity through the LPA receptor, LPA1. In fibroblasts isolated from lpa1−/− mice, but not from wild-type nor lpa2−/−, cell motility stimulated with LPA and ATX was completely absent. In the lpa1−/− cells, LPA-stimulated lamellipodia formation was markedly diminished with a concomitant decrease in Rac1 activation. LPA stimulated the motility of multiple human cancer cell lines expressing LPA1 and the motility was attenuated by an LPA1-selective antagonist, Ki16425. The present study suggests that ATX and LPA1 represent potential targets for cancer therapy.
INTRODUCTION

Cell migration is an important cellular function for many physiological processes such as embryonic morphogenesis, wound healing, immune-cell trafficking, and brain development (1). In addition to physiological functions, cancer cells use migration mechanisms that are similar to those that occur in non-neoplastic cells (2). The principles of cell migration were initially investigated in non-neoplastic fibroblasts, keratinocytes and myoblasts, and additional studies on tumor cells identified the same basic mechanisms. Understanding more about the cellular and molecular basis of different cell migration/invasion mechanisms will help us to explain how cancer cells disseminate and should lead to new treatment strategies.

Lysophosphatidic acid (LPA, 1- or 2-acyl-sn-glycerol-3-phosphate) is a naturally-occurring phospholipid. It evokes a variety of biological responses, including platelet aggregation, smooth-muscle contraction, neurite retraction and cell proliferation (3, 4). LPA stimulates cell migration in many cell types in vitro, including fibroblasts, gliomas, T lymphomas, and colorectal cancer cells (5-7), indicating a potential role of LPA in cellular migration in both physiological and pathological conditions (8). A role for LPA signaling in cancer cell migration received further support from the identification of autotaxin (ATX), a protein previously implicated in neoplastic invasion and metastasis (9), as a major biosynthetic enzyme for LPA. ATX was found to be identical to an LPA-producing enzyme in blood, lysophospholipase D (lysoPLD), which converts lysophosphatidylcholine (LPC) to LPA (10, 11). ATX also shows properties of a nucleotide pyrophosphatase/phosphodiesterase, which might also explain its bioactivities. It has been shown that LPA- and ATX-stimulated cell motility is attenuated by treating cells with pertussis toxin (PTX) (8, 12-13), suggesting that G protein-coupled receptors (GPCRs) coupled with G\textsubscript{i0} are involved. LPA elicits most of the cellular events via signal transduction cascades down stream of its specific GPCRs, LPA\textsubscript{1}/Edg-2, LPA\textsubscript{2}/Edg-4, LPA\textsubscript{3}/Edg-7, which belong to the Edg (endothelial cell differentiation gene) family, and LPA\textsubscript{4}/GPR23, a non-Edg family LPA receptors (4, 14-17). Non-GPCR pathways have been also proposed (18, 19). Several
experiments have demonstrated that these GPCRs can mediate mitogen-activated protein kinase activation, phospholipase C activation and calcium mobilization through PTX-sensitive ($G_{i/o}$) and -insensitive G proteins ($G_{12/13}$ and $G_{q/11/14}$) (4). However the LPA receptor subtype involved in LPA-induced cell motility was remained to be identified.

In this study, we explored the role of each LPA receptor in LPA- and ATX-induced cell migration. Our results clearly indicate that LPA- and ATX-induced cell motility is driven by LPA$_1$ activation. We also suggest that a crucial role of Rac1 activation in LPA$_1$-mediated cell migration.
EXPERIMENTAL PROCEDURES

Reagents
1-oleoyl-LPA (18:1) was purchased from Avanti Polar Lipids Inc. (Alabaster, AL). Other chemicals were purchased from Sigma-Aldrich. Recombinant autotaxin/lysoPLD protein was prepared as described previously (10).

Cell culture
Mouse skin fibroblast (MSF) cells were prepared from skin of newborn mice generated by wild-type or knockout (lpa1−/− single, lpa2−/− single and lpa1−/− lpa2−/− double) intercrosses as described previously (20). MSF cells were cultured in minimum essential medium (Sigma) supplemented with 10% fetal bovine serum (FBS), and cells from the first to the fifth passages were used for all experiments. All cancer cell lines used in this study were cultured in RPMI 1640 (Sigma) supplemented with 5% FBS as described previously (21).

Chemotaxis assay
Cell migration was measured in a modified Boyden chamber as described previously (10). In brief, polycarbonate filters with 5 μm pores (MSF cells) or 8 μm pores (carcinoma cell lines) (Neuro Probe, Inc., Gaithersburg, MD) were coated with 0.001% of fibronectin (Sigma). Cells (1x10^5 cells in 200 μl per well) were loaded into upper chambers and incubated at 37 °C for 3 h to allow migration. The cell migration to the bottom side of the filter was evaluated by measuring optical densities at 590 nm. For PTX and Ki16425 treatment, cells were preincubated with 10 ng ml⁻¹ of PTX for 24 h and 1 μM of Ki16425 for 30 min, respectively.

Quantitative real-time RT-PCR
Total RNA from cells was extracted using ISOGEN (Nippongene, Toyama, Japan) and reverse-transcribed using SuperScript First-strand Synthesis System for RT-PCR (Invitrogen, Carlsbad, CA). Oligonucleotide primers for PCR were designed using Primer Express Software (Applied Biosystems, Foster City, CA). The sequences of the oligonucleotides used in PCR reaction were as follows. LPA₁ (mouse)-forward gagaatcgagacccatgat, LPA₁ (mouse)-reverse acatccagcataaaacagcaac, LPA₁ (human)-forward aatcgggataccatgatgagtctt, LPA₁ (human)-reverse ccaggtcagcacagtataaa, LPA₂ (mouse)-forward accacactcagcctgaagcag, LPA₂ (mouse)-reverse cagggagcagcagcctgg, LPA₂ (human)-forward cgctcagctgtgaagact, LPA₂ (human)-reverse tgctcagctcagctaaac, LPA₃ (mouse)-forward gcctcataagctgaagac, LPA₃ (mouse)-reverse aggcgtcagacgaca, LPA₃ (human)-forward aggacacccatgaagctaatgaa, LPA₃ (human)-reverse ggcgtcagagagcagcagc, LPA₄ (mouse)-forward cagtcctcagttgttcttc, LPA₄ (mouse)-reverse gaggggccaggtttgtgtat, LPA₄ (human)-forward cctagtcctcagtggcggtatt, LPA₄ (human)-reverse ccttcaagcaggtttgtt. GAPDH (mouse/human)-forward gccaaggtcatccatgacaact, GAPDH (mouse/human)-reverse gcctcagtcagctgcgtt. PCR reactions were performed using an ABI Prism 7000 Sequence Detection System (Applied Biosystems). The transcript number of mouse GAPDH was quantified, and each sample was normalized on the basis of GAPDH content.

Intracellular calcium mobilization

A-2058 cells were incubated with 5 µM fura-2 acetoxymethyl ester (Dojin, Tokyo, Japan) in calcium ringer buffer (150 mM NaCl, 4 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂, 5.6 mM glucose, 0.1% BSA, and 5 mM HEPES (pH 7.4)) at 37 °C for 30 min. Following stimulation with LPA, cytosolic calcium was measured by monitoring fluorescence intensity at an emission wavelength of 500 nm, and excitation wavelengths of 340 and 380 nm using a CAF-110 (JACS, Tokyo, Japan).
Fluorescence microscopy

MSF cells were seeded onto glass coverslips, grown in the presence of serum to subconfluence, and starved for 24 h by replacing the medium with serum-free medium containing 0.1 % BSA. Then the cells were treated with 1 μM LPA in serum-free medium for 3 h and stained for F-actin with BODIPY FL phallacidin (Molecular Probes) according to the manufacture’s protocol.

Rac1 and RhoA activity assays

Measurement of Rac1 and RhoA activities was performed as described previously (22). Cells starved for 24 h were stimulated with LPA (1 μM) and lysed for 5 min in ice-cold cell lysis buffer containing GST-aPAK or GST-Rhotekin. The cell lysates were incubated with Glutathione-Sepharose 4B (Amersham Pharmacia) for 60 min at 4 °C. After the beads had been washed with the cell lysis buffer, the bound proteins were analyzed by western blotting using anti-Rac1 antibody (Transduction Laboratories) or anti-RhoA antibody (Santa Cruz Biotechnology).
RESULTS
To determine whether LPA receptors are required for LPA-dependent cell motility and, if so, which receptor subtype and signaling cascade is utilized, we generated mouse skin fibroblasts (MSF cells) isolated from newborn mice. The MSF cells expressed LPA_1, LPA_2 and LPA_4 with undetectable level of LPA_3 as judged by quantitative real-time RT-PCR (Fig. 1B). In the Boyden chamber assay, MSF cells migrated in response to LPA and the response was PTX-sensitive (Fig. 1A). We therefore examined LPA-induced cell motility in MSF cells isolated from previously established LPA receptor knockout mice (20, 23). The migratory response was completely abolished in MSF cells isolated from lpa_1^-/- mice (Fig. 2A). MSF cells from lpa_2^-/- mice migrated normally in response to LPA (Fig. 2A). The lpa_1^-/- MSF cells migrated normally in response to platelet-derived growth factor, a potent inducer of migration for fibroblasts (Fig. 2B), indicating that the lpa_1^-/- cells have defects in their response to LPA but not migration per se. We also found that ATX stimulated the migration of MSF cells (Fig. 3) in a PTX-sensitive manner (data not shown). The migratory response induced by ATX also disappeared in MSF cells from lpa_1^-/- mice but not from wild-type nor lpa_2^-/- mice (Fig. 3). These data demonstrated that of the three LPA receptors expressed in the MSF cells, LPA_1 is at least essential for LPA-stimulated cell migration. They also show that the motility effects of ATX are mediated by LPA signaling.

We next examined whether LPA_1 is involved in LPA- or ATX-induced cell motility of carcinoma cells by using various carcinoma cell lines that differentially express LPA receptors. LPA stimulated cell migration of multiple carcinoma cell lines including MDA-MB-231 (breast cancer), PC-3 (prostate cancer), A-2058 (melanoma), A549 (lung cancer), ACHN (renal cancer), SF295 (glioblastoma) and SF539 (glioblastoma) (Fig. 4). Interestingly, these cells were found to express LPA_1 endogenously as judged by quantitative real-time RT-PCR (Fig. 4). LPA did not support the migration of MCF7 (breast cancer), HT-29 (colorectal cancer), KM-12 (colorectal cancer), OVCAR-4 (ovarian cancer), OVCAR-8 (ovarian cancer), NCI-H522 (lung cancer), LNCaP (prostate cancer) and HeLa (cervical cancer) cells, and these cells did not appreciably
express LPA₁ (Fig. 4). There was no obvious correlation between LPA-induced cell motility and expression of the three other LPA receptors, LPA₂, LPA₃ or LPA₄ (Fig. 4). ATX also induced migratory effects in LPA₁-expressing cells (Fig. 5), but not in cells that did not express LPA₁ (data not shown). Recently, an LPA₁-selective antagonist Ki16425 (Ki values were 0.25 μM for LPA₁, 5.60 μM for LPA₂ and 0.36 μM for LPA₃) was developed (24). It has not been tested whether Ki16425 affects the activation of LPA₄. We then monitored intracellular calcium mobilization in HeLa cells transiently transfected with human and mouse LPA₄ cDNA and found that it was not inhibited by 1 μM of Ki16425 (data not shown). Ki16425 inhibited the migratory response of LPA₁-expressing cells to both LPA and ATX (Fig. 4 and 5). Since Ki16425 is also a weak antagonist for LPA₃, it is possible that LPA₃ could be involved in the LPA- or ATX-stimulated cell motility of LPA₃-expressing cells. However, carcinoma cell lines expressing LPA₃ but not LPA₁ (OVCAR-8 and LNCaP) did not migrate in response to LPA (Fig. 4). In addition, OMPT, an LPA₃-selective agonist we recently developed (25), induced a smaller migratory response in A-2058 cells that express both LPA₁ and LPA₃, although LPA stimulated migration effectively (Fig. 6A). OMPT did activate intracellular calcium mobilization more effectively than LPA (Fig. 6B), indicating that OMPT activates LPA₃ in A-2058 cells. These results argue against the possibility that LPA₃ mediates LPA- or ATX-induced cell motility stimulating activity. Thus, it can be concluded that LPA and ATX stimulate cell motility through LPA₁, but not through other LPA receptors, at least for the range of neoplastic cells examined here.

It is generally accepted that locomotion in cellular migration involves reorganization of the actin cytoskeleton as is observed in lamellipodia and stress fiber formation (26, 27). LPA signaling stimulates cytoskeletal reorganization in various cell type through activation of Rho GTPases. However, the molecular mechanisms underlined, particularly at receptor level, remained to be solved. We recently showed that LPA-induced stress fiber formation in mouse embryonic meningeal fibroblast cells (MEMFs) requires either LPA₁ or LPA₂ activation based on the observation that it was severely affected in MEMFs from lpa₁⁻/⁻ lpa₂⁻/⁻ mice but not in MEMFs
from wild-type, \textit{lpa}_1^{-/-} \text{ and } \textit{lpa}_2^{-/-} \text{ mice} (20). In the present study, we tried to confirm the same effect in MSF cells derived from each LPA receptor knockout mice. However, we could not evaluate this in the MSF cells because abundant stress fibers were formed even in the absence of LPA. By contrast, we found that LPA-induced lamellipodia formation in MSF cells requires only LPA\textsubscript{1} expression. In wild-type and \textit{lpa}\textsubscript{2}^{-/-} cells, LPA increased the number of cells with ruffling membranes (lamellipodia formation). In contrast, LPA did not affect the lamellipodia formation in both \textit{lpa}\textsubscript{1}^{-/-} and \textit{lpa}\textsubscript{1}^{-/-}\textit{lpa}\textsubscript{2}^{-/-} MSF cells (Fig. 7A and 7B).

The molecular control of actin filament assembly is dependent on the Rho family of small GTPases, particularly RhoA, Rac\textsubscript{1} and Cdc42 (28). Rac\textsubscript{1} regulates lamellipodia, while RhoA regulates the formation of contractile actin-myosin filaments to form stress fibers (28). LPA-stimulated migration of MSF cells was efficiently blocked by pretreatment of the cells with Y-27632 (data not shown), an inhibitor of Rho kinase that inactivates the RhoA pathway. We therefore measured the LPA-induced activation of the two small GTPases, Rac\textsubscript{1} and RhoA in MSF cells. Rac\textsubscript{1} and RhoA were measured with GST-PAK and GST-Rhotekin pull-down assays, respectively. When MSF cells from wild-type and \textit{lpa}\textsubscript{2}^{-/-} mice were stimulated with 1 \(\mu\)M LPA, a GTP-bound form of Rac\textsubscript{1} was dramatically increased (Fig. 8). By contrast, Rac\textsubscript{1} activation was almost completely abolished in both \textit{lpa}\textsubscript{1}^{-/-} and \textit{lpa}\textsubscript{1}^{-/-}\textit{lpa}\textsubscript{2}^{-/-} MSF cells (Fig. 8). While RhoA activation in response to LPA was obvious in wild-type and \textit{lpa}\textsubscript{2}^{-/-} MSF cells, it appeared to be reduced in \textit{lpa}\textsubscript{1}^{-/-} MSF cells, and markedly reduced in \textit{lpa}\textsubscript{1}^{-/-}\textit{lpa}\textsubscript{2}^{-/-} MSF cells (Fig. 8). These results indicate that LPA\textsubscript{1} has a major role in both Rac\textsubscript{1} and RhoA activation induced by LPA stimulation (Fig. 9). The Rac\textsubscript{1} activation is predominantly dependent on LPA\textsubscript{1} whereas RhoA activation is less dependent on LPA\textsubscript{1}. LPA\textsubscript{2} does contribute to the activation of RhoA in the absence of LPA\textsubscript{1} expression. In addition, RhoA can be activated to some extent (Fig. 8) in the absence of LPA\textsubscript{1} and LPA\textsubscript{2}, indicating the presence of other LPA receptors and/or indirect mechanisms of RhoA activation (Fig. 9).
DISCUSSION

LPA is a multifunctional signaling molecule with diverse activities including stimulation of cell motility. Recent identification of lysophospholipase D (lysoPLD), an LPA-producing enzyme, as autotaxin (ATX), a cell-motility stimulating factor of cancer cells (10, 11), indicated that the activity is one of the intrinsic functions of LPA. In this study we showed that among the four LPA receptors identified so far LPA1 has a crucial role in LPA-induced cell migration of fibroblast cells (Fig. 2) and multiple cancer cells (Fig. 4) based on the observation that inactivation of LPA1 either by gene-targeting technique or by a receptor-selective antagonist resulted in loss of migratory response. In addition we observed that the migratory response induced by ATX again disappeared in these cells (Fig. 3 and 5). These results clearly show that ATX exerts its function through LPA production and the following LPA1 activation. Both LPA1 and ATX are highly expressed in brain (14, 29). Recently it was reported that oligodendrocyte express LPA1 and ATX during myelination (30, 31). We also found that LPA1 and ATX are highly enriched within certain regions of developing mouse brain such as olfactory bulb (Fukaya, M., Watanabe, M., Aoki, J., and Arai, H., unpublished result). The colocalization of the two genes suggests that they function co-operatively also in physiological condition.

The migratory responses were found to be PTX-sensitive (Fig. 1A). By contrast, in a previous report, LPA was found to stimulate cell motility of other cell types such as lymphoma cells in a PTX-insensitive manner (6). Because lymphocytes express LPA2 predominantly with no detectable expression of LPA1 (data not shown), it is possible that LPA2 is involved in LPA-induced migratory response of non-carcinoma neoplasms such as lymphoma cells. Splenocytes and thymocytes isolated from wild-type, lpa1−/−, lpa2−/− and lpa1−/−lpa2−/− mice did not show a migratory response to LPA in our system (data not shown). In addition, in the absence of selective agonists or antagonists for LPA2, we could not test the migratory effect of LPA2 on lymphoma cell migration. Further study is necessary to show the role of LPA2 in migratory response of lymphoma cells.
We previously showed that LPA₁ and LPA₂ had redundant functions in mediating multiple endogenous LPA responses including PLC activation, Ca²⁺ mobilization, cell proliferation and stress fiber formation in mouse embryonic fibroblasts (20). In this study, we demonstrated that LPA-induced lamellipodia formation was severely affected in lpa₁⁻/⁻ MSF cells (Fig. 7A and 7B). In addition, we showed that LPA activates Rac1 in LPA₁-dependent manner, whereas it activates RhoA either in LPA₁-, LPA₂-dependent or LPA₁-, LPA₂-independent pathway (Fig. 8). Thus, LPA₁ is able to activate both Rac1 and RhoA regardless of LPA₂ expression (Fig. 9). Because the activation of both RhoA and Rac1 is essential for LPA-stimulated cell migration (12), the lack of Rac1 activation in lpa₁⁻/⁻ MSF cells explains why the cells could not migrate in response to LPA. Many reports have shown that G₁α and G₁₂/₁₃ regulate the activation of Rac1 and RhoA, respectively (12, 32). Thus, our results again suggest that LPA₁ couples with both G₁α and G₁₂/₁₃ whereas LPA₂ mainly couples with G₁₂/₁₃ as we indicated previously using cells transfected with each LPA receptor (Fig. 9) (20, 33). van Leeuwen et al. recently showed that LPA₁, when overexpressed in B103 neuroblastoma cells, mediates LPA-induced cell migration through concomitant activation of Rac1 (12). However, the role of other LPA receptors in cell motility remained to be solved, which we solved in this study (Fig. 2, 8). We showed that multiple carcinoma cells utilize the same mechanism for their LPA-induced cell motility (Fig. 4). It is well accepted that cell motility is closely linked to the metastatic and invasive potential of cancer cells. In addition, the activating pathway of both Rac1 and RhoA were also implicated in tumor invasion and metastasis (34, 35). Furthermore, there is accumulating evidence that elevated expression of ATX is observed in various cancer tissues (36, 37) and that the expression is closely linked to the invasive and metastatic potency of cancer cells (38). We therefore propose that both ATX and LPA₁ are the potential targets for cancer therapy.
FIGURE LEGENDS

Fig. 1. **LPA induces PTX-sensitive cell motility in mouse skin fibroblasts.** (A) LPA stimulates cell motility of MSF cells in a pertussis toxin (PTX)-sensitive manner. MSF cells were pretreated with PTX (100 ng ml\(^{-1}\), 24 h) and LPA-induced cell motility was evaluated using Boyden camber assay. (B) Expression of each LPA receptor mRNA in MSF cells. The level of LPA receptor mRNA in MSF cells was measured using quantitative real-time RT-PCR, and is expressed as a relative value to GAPDH mRNA.

Fig. 2. **LPA\(_1\) is essential for LPA-induced cell motility in mouse skin fibroblasts.** LPA (A)- and PDGF (B)-induced migration of MSF cells isolated from *lpa\(_1\)*\(^{-/-}\) (closed circles), *lpa\(_2\)*\(^{-/-}\) (open circles) and wild-type (open triangles) mice. Results shown are representative of at least three independent experiments. Error bars indicate the s.d. of the mean.

Fig. 3. **LPA\(_1\) is essential for ATX-induced cell motility in mouse skin fibroblasts.** ATX-induced migration of MSF cells isolated from *lpa\(_1\)*\(^{-/-}\) (closed circles), *lpa\(_2\)*\(^{-/-}\) (open circles) and wild-type (open triangles) mice. Results shown are representative of at least three independent experiments. Error bars indicate the s.d. of the mean.

Fig. 4. **LPA\(_1\) is involved in LPA-induced cell motility in multiple carcinoma cell lines.** In each cell line, upper panel shows migratory response to LPA either in the absence (open circles) or presence (closed circles) of an LPA\(_1\)-selective antagonist, Ki16425 (1 \(\mu\)M) evaluated by the Boyden Chamber assay. Lower panel shows expression of each LPA receptor mRNA (*lpa\(_1\)*, *lpa\(_2\)*, *lpa\(_3\)* and *lpa\(_4\)*) measured using quantitative real-time RT-PCR. The LPA-induced migratory responses are appear to be parallel with the expression of LPA\(_1\).
FIG. 5. **Ki16425 inhibits ATX-induced cell motility of carcinoma cell expressing LPA1.**
ATX stimulates cell motility of three LPA1-expressing carcinoma cell lines (MDA-MB-231, PC-3 and A-2058) and the motility is suppressed by an LPA1-selective antagonist, Ki16425.

FIG. 6. **LPA3 is not involved in LPA-induced cell motility.** (A) OMPT did not stimulate cell motility of LPA3-expressing cell, A-2058. (B) OMPT induced an intracellular calcium mobilization of the cells more efficiently than LPA.

FIG. 7. **LPA induces lamellipodia formation through LPA1.** (A) Fluorescence microscopy of BODIPY FL phallacidin-stained MSF cells from wild-type, *lpa1*+/−, *lpa2*+/− and *lpa1*−/−*lpa2*−/− mice before (upper panels) or after (lower panels) the LPA stimulation (1 µM, 3 h). The lamellipodia formation (arrows) were observed in wild-type, and *lpa2*−/− MSF cells, but rarely observed in *lpa1*−/− MSF cells. Bar, 20 µm. (B) Percentage of wild-type, *lpa1*+/−, *lpa2*+/− and *lpa1*−/−*lpa2*−/− MSF cells with lamellipodia after stimulation with 1 µM LPA.

FIG. 8. **LPA-induced activation of Rac1 and RhoA in MSF cells.** MSF cells were serum starved for 24 h and stimulated with 1 µM of LPA. Activated RhoA and Rac1 were isolated using GST-PAK (Rac1, upper panel) or GST-Rhotekin (RhoA, lower panel) coupled to glutathione-Sepharose beads. Rac1 and RhoA bound to the beads were detected by Western blotting using specific antibodies.

FIG. 9. **Model for LPA- or ATX-induced cell motility.** ATX activates Rac1 and RhoA through G_{i} and G_{12/13}, respectively, by producing LPA. The activation of Rac1 and RhoA results in membrane ruffling and stress fiber formation, which finally lead to activation of cell motility. The Rac1 activation is predominantly dependent on LPA1 whereas RhoA activation is induced by
either LPA$_1$ or LPA$_2$ activation. LPA$_1$- and LPA$_2$-independent pathway also contributes partially to the RhoA activation.
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Acknowledgements

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**FIG. 1.**
**FIG. 2.** LPA$_1$ is essential for LPA-induced cell motility in mouse skin fibroblasts. LPA (A)- and PDGF (B)-induced migration of MSF cells isolated from *lpa$_1^{-/-}$* (closed circles), *lpa$_2^{-/-}$* (open circles) and wild-type (open triangles) mice. Results shown are representative of at least three independent experiments. Error bars indicate the s.d. of the mean.
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ATX stimulates cell motility of three LPA$_1$-expressing carcinoma cell lines (MDA-MB-231, PC-3 and A-2058) and the motility is suppressed by an LPA$_1$-selective antagonist, Ki16425.
**FIG. 6.** LPA$_3$ is not involved in LPA-induced cell motility. (A) OMPT did not stimulate cell motility of LPA$_3$-expressing cell, A-2058. (B) OMPT induced an intracellular calcium mobilization of the cells more efficiently than LPA.
FIG. 7A.
FIG. 7B. **LPA induces lamellipodia formation through LPA₁.** (A) Fluorescence microscopy of BODIPY FL phallacidin-stained MSF cells from wild-type, *lpa₁⁻/⁻*, *lpa₂⁻/⁻* and *lpa₁⁻/⁻ lpa₂⁻/⁻* mice before (upper panels) or after (lower panels) the LPA stimulation (1 µM, 3 h). The lamellipodia formation (arrows) were observed in wild-type, and *lpa₂⁻/⁻* MSF cells, but rarely observed in *lpa₁⁻/⁻* MSF cells. Bar, 20 µm. (B) Percentage of wild-type, *lpa₁⁻/⁻*, *lpa₂⁻/⁻* and *lpa₁⁻/⁻ lpa₂⁻/⁻* MSF cells with lamellipodia after stimulation with 1 µM LPA.
**FIG. 8.** **LPA-induced activation of Rac1 and RhoA in MSF cells.** MSF cells were serum starved for 24 h and stimulated with 1 µM of LPA. Activated RhoA and Rac1 were isolated using GST-PAK (Rac1, upper panel) or GST-Rhotekin (RhoA, lower panel) coupled to glutathione-Sepharose beads. Rac1 and RhoA bound to the beads were detected by Western blotting using specific antibodies.
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Lysophosphatidic acid and autotaxin stimulate cell motility of neoplastic and non-neoplastic cells through LPA1

Kotaro Hama, Junken Aoki, Masahiro Fukaya, Yasuhiro Kishi, Teruyuki Sakai, Rika Suzuki, Hideo Ohta, Takao Yamori, Masahiko Watanabe, Jerold Chun and Hiroyuki Arai

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