

Lysophosphatidic Acid and Autotaxin Stimulate Cell Motility of Neoplastic and Non-neoplastic Cells through LPA₁*

Received for publication, December 19, 2003, and in revised form, January 20, 2004
Published, JBC Papers in Press, January 26, 2004, DOI 10.1074/jbc.M313927200

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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, which 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 or *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.

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 (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 lysophospholipase D, an LPA-producing enzyme in blood that converts lysophosphatidylcholine 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_{i/o} are involved. LPA elicits most of the cellular events via signal transduction cascades downstream of its specific GPCRs, LPA₁/Edg-2, LPA₂/Edg-4, LPA₃/Edg-7, which belong to the Edg (endothelial cell differentiation gene) family, and LPA₄/GPR23, a non-Edg family LPA receptor (4, 14–17). Non-GPCR pathways have also been 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 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 LPA₁ activation. We also suggest a crucial role of Rac1 activation in LPA₁-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. Recombinant ATX/lysophospholipase D 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 knock-out (*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, and cells from the first to the fifth passages were used for all experiments. All

* This work was supported in part by research grants from the Ministry of Education, Culture, Sports, Science, and Technology and the Human Frontier Special Program. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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¹ The abbreviations used are: LPA, lysophosphatidic acid; ATX, autotaxin; OMPT, 1-oleoyl-2-O-methyl-*rac*-glycerophosphothionate; PTX, pertussis toxin; MSF, mouse skin fibroblast; GAPDH, glyceraldehyde-phosphate dehydrogenase; Edg, endothelial cell differentiation gene; GST, glutathione S-transferase.

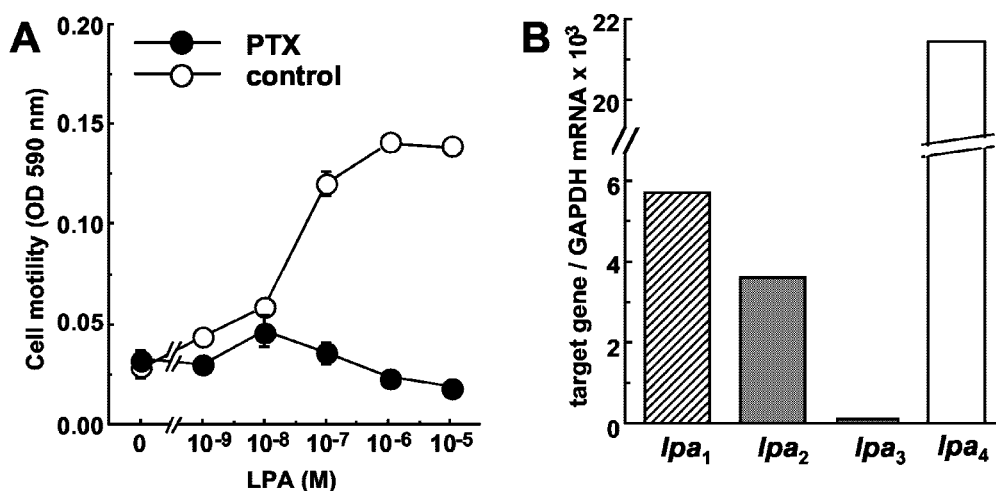


FIG. 1. LPA induces PTX-sensitive cell motility in mouse skin fibroblasts. A, LPA stimulates cell motility of MSF cells in a PTX-sensitive manner. MSF cells were pretreated with PTX (100 ng ml⁻¹, 24 h), and LPA-induced cell motility was evaluated using the Boyden chamber assay. B, expression of each 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.

cancer cell lines used in this study were cultured in RPMI 1640 (Sigma) supplemented with 5% fetal bovine serum 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- μ m (MSF cells) or 8- μ m pores (carcinoma cell lines) (Neuro Probe, Inc., Gaithersburg, MD) were coated with 0.001% of fibronectin (Sigma). Cells (1×10^5 cells in 200 μ l/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 μ M 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 the SuperScript first-strand synthesis system for RT-PCR (Invitrogen). 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 gaggaatcgggacaccatgat; LPA₁ (mouse)-reverse acatccagaataacaagaccatc; LPA₁ (human)-forward aatcgggataccatgatgagtctt; LPA₁ (human)-reverse ccaggatccagcagatgataaa; LPA₂ (mouse)-forward gaccacactcagcctagtcaagac; LPA₂ (mouse)-reverse ctta-cagctcagcgcaccca; LPA₂ (human)-forward cgctcagcctggtaagact; LPA₂ (human)-reverse ttgcaggactcacagcctaacc; LPA₃ (mouse)-forward gctc-catgaagtaatgaagaca; LPA₃ (mouse)-reverse agccgctcagcagcaga; LPA₃ (human)-forward aggacaccatgaagtaagtaaa; LPA₃ (human)-reverse gccgctcagcagcagaa. LPA₄ (mouse)-forward catgctcctgtttgctctc; LPA₄ (mouse)-reverse gagagggccaggttggtgat. LPA₄ (human)-forward ctactctcagctggcgggtatt; LPA₄ (human)-reverse ctccaagcaggttggtggt. GAPDH (mouse/human)-forward gccaaggtcatccatgacaact; GAPDH (mouse/human)-reverse gaggggcatccacagtctt. 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 μ M fura-2 acetoxyethyl 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% bovine serum albumin, 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% bovine serum albumin. Then the cells were treated with 1 μ M LPA in serum-free medium for 3 h and stained for F-actin with BODIPY FL phalloidin (Molecular Probes, Inc., Eugene, OR) according to the manufacturer'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 μ M) and lysed for 5 min in ice-cold cell

lysis buffer containing GST- α PAK or GST-Rhotekin. The cell lysates were incubated with glutathione-Sepharose 4B (Amersham Biosciences) 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 (BD Biosciences) 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 are utilized, we generated MSF cells isolated from newborn mice. The MSF cells expressed LPA₁, LPA₂, and LPA₄ with an undetectable level of LPA₃ 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 knock-out 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 or *lpa*₂^{-/-} mice (Fig. 3). These data demonstrated that of the three LPA receptors expressed in the MSF cells, LPA₁ 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 LPA₁ 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 LPA₁ 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

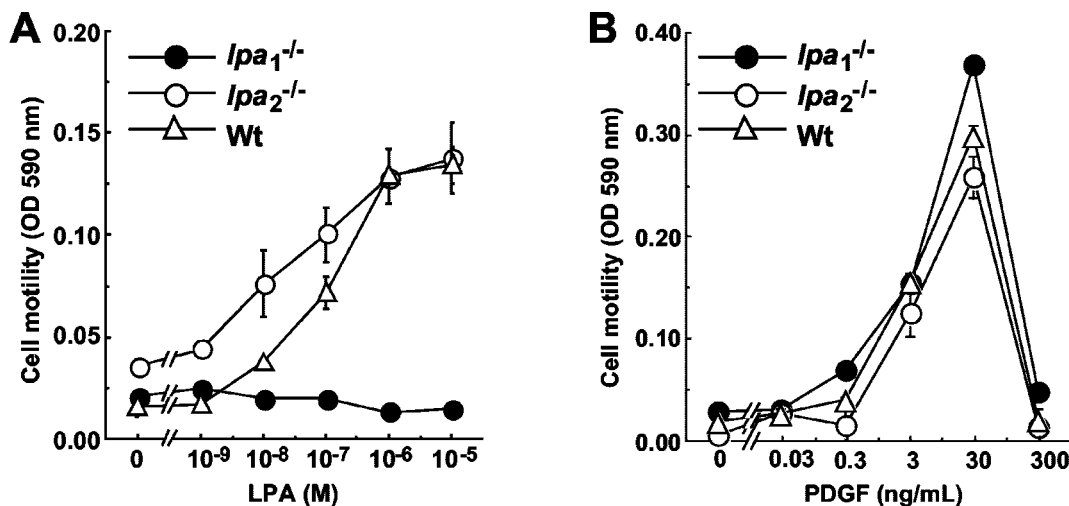


FIG. 2. LPA₁ is essential for LPA-induced cell motility in mouse skin fibroblasts. LPA-induced (A) and platelet-derived growth factor-induced (B) migration of MSF cells isolated from *lpa*₁^{-/-} (filled circles), *lpa*₂^{-/-} (open circles), and wild-type (open triangles) mice. Results shown are representative of at least three independent experiments. Error bars indicate the S.D. of the mean.

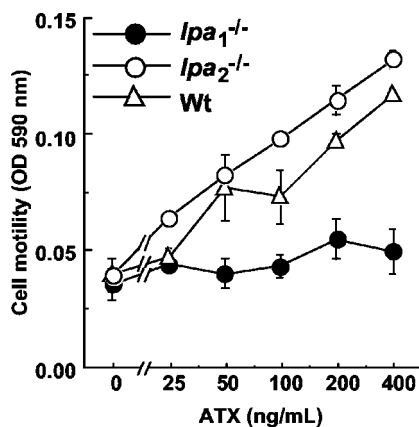


FIG. 3. LPA₁ is essential for ATX-induced cell motility in mouse skin fibroblasts. ATX-induced migration of MSF cells isolated from *lpa*₁^{-/-} (filled circles), *lpa*₂^{-/-} (open circles), and wild-type (open triangles) mice. Results shown are representative of at least three independent experiments. Error bars indicate the S.D. of the mean.

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, (K_i values were 0.25 μ M for LPA₁, 5.60 μ M for LPA₂, and 0.36 μ M for LPA₃) were 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 μ M Ki16425 (data not shown). Ki16425 inhibited the migratory response of LPA₁-expressing cells to both LPA and ATX (Figs. 4 and 5). Because 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 cal-

cium 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 types 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 *lpa*₁^{-/-}*lpa*₂^{-/-} mice but not in MEMFs from wild-type, *lpa*₁^{-/-}, and *lpa*₂^{-/-} mice (20). In the present study, we tried to confirm the same effect in MSF cells derived from each LPA receptor knock-out mouse. 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 LPA₁ expression. In wild-type and *lpa*₂^{-/-} cells, LPA increased the number of cells with ruffling membranes (lamellipodia formation). In contrast, LPA did not affect the lamellipodia formation in *lpa*₁^{-/-} and *lpa*₁^{-/-}*lpa*₂^{-/-} MSF cells (Fig. 7, A and B).

The molecular control of small filament assembly is dependent on the Rho family of small GTPases, particularly RhoA, Rac1, and Cdc42 (28). Rac1 regulates lamellipodia, whereas 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, Rac1 and RhoA, in MSF cells. Rac1 and RhoA were measured with GST-PAK and GST-Rhotekin pull-down assays, respectively. When MSF cells from wild-type and *lpa*₂^{-/-} mice were stimulated with 1 μ M LPA, a GTP-bound form of Rac1 was dramatically increased (Fig. 8). By contrast, Rac1 activation was almost completely abolished in both *lpa*₁^{-/-} and *lpa*₁^{-/-}*lpa*₂^{-/-} MSF cells (Fig. 8). Although RhoA activation in response to LPA was obvious in wild-type and *lpa*₂^{-/-} MSF cells, it appeared to be

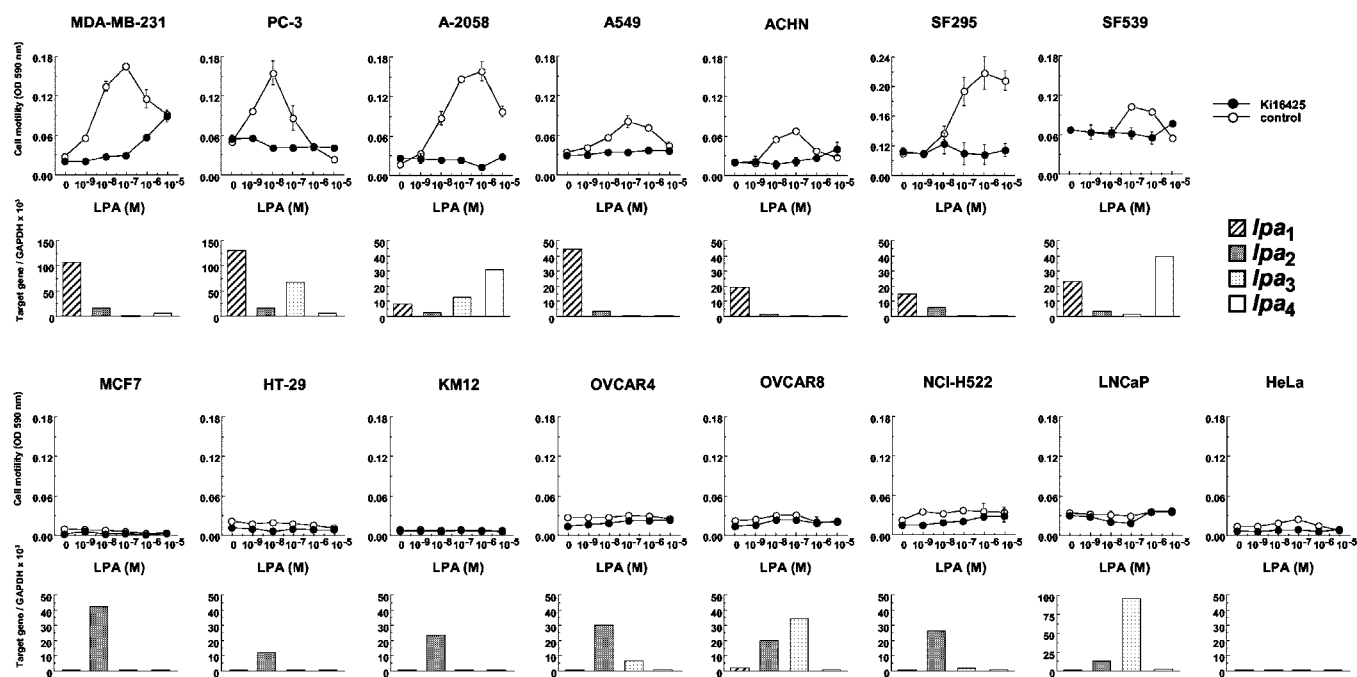


FIG. 4. LPA₁ is involved in LPA-induced cell motility in multiple carcinoma cell lines. In each cell line, the upper panel shows migratory response to LPA either in the absence (open circles) or presence (filled circles) of an LPA₁-selective antagonist, Ki16425 (1 μ M), evaluated by the Boyden chamber assay. The 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 appear to be parallel with the expression of LPA₁.

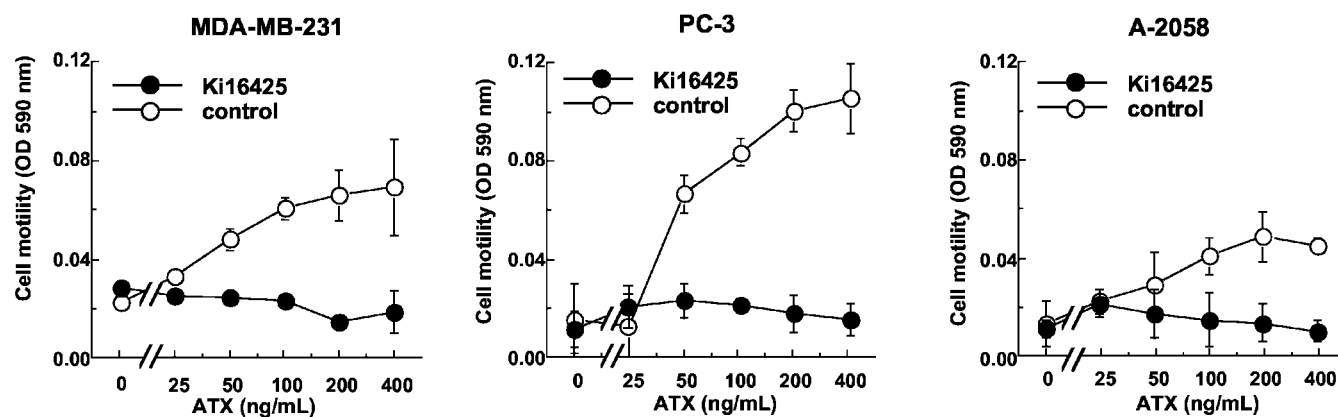


FIG. 5. Ki16425 inhibits ATX-induced cell motility of carcinoma cell-expressing LPA₁. ATX stimulates cell motility of three LPA₁-expressing carcinoma cell lines (MDA-MB-231, PC-3, and A-2058), and the motility is suppressed by an LPA₁-selective antagonist, Ki16425.

reduced in *lpa*₁^{-/-} MSF cells and markedly reduced in *lpa*₁^{-/-} *lpa*₂^{-/-} MSF cells (Fig. 8). These results indicate that LPA₁ has a major role in both Rac1 and RhoA activation induced by LPA stimulation (Fig. 9). The Rac1 activation is predominantly dependent on LPA₁, whereas RhoA activation is less dependent on LPA₁. LPA₂ does contribute to the activation of RhoA in the absence of LPA₁ expression. In addition, RhoA can be activated to some extent (Fig. 8) in the absence of LPA₁ and LPA₂, 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, an LPA-producing enzyme, as 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 (Figs. 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 oligodendrocytes 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.² The colocalization of the two genes suggests that they also function co-operatively 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

² M. Fukaya, M. Watanabe, J. Aoki, and H. Arai, unpublished result.

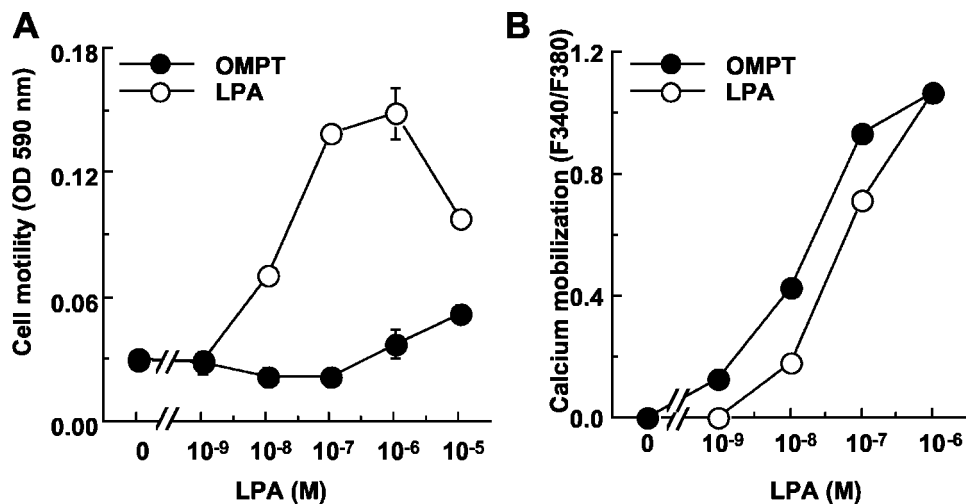


FIG. 6. LPA₃ is not involved in LPA-induced cell motility. A, OMPT did not stimulate cell motility of LPA₃-expressing cell, A-2058. B, OMPT induced an intracellular calcium mobilization of the cells more efficiently than LPA.

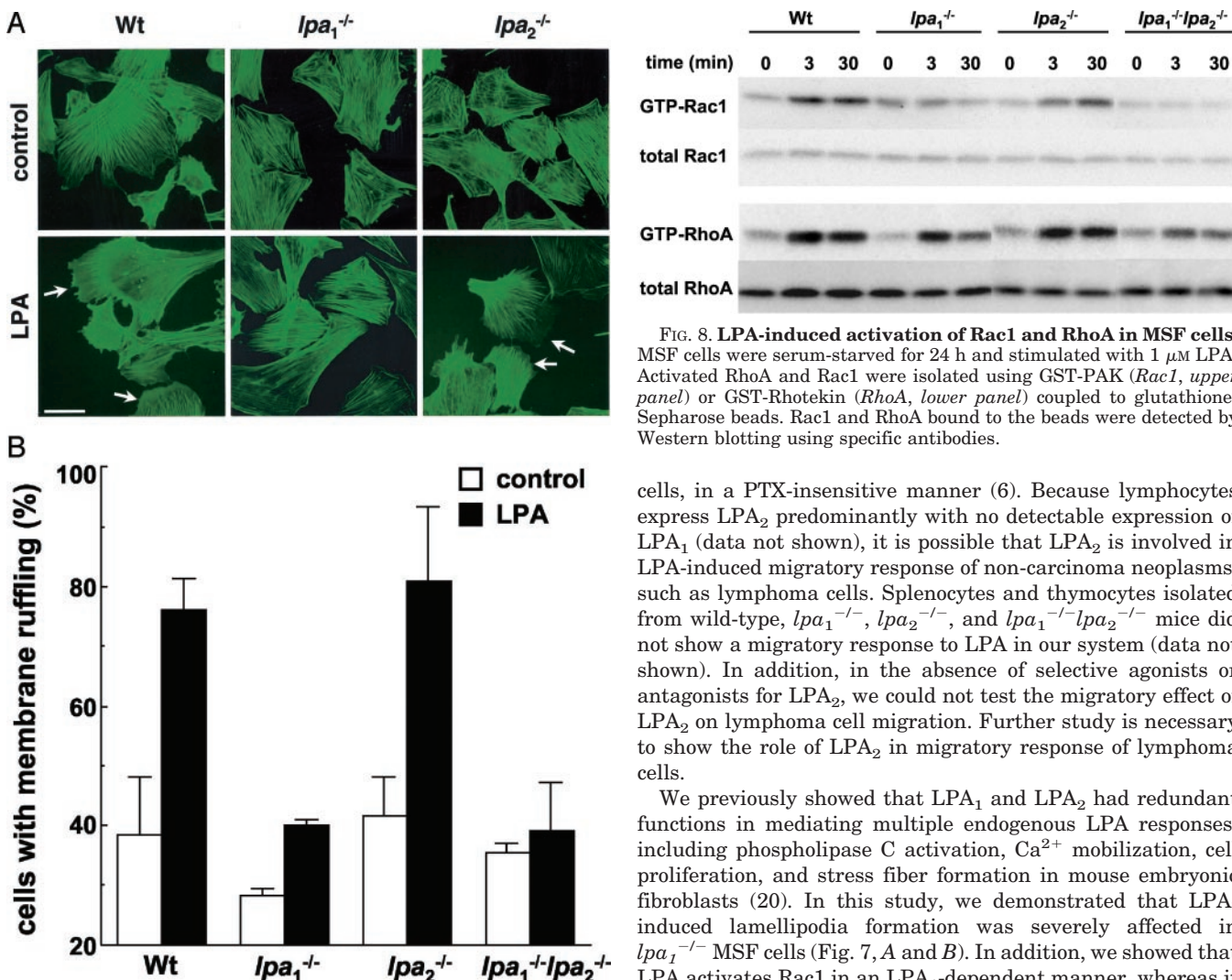


FIG. 7. LPA induces lamellipodia formation through LPA₁. A, fluorescence microscopy of BODIPY FL phalloidin-stained MSF cells from wild-type, *lpa*₁^{-/-}, and *lpa*₂^{-/-} mice before (upper panels) or after (lower panels) LPA stimulation (1 μM, 3 h). The lamellipodia formation (arrows) was observed in wild-type and *lpa*₂^{-/-} MSF cells but rarely observed in *lpa*₁^{-/-} MSF cells. Bar, 20 μm. B, percentage of wild-type, *lpa*₁^{-/-}, *lpa*₂^{-/-}, and *lpa*₁^{-/-}*lpa*₂^{-/-} MSF cells with lamellipodia after stimulation with 1 μM LPA.

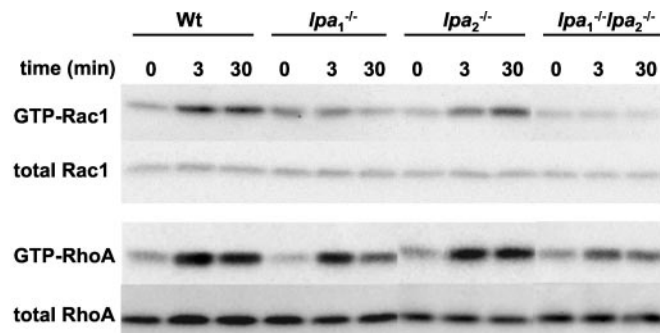


FIG. 8. LPA-induced activation of Rac1 and RhoA in MSF cells. MSF cells were serum-starved for 24 h and stimulated with 1 μM LPA. Activated RhoA and Rac1 were isolated using GST-PAK (*Rac1*, upper panel) or GST-Rhotekin (*RhoA*, lower panel) coupled to glutathione-Sepharose beads. Rac1 and RhoA bound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

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, *lpa*₁^{-/-}, *lpa*₂^{-/-}, and *lpa*₁^{-/-}*lpa*₂^{-/-} 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 phospholipase C 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 *lpa*₁^{-/-} MSF cells (Fig. 7, A and B). In addition, we showed that LPA activates Rac1 in an 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 *lpa*₁^{-/-} MSF cells explains why the cells could not migrate in response to LPA. Many reports have

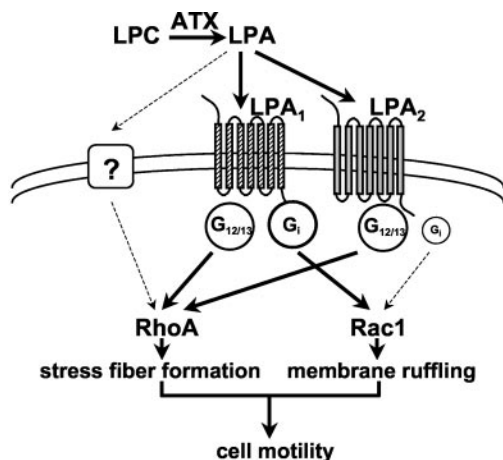


FIG. 9. Model for LPA- or ATX-induced cell motility. ATX activates Rac1 and RhoA through G_i and $G_{12/13}$, respectively, by producing LPA. The activation of Rac1 and RhoA results in membrane ruffling and stress fiber formation, which finally lead to activation of cell motility. The Rac1 activation is predominantly dependent on LPA₁, whereas RhoA activation is induced by either LPA₁ or LPA₂ activation. LPA₁- and LPA₂-independent pathways also contribute partially to RhoA activation.

shown that $G_{i/o}$ and $G_{12/13}$ regulate the activation of Rac1 and RhoA, respectively (12, 32). Thus, our results again suggest that LPA₁ couples with both $G_{i/o}$ and $G_{12/13}$, whereas LPA₂ mainly couples with $G_{12/13}$ as we indicated previously using cells transfected with each LPA receptor (Fig. 9) (20, 33). van Leeuwen *et al.* (12) recently showed that LPA₁, when overexpressed in B103 neuroblastoma cells, mediates LPA-induced cell migration through concomitant activation of Rac1. However, the role of other LPA receptors in cell motility remained to be solved, which we did in this study (Figs. 2 and 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 pathways 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.

Acknowledgments—We thank Dr. M. Negishi (Kyoto University) for advice on GST-pull-down assay and for the gift of GST-PAK and GST-RBD constructs and Drs. S. Ishii and T. Shimizu (University of Tokyo) for the generous gift of LPA₄ cDNA.

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