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# Lysophosphatidic Acid Stimulates SKOV-3 Cell Migration through the Generation of Reactive Oxygen Species *via* the mTORC2/Akt1/NOX Signaling Axis

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Reactive oxygen species (ROS) play an essential role in a variety of cellular physiological phenomena. The present study assessed the signaling axis that mediates the lysophosphatidic acid (LPA)-induced migration of SKOV-3 cells. Insulin-like growth factor-1 (IGF-1) stimulated SKOV-3 cell migration in a time- and dose-dependent manner. Similarly, LPA stimulated SKOV-3 cell migration and the phosphorylation of Akt in a time- and dose-dependent manner. The pharmacological inhibition of LPA receptors ($LPA_1$/$LPA_3$) significantly suppressed LPA-induced SKOV-3 cell migration. However, IGF-1-induced SKOV-3 cell migration was not affected by the inhibition of $LPA_1$ and $LPA_3$. Pharmacological inhibition of phosphoinositide 3-kinase (PI3K) or Rho-associated kinase (ROCK) significantly suppressed LPA-induced migration, whereas the inhibition of MAPK kinase (MEK) had no effect. Inhibition of PI3K or ROCK completely suppressed LPA-induced ROS generation, and suppression of nicotinamide adenine dinucleotide phosphate oxidase (NOX) or chelation of ROS by N-acetylcysteine (NAC) blocked LPA-induced SKOV-3 cell migration. LPA-induced ROS generation was suppressed by silencing Rictor or Akt1 but not Raptor or Akt2. Silencing Rictor or Akt1 significantly suppressed LPA-induced SKOV-3 cell migration, whereas silencing Raptor or Akt2 had no effect. Finally, the overexpression of the constitutively active form Akt1 (CA-Akt1) significantly enhanced the LPA-induced migration of SKOV-3 cells. Given these results, we suggest that LPA stimulates SKOV-3 cell migration by ROS generation, which is mediated by the mTORC2/Akt1/NOX signaling axis.

**Key words :** Akt, migration, mTOR, NOX, ROS

## Introduction

Control of cell migration in response to various extracellular stimuli has been known to be important in the proper gastrulation during embryogenesis [37]. It has been reported that cell migration is mainly mediated by growth factors such as platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), and insulin-like growth factor-1 (IGF-1). Growth factors induce the activation of several signaling mediators such as phosphoinositide 3-kianse (PI3K), Ras, and phospholipase C-γ (PLC-γ) [18]. Among the signaling molecules of growth factor receptors, PI3K is the major responsible mediator that regulate cell migration [15]. Mammalian target of rapamycin (mTOR) which is a serine/threonine kinase comprises two distinct molecular complexes with either Raptor (mTORC1) or Rictor (mTORC2), and is the major downstream target of PI3K [20]. mTORC1 controls cellular growth through the regulation of protein translation *via* S6 kinase (S6K) and mTORC2 activates Akt thereby regulates variety of Akt-mediated cellular responses [23].

Akt is a serine/threonine kinase that is activated by PI3K [10]. Akt is activated by phosphorylation at Thr308 which is mediated by phosphatidylinositol 3-phosphate-dependent kinase 1 (PDK1) and maintained active form by phosphorylation at Ser473 which is mediated by mTORC2 [35]. Akt consists of three isoforms (Akt1, Akt2, and Akt3) that are encoded by three different genes, and share more than 80% in amino acid sequence [3]. Even though Akt isoforms share high sequence homology, isoform-specific and non-redundant function has been reported. For example, mice lacking Akt1 isoform show smaller organism size than wild-type throughout their life span whereas mice lacking Akt2 isoform show type 2 diabetic-like syndrome with normal organism size [7, 8]. It also has been reported that growth factor-induced cell migration and insulin-dependent glucose uptake are exclu-

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sively mediated by Akt1 and Akt2 isoform, respectively [1, 21]. However, it is still not clear whether each Akt isoform has distinct function in cell migration induced by G protein-coupled receptor (GPCR) signaling cascade.

Lysophosphatidic acid (LPA, or 2-acyl-*sn*-glycero-3-phosphate) is a phospholipid ligand that normally exists in serum and body fluid, and regarded as a biomarker for ovarian cancer since high level of LPA was detected in the plasmas and ascetic fluids of ovarian cancer patients [47]. LPA receptor is categorized into GPCR and consists $LPA_1$/Edg-2, $LPA_2$/Edg-4, and $LPA_3$/Edg-7 [9]. Occupation of LPA to its cognate receptor leads to the activation of several downstream signaling molecules such as mitogen-activated protein kinase (MAPK), c-Jun N-terminal kinase (JNK), p38 MAPK, and PI3K [2, 27, 28, 39]. Although it has been reported that LPA induces ovarian cancer cell migration [14, 17], the downstream signaling mechanism of ovarian cancer cell migration is yet to be elucidated.

Reactive oxygen species (ROS) is a highly bioactive molecules and have been studied in various types of cancers [31]. ROS are generated as byproducts of a variety of cellular processes such as nucleic acid metabolism by xanthine oxidase, lipid metabolism by cyclooxygenase (COX), and leakage of electron from respiratory chain in mitochondria [4, 38]. In addition to the generation of ROS as byproduct of cellular process, ROS also can be generated actively by nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX) [44]. NOX is activated by extracellular stimuli and comprises NOX1-5 and dual oxidase (DUOX)1-2. NOX1 is fully activated by complex formation with NOXO1, $p22^{phox}$, $p47^{phox}$ whereas NOX2 forms complex with $p47^{phox}$, $p67^{phox}$, $p22^{phox}$, and Rac [5, 24]. NOX3 requires $p22^{phox}$, NOXO1, and NOXA1 for the activation in the physiological condition [6]. NOX4 requires only $p22^{phox}$ and shows constitutively active status in the absence of additional regulatory factors [32]. NOX5, DUOX1, and DUOX2 do not require $p22^{phox}$ instead requires intracellular calcium for activation [12, 33, 42].

It has been reported that LPA itself is produced from ovarian cancer cells and stimulates subsequent signaling cascades in a ROS-dependent manner [36]. However, the signaling cascades leading to the generation of ROS and subsequent physiological responses still remain unclear. In the present study, we demonstrated that LPA activated Akt1 isoform through the mTORC2 and leads to the generation of ROS, which is necessary for ovarian cancer cell migration.

## Materials and Methods

### Materials

All cell culture media were purchased from Hyclone Laboratories Inc. (Logan, UT, USA). Anti-actin antibody was purchased from MP Biomedicals (Aurora, OH, USA). Anti-Akt, Anti-P-Akt (Ser473), Anti-P-Akt (Thr308) antibodies were purchased from Cell Signaling Technology (Danvers, MA, USA). Linsitinib was purchased from Selleck Chemicals LLC (Houston, TX, USA). LY294002, Y27632, PD98059, and Ki16425 were obtained from Calbiochem (Darmstadt, Germany). ChemoTx membrane (8-μm pore size) was obtained from Neuro Probe Inc. (Gaithersburg, MD, USA). Infrared dye (IRDye)700- and IRDye800-conjugated rabbit/mouse secondary antibodies were obtained from Li-COR Bioscience (Lincoln, NE, USA). Recombinant GF-1, LPA, anti-FLAG antibody, and all other high quality reagents were purchased from Sigma-Aldrich (St Louis, MO, USA), unless otherwise indicated.

### ROS generation assay

To measure ROS generation, SKOV-3 cells were grown in 48-well plates, after being starved for 12 hr, cells were incubated with 2',7'-dichlorofluorescein diacetate (DCF-DA, 20 μM) for 1 hr and then stimulated with IGF-1 or LPA under the indicated conditions. Cells were washed three times with phosphate-buffed saline (PBS). Fluorescence was detected using a fluorescence microscope at 10× magnification (Axiovert 200, Carl Zeiss, Jena, Germany). Pixel intensity in a field was measured by MetaMorph software (Molecular Devices, Sunnyvale, CA, USA).

### Migration assay

SKOV-3 cells were grown and serum-starved for 12 hr before plating on the ChemoTx membrane. Cells were detached with trypsin-EDTA and washed with serum-free RPMI. For the migration assay, the bottom side of the ChemoTx membrane was coated with type I collagen for 30 min, and SKOV-3 ($1\times10^5$) serum-starved cells (in 50 μl) were placed on the top side of the ChemoTx membrane. Migration was induced by submerging the ChemoTx membrane in serum-free medium either in the presence or absence of IGF-1 for 12 hr. The ChemoTx membrane was fixed with 4% paraformaldehyde, and non-migratory cells on the top side of the membrane were removed by gently wiping with a cotton swab. The membrane was stained with DAPI and migrating cells were counted under the fluorescence microscope at 10×

magnification (Axiovert200, Carl Zeiss, Jena, Germany).

### Western blotting

Cells were lysed in 20 mM Tris-HCl, pH 7.4, 1 mM EGTA/ EDTA, 1% Triton X-100, 1 mM $Na_3VO_4$, 10% glycerol, 1 μg/ml leupeptin and 1 μg/ml aprotinin. After centrifugation at 12,000 rpm, thirty micrograms of total protein were loaded into 10% polyacrylamide gels and transferred onto nitrocellulose membranes. Membranes were incubated with the indicated primary antibodies and IRDye-conjugated secondary antibodies, and protein bands were visualized with infrared image analyzer (Li-COR Bioscience).

### Short hairpin RNA construct

To silence Akt1, Akt2, Rictor or Raptor, oligonucleotides tagged with a 5′-end *AgeI* site and a 3′-end *EcoRI* site were designed for sh-Akt1 (5′-cgagtttgagtacctgaagct-3′), sh-Akt2 (5′-cgacccaacacctttgtcata-3′), sh-Rictor (5′-caccaccaaagcaacc tatag-3′), and sh-Raptor (5′-cacctcactttatttccatgt-3′) and both sense and anti-sense oligonucleotides were synthesized (XENOTECH, Daejeon, Korea). Both complementary oligonucleotides were mixed and heated at 98℃ for 5 min and cooled to room temperature. Annealed nucleotides were subcloned into the *AgeI/EcoRI* site of a pLKO.1 lentiviral vector.

### Plasmid construction

FLAG-tagged murine wild type Akt1 and constitutively active form of Akt1 (CA-Akt1) were introduced into retroviral vector (pMIGR2) as described in previous reports [1, 22].

### Retroviral gene expression

Generation of retroviral particles for the expression of genes and their infection were performed essentially, as described previously [1].

### Lentiviral knockdown

For gene silencing, HEK293-FT packaging cells (Invitrogen) were grown to ~70% confluence in 100-mm cell culture dishes. Cells were triple transfected with 20 μg of pLKO.1 lentiviral vector containing sh-Akt1, sh-Akt2, sh-Rictor, or sh-Raptor 5 μg of Δ8.9, and 5 μg of pVSV-G using a calcium phosphate method. Medium was replaced with fresh medium 8 hr post-transfection. Lentiviral supernatants were harvested 24 hr and 48 hr post-transfection and passed through 0.45-μm filters. Cell-free viral culture supernatants were used to infect contractile VSMCs in the presence of 8 μg/ml of polybrene (Sigma-Aldrich). Infected cells were isolated by selection with 10 μg/ml puromycin for 2 days.

### Statistical analysis

Data were analyzed and plotted using GraphPad Prism. The unpaired Student's *t*-test (two tailed) was used to determine the significances of intergroup differences. Multiple sets of data were analyzed by analysis of variance (One-way ANOVA) with Tukey's multiple comparison test. The results are expressed as means ± SEMs, and $P$ values of less than 0.05 were considered significant.

## Results

### LPA induces SKOV-3 cell migration

IGF-1 stimulated SKOV-3 cell migration in a time- and dose-dependent manner (Fig. 1A, Fig. 1B). EC50 of IGF-1 was 4 ng/ml and saturated at 10 ng/ml. Likewise, LPA promoted SKOV-3 cell migration in a time- and dose-dependent manner (Fig. 1C, Fig. 1D). The EC50 of LPA was about 0.1 nM and saturated at 1 nM. In addition, stimulation of SKOV-3 cells with LPA (1 μM) resulted in the phosphorylation of Akt in a time- and dose-dependent manner, indicating the involvement of Akt in LPA-induced migration of SKOV-3 cells.

### LPA stimulates SKOV-3 cell migration via PI3K/Akt and ROS generation

Since both IGF-1 and LPA stimulated SKOV-3 cell migration, we next examined possible involvement of IGF-1 in LPA-stimulated SKOV-3 cell migration. As shown in Fig. 2A, pharmacological inhibition of LPA1 and LPA3 receptors by Ki16425 (10 μM) significantly blocked LPA-induced migration of SKOV-3 cells. However, Ki16425 could not block IGF-1-induced SKOV-3 cell migration. Likewise, inhibition of IGF-1 receptor by linsitinib (10 μM) selectively blocked IGF-1-induced SKOV-3 cell migration but not LPA-induced SKOV-3 cell migration. LPA-induced SKOV-3 cell migration was completely blocked by the inhibition of PI3K (LY294002, 10 μM) or Rho-associated kinase (ROCK) (Y27632, 10 μM), however, inhibition of ERK by PD98059 (10 μM) had no effect (Fig. 2B). In line with this LPA-induced generation of ROS was completely blocked by the inhibition of PI3K or ROCK, whereas inhibition of ERK had no effect on the generation of ROS (Fig. 2C). Moreover, LPA-induced SKOV-3 cell migration was completely blocked by the chelation of intracellular ROS by N-acetylcysteine (10 μM) (Fig. 2D).

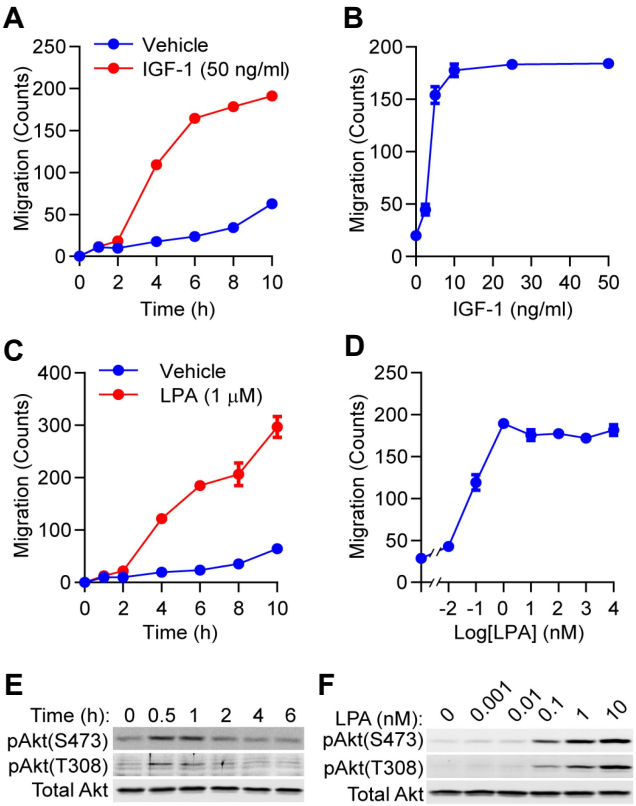


Fig. 1. IGF-1- and LPA-dependent migration of SKOV-3 cells. (A) SKOV-3 cells were stimulated with IGF-1 (50 ng/ml) for the indicated time points. Migrated cells were measured as described in "Materials and Methods". (B) SKOV-3 cells were stimulated with indicated doses of IGF-1 for 6 hrs. Migrated cells were measured as described in "Materials and Methods". SKOV-3 cells were stimulated with LPA for the indicated times (C) or doses (D), and migrated cells were measured as described in "Materials and Methods". SKOV-3 cells were stimulated with LPA for the indicated times (E) or doses (F), and phosphorylation at Ser473 or Thr308 was examined by Western blotting.

Pharmacological inhibition of NOX by DPI (10 μM) or apocynin (10 μM) suppressed LPA-induced SKOV-3 cell migration, however, inhibition of xanthine oxidase by allopurinol (All, 10 μM), COX by indomethacin (Indo, 10 μM), or mitochondrial respiratory chain by rotenone (Rote, 10 μM) had no effect on LPA-induced migration of SKOV-3 cells (Fig. 2E).

### Rictor and Akt1 is required for LPA-induced ROS generation

Since previous results showed that inhibition of PI3K blocked LPA-induced ROS generation, we next examined that effect of mTOR complexes on the LPA-induced ROS generation. As shown in Fig. 3A, inhibition of mTORC2 by silencing Rictor significantly suppressed LPA-induced ROS generation whereas inhibition of mTORC1 did not affect LPA-induced ROS generation (Fig. 3A, Fig. 3B). In addition, silencing of Akt1 significantly suppressed LPA-induced ROS generation whereas silencing of Akt2 had no effect (Fig. 3C, Fig. 3D), indicating that mTORC2/Akt1 signaling axis plays an essential role in LPA-induced ROS generation in SKOV-3 cells.

### mTORC2/Akt1 is necessary for LPA-induced SKOV-3 cell migration

Since mTORC2/Akt1 is necessary for the ROS generation and subsequent migration of SKOV-3 cells, we assessed the effect of mTORC2 and Akt1 in LPA-induced SKOV-3 cell migration. As shown in Fig. 4A, Fig. 4B, inhibition of mTORC2 by silencing of Rictor significantly blocked LPA-induced SKOV-3 cell migration, however, inhibition of mTORC1 by silencing of Raptor did not affect. In addition, silencing of Akt1 significantly suppressed LPA-induced migration of SKOV-3 cells whereas silencing of Akt2 had no effect (Fig. 4C, Fig. 4D). Ectopic overexpression of Akt1 augmented LPA-induced SKOV-3 cell migration, and furthermore overexpression of constitutively active form of Akt1 markedly enhanced basal level of SKOV-3 cell migration.

## Discussion

In the present study, we explored signaling cascades that are involved in ROS generation and migration in SKOV-3 cells. Particularly, we reported signaling specificity in the regulation of ROS generation and migration of SKOV-3 cells. For example, LPA receptor can activate PI3K/Akt signaling cascade without transactivation of IGF-1 receptor, mTORC2

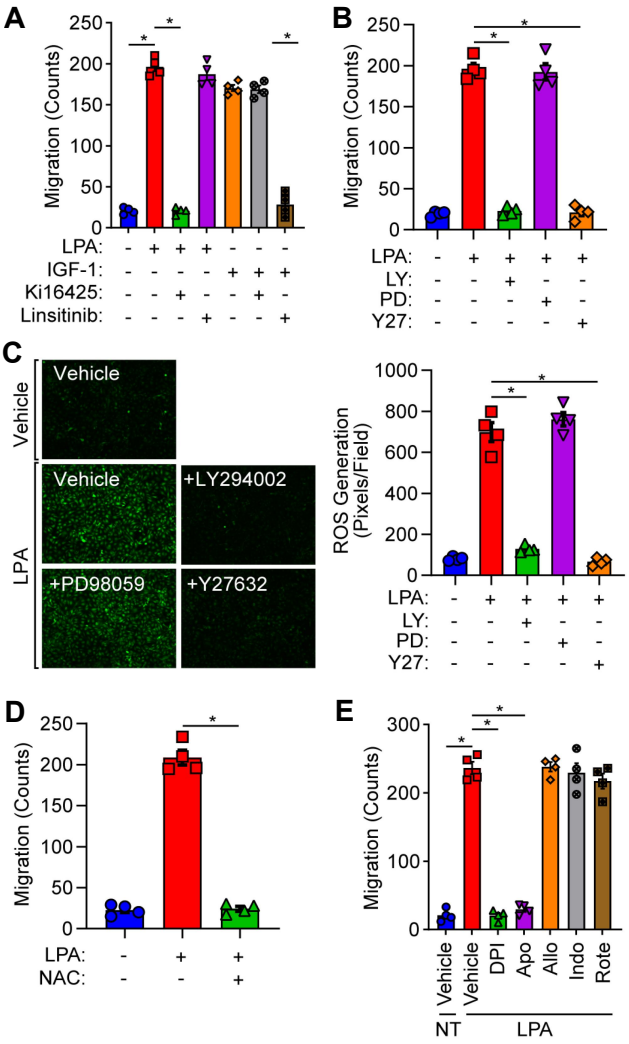


Fig. 2. ROS-dependent migration of SKOV-3 cells. (A) SKOV-3 cells pretreated with either LPA receptor antagonist (Ki16425, 10 μM) or IGF-1R antagonist (Lisitinib, 10 μM), and treated with LPA or IGF-1. Migrated cells were measured as described in "Materials and Methods". (B) SKOV-3 cells were pretreated with PI3K inhibitor (LY, 10 μM), MAPK inhibitor (PD, 10 μM), ROCK inhibitor (Y27, 10 μM) followed by stimulation with LPA for 6 hrs. Migrated cells were measured as described in "Materials and Methods". (C) SKOV-3 cells were pretreated with PI3K inhibitor (LY, 10 μM), MAPK inhibitor (PD, 10 μM), ROCK inhibitor (Y27, 10 μM) followed by stimulation with LPA for 30 min. ROS was measured as described in "Materials and Methods". (D) SKOV-3 cells were pretreated with NAC (10 μM) for 30 min and LPA-induced migration was measured. (E) SKOV-3 cells were pretreated with NOX inhibitor (Apo or DPI, 10 μM of each), xanthine oxidase inhibitor (All, 10 μM), COX inhibitor (Indo, 10 μM), or mitochondrial respiratory chain inhibitor (Rote, 10 μM), and LPA-induced migration was measured as described in "Materials and Methods". *$p$<0.05. The unpaired Student's $t$-test (two tailed) was used to determine the significances of intergroup difference. Results are represented as means ±SEMs.

rather than mTORC1 plays an essential role in ROS generation and migration, and Akt1 rather than Akt2 is important for ROS generation and subsequent migration of SKOV-3 cells.

In general, it has been known that GPCR regulates certain downstream signaling cascades such as cAMP signaling by modulation of Gs or Gi, and calcium signaling by modulation of PLC-β. However, it is also well known that activation of GPCR often activates signaling molecules that are involved in growth factor receptor signaling such as PI3K/Akt and Ras/ERK signaling pathways [11]. Likewise, our results also showed that PI3K/Akt signaling cascades are important for LPA-induced ROS generation and migration of SKOV-3 cells (Fig. 2 - Fig.4). Plausible explanation for this mechanism might be transactivation of growth factor receptors [19]. For example, TrkB receptor is transactivated by oxytocin receptor and epidermal growth factor receptor (EGFR) is transactivated by angiotensin receptor 1 (AT1) [29, 41]. However, our results showed that inhibition of IGF-1 receptor did not affect LPA-induced migration of SKOV-3 cells (Fig. 2A), indicating that LPA did not induce SKOV-3 migration *via* transactivation of IGF-1 receptor. Another possible mechanism for PI3K activation by GPCR could be direct activation of PI3K by Src. Indeed, it has been reported that dopamine D2 receptor directly activates PI3K and P2Y2 receptor activates Src, proline-rich tyrosine kinase 2, and subsequently growth factor receptors [26, 30].Recently, structural analysis of GPCR complex revealed that recruitment of β-arrestin to the GPCR provides platform for the activation of Src, PI3K/Akt, protein phosphatase 2A, and MAPK activation [45]. Therefore, it is reasonable to assume that LPA activates PI3K *via* the recruitment of β-arrestin.

It is noteworthy that there is overt specificity of each signaling molecule in the regulation of LPA-induced SKOV-3 cell migration. In our data, it clear that mTORC2 rather than mTORC1 is involved in the SKOV-3 cell migration as well

as ROS generation (Fig. 3, Fig.4). mTOR interacts with Raptor resulting in the formation of mTORC1, and regulates protein translation by sensing nutritional condition of the cells [20]. On the other hand, mTOR interacts with Rictor, forms molecular complex (mTORC2), and phosphorylates Akt at Ser473 residue [34]. In addition, it has been reported that disruption of mTORC2 negates migration of cancer cells [16, 46]. Therefore, it is reasonable to suggest that mTORC2/Akt signaling axis regulates LPA-induced SKOV-3 cell migration.

It is also deciphered that there is an isoform specificity of Akt in the regulation of LPA-induced SKOV-3 cell migration and ROS generation (Fig. 3, Fig. 4). Akt1 isoform plays an essential role in LPA-induced migration and ROS generation, however, there was no substantial effect of Akt2 in the regulation of LPA-induced migration and ROS generation. Several other reports also have demonstrated that there is an isoform specificity of Akt. For instance, ablation of each Akt isoform in mice shows different phenotypes; ablation Akt1 shows smaller organism size, mice lacking Akt2 displays type 2 diabetes syndrome, disruption of Akt3 results in smaller brain size [7, 8, 13]. In addition, it has been reported that linker region of Akt1 isoform provides specificity in PDGF-induced fibroblast cell migration [21]. The exact molecular network between mTORC and Akt is still ambiguous. However, recent report suggest that Akt1 rather than Akt2 preferentially forms molecular complex with mTORC2 and subsequently regulates cancer cell migration [22]. Therefore, it is reasonable to suggest that selective activation of Akt1 by mTORC2 regulates LPA-induced SKOV-3 cell migration and ROS generation.

Plethora of reports provide evidences that ROS regulates a variety of cell migration [40]. In the present study, we suggest that mTORC2/Akt1 signaling axis plays an essential role in the ROS generation (Fig. 3A). However, the molecular mechanism linking mTORC2/Akt1 to ROS generation is still unclear. Nevertheless, we could assume that NOX system could be involved in the LPA-induced ROS generation since chelation of ROS by NAC or inhibition of NOX enzyme completely blocked LPA-induced migration of SKOV-3 cells (Fig. 2D, Fig. 2E). In line with this, it has been reported that LPA modulates intracellular redox status through the regulation of NOX [25, 43]. Hence, it seems likely that LPA regulates SKOV-3 cell migration through the activation of NOX although the regulatory mechanism of NOX activation by mTORC2/Akt1 is still unclear. Further studies dealing with this regulatory mechanism would provide basis for the development of anti-cancer therapeutics.

In conclusion, LPA induces ROS generation and subsequently regulates migration of SKOV-3 cells. Mechanically, LPA activates PI3K and mTOR in a growth factor-independent manner. mTORC2 rather than mTORC1 specifically activates Akt1 isoform possibly through molecular complex formation. Activation of Akt1 leads to enhancement of NOX1 activity thereby produce ROS in SKOV-3 cells. Further investigation on the Akt1-dependent activation of NOX would provide key knowledge for the development of therapeutics for ovarian cancer.

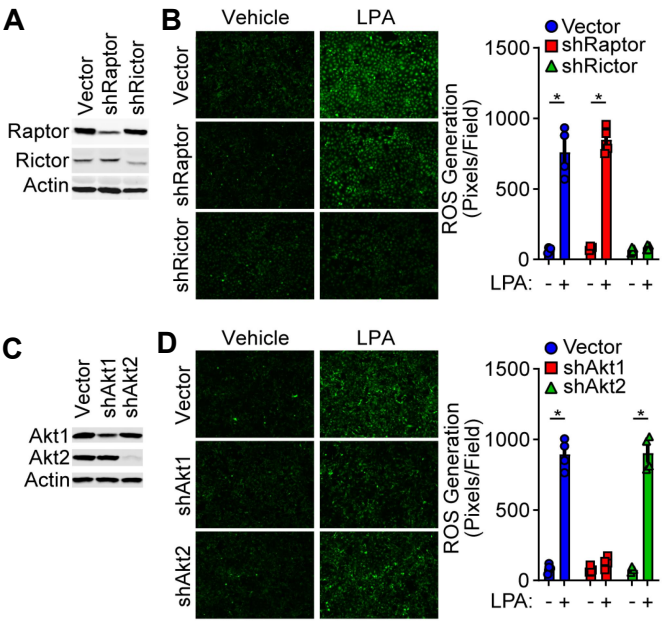


Fig. 3. mTORC2 and Akt1 are involved in the LPA-induced ROS generation. (A) Raptor or Rictor was silenced in SKOV-3 cells and expression of Raptor and Rictor was verified by western blotting. (B) After silencing of either Raptor or Rictor, LPA-induced ROS generation was determined. (C) Akt1 or Akt2 was silenced in SKOV-3 cells and expression of Akt1 and Akt2 was verified by Western blotting. (D) After silencing of either Akt1 or Akt2, LPA-induced ROS generation was determined. *$p$<0.05. The unpaired Student's $t$-test (two tailed) was used to determine the significances of intergroup difference. Results are represented as means ± SEMs.

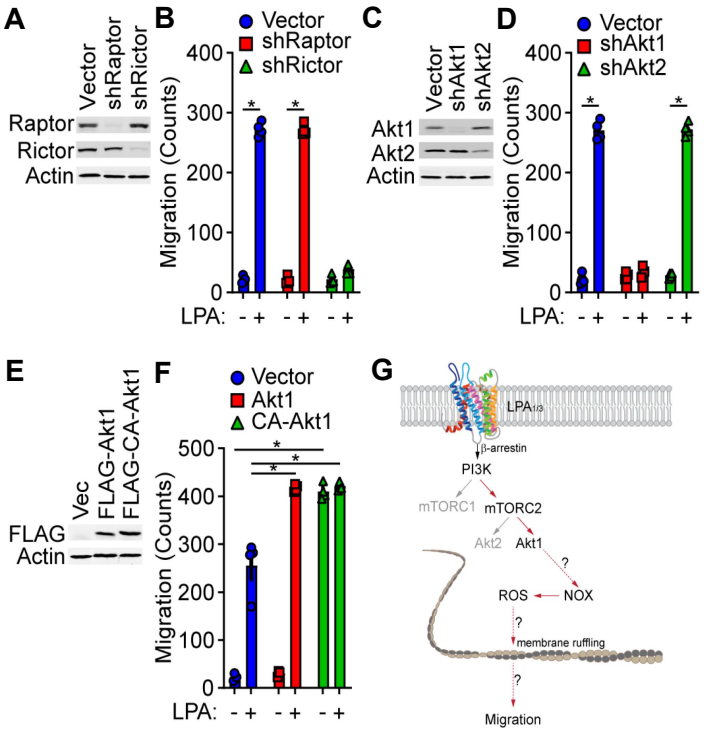


Fig. 4. mTORC2 and Akt1 are involved in the LPA- induced migration of SKOV-3 cells. (A) Raptor or Rictor was silenced in SKOV-3 cells and expression of Raptor and Rictor was verified by Western blotting. (B) After silencing of either Raptor or Rictor, LPA-induced migration of SKOV-3 cells was determined. (C) Akt1 or Akt2 was silenced in SKOV-3 cells and expression of Akt1 and Akt2 was verified by Western blotting. (D) After silencing of either Akt1 or Akt2, LPA-induced migration of SKOV-3 cells was determined. (E) Either FLAG- tagged wild type Akt1 or constitutively active form of Akt1 (CA-Akt1) was overexpressed in SKOV-3 cells, and expression of each Akt type was verified by Western blotting. (F) After expression of wild type Akt1 or constitutively active form (CA-Akt1) of Akt was expressed in SKOV-3 cells, LPA-induced migration was determined. **P*<0.05. The unpaired Student's *t*-test (two tailed) was used to determine the significances of intergroup difference. Results are represented as means ±SEMs. (G) Illustration of LPA-induced migration of SKOV-3 cells *via* the PI3K/mTORC2/ Akt1/NOX signaling axis.

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## The Conflict of Interest Statement

The authors declare that they have no conflicts of interest with the contents of this article.

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## 초록 : 리소포스타티드산은 SKOV-3 난소암세포의 mTORC2/Akt1/NOX 신호전달 기전을 통해 활성산소를 형성하고 이를 통해 세포의 이동을 촉진

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활성산소는 세포의 다양한 생리활성에 중요한 역할을 수행한다. 본 연구에서는 리소포스파티드산에 의해 유도되는 SKOV-3 세포의 이동을 조절하는 신호전달 기전 연구를 수행하였다. IGF-1 및 LPA는 처리시간 그리고 용량 의존적으로 SKOV-3 세포의 이동을 촉진시켰으며, 리소포스파티드산은 이에 따라 Akt의 인산화도 촉진하였다. 리소포스파티드산에 의한 세포이동은 리소포스파티드산 수용체 억제제에 의해 길항되었으나 IGF-1에 의한 세포이동에는 영향이 없었다. PI3K 및 ROCK의 억제는 리소포스파티드산에 의한 세포의 이동을 길항하였으나 MAPK 억제제에 의해서는 길항되지 않았다. 리소포스파티드산에 의해 형성되는 활성산소는 PI3K 및 ROCK의 억제제에 의해 길항되었으며 활성산소를 킬레이트화하면 리소포스파티드산에 의한 세포의 이동이 억제되었다. 또한 리간드에 의해 활성산소를 형성하는 NOX를 억제하면 리소포스파티드산에 의한 세포의 이동도 억제 되는 것이 관찰되었다. Rictor 및 Akt1의 발현을 억제하면 활성산소 및 세포의 이동이 저해되었으나 Raptor 및 Akt2의 발현조절은 모두 영향이 없는 것으로 관찰되었다. 마지막으로 우성활성화형태인 Akt1의 과발현은 리소포스파티드산의 자극이 없어도 SKOV-3 세포의 이동을 촉진하는 것으로 관찰되었다. 이러한 결과들을 바탕으로 리소포스파티드산은 mTORC2/Akt1/NOX 신호전달 기전을 통해 활성산소를 형성하고 SKOV-3 난소암세포의 이동을 촉진한다는 것을 제안한다.