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Application of *in Utero* Electroporation of G-Protein Coupled Receptor (GPCR) Genes, for Subcellular Localization of Hardly Identifiable GPCR in Mouse Cerebral Cortex

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Lysophosphatidic acid (LPA) is a lipid growth factor that exerts diverse biological effects through its cognate receptors (LPA_1-LPA_6). LPA_1, which is predominantly expressed in the brain, plays a pivotal role in brain development. However, the role of LPA_1 in neuronal migration has not yet been fully elucidated. Here, we delivered LPA_1 to mouse cerebral cortex using *in utero* electroporation. We demonstrated that neuronal migration in the cerebral cortex was not affected by the overexpression of LPA_1. Moreover, these results can be applied to the identification of the localization of LPA_1. The subcellular localization of LPA_1 was endogenously present in the perinuclear area, and overexpressed LPA_1 was located in the plasma membrane. Furthermore, LPA_1 in developing mouse cerebral cortex was mainly expressed in the ventricular zone and the cortical plate. In summary, the overexpression of LPA_1 did not affect neuronal migration, and the protein expression of LPA_1 was mainly located in the ventricular zone and cortical plate within the developing mouse cerebral cortex. These studies have provided information on the role of LPA_1 in brain development and on the technical advantages of *in utero* electroporation.

INTRODUCTION

Lysophosphatidic acid (LPA; 1-acyl-sn-glycerol-3-phosphate), which is a phospholipid growth factor, exerts diverse biological effects on neuronal cells, including proliferation, differentiation, survival, morphological change, and migration (Choi et al., 2010; Ishii et al., 2004; Moolenaar et al., 2004; Yung et al., 2014). LPA signals through at least six specific membrane-bound G protein-coupled receptors (GPCRs) that are designated as LPA_1/Vzg-1/Edg2, LPA_2/Edg4, LPA_3/Edg7, LPA_4/p2y9/GPR23, LPA_5/GPR92, and LPA_6/p2y5 (An et al., 1998; Bandoh et al., 1999; Hecht et al., 1996; Kotarsky et al., 2006; Lee et al., 2006; Noguchi et al., 2003; Pasternack et al., 2008). The lysophosphatidic acid receptor-1 (LPA_1), which was the first LPA receptor identified (Hecht et al., 1996), was found to be involved in LPA signaling in the development of the central nervous system through studies involving the targeted deletion of LPA_1 (Contos et al., 2000; Estivill-Torrus et al., 2008; Matas-Rico et al., 2008). This receptor was shown to be expressed in the neurogenic region of the embryonic neocortical region, which is called the ventricular zone (VZ) (Hecht et al., 1996).

Following the identification of LPA_1, studies were directed towards understanding the role of LPA in the cortical development of mice. When LPA was not present in the VZ, neural progenitor cells underwent proliferation, differentiation, and cell death. In the presence of LPA, cell rounding and retraction fiber formation were observed in mouse cortical primary cells (Fukushima et al., 2000). In addition, LPA signaling regulated the formation of cerebral cortical folds that resemble gyri by affecting proliferation, differentiation, and cell survival during embryonic development (Kingsbury et al., 2003). The mouse embryonic cerebral cortex exhibited gene expression profiles of two LPA receptors (LPA_1 and LPA_2), suggesting important roles of LPA_1 and LPA_2 in cerebral cortical development (Kingsbury et al., 2003). However, in many LPA studies, it has been difficult to localize the LPA receptor *in vivo*.

Formerly, studies of the functional consequence and expression pattern of the LPA_1 receptor were performed with *in situ* hybridization and reverse transcription polymerase chain reaction analysis (Cheng et al., 2009; Hecht et al., 1996; Kim et al., 2006; Spohr et al., 2008; Sun et al., 2010).

Since LPA1 receptors, like all G protein-couple receptors, cannot be easily visualized by an antibody, it is hard to discern the actual localization of the protein. Some papers have used LPA_1 antibodies to show the subcellular localization and expression of LPA_1. These studies examined antibody specificity by blocking the signal with the overexpression of antisense LPA receptors or LPA receptor peptides (Gobeil et al., 2003; Liszewska et al., 2009; Moughal et al., 2004; Waters et al., 2006;

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Zheng et al., 2001). However, these studies were not conclusive.

In this study, we ectopically overexpressed LPA_1 in the developing mouse cerebral cortex using *in utero* electroporation. LPA_1 overexpressed mice did not show obvious abnormalities in cerebral cortical development. In addition, we examined the subcellular localization and tissue distribution of LPA_1 in embryonic brain. The subcellular localization of LPA_1 in LPA_1-overexpressing cells was mainly located in the plasma membrane and endogenously located in the perinuclear area. The tissue distribution of LPA_1 was mainly observed in the VZ and cortical plate (CP) of the embryonic cortex. Our studies revealed the subcellular localization of LPA_1 *in vivo* and showed the technical advantage of antibody validation using *in utero* electroporation.

MATERIALS AND METHODS

Animal

Pregnant mice of C57BL/6N strain were purchased from Orient Bio (Korea). Stage E0.5 was defined as noon on the day of the vaginal plug. Fetuses at E13.5, E15.5, and E17.5 were used for experiments.

Materials

All chemicals used were of analytical grade if not stated otherwise. Antibody to β-actin was obtained from Cell Signaling Technology (USA). Antibodies to LPA_1, and green fluorescent protein (GFP) were purchased from Abcam (UK). Dulbecco's modified Eagle's Medium (DMEM), Opti-MEM I reduced-serum medium, 100 unit penicillin/100 μg streptomycin, fetal bovine serum (FBS), lipofectamine 2000, and DAPI were obtained from Invitrogen (USA).

Cell culture

TR cells which is derived from neocortical neuroblast cells infected with the oncogenes Large T and *vras* (Chun and Jaenisch, 1996) were maintained as monolayer cultures in Opti-MEM I reduced-serum medium supplemented with 2.5% heat-inactivated fetal bovine serum, 20 mM glucose, 55 μM 2-mercaptoethanol, and 100 unit penicillin/ 100 μg streptomycin. B103 rat neuroblastoma cells were maintained in DMEM supplemented with 10% FBS. B103 cells were transiently transfected with pCAGIG or pCAGIG-LPA1 expression plasmid using the Lipofectamine 2000 reagent. After 24 h, the fluorescent images were acquired with an inverted microscope (IX70; Olympus).

Isolation of total protein and Western blot analysis

TR cells were lysed in lysis buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1 mM ethylene glycol tetraacetic acid (EGTA), 1 mM ethylenediaminetetraacetic acid (EDTA), 1% Triton X-100, 1 mM Na3VO4, 5 mM NaF, and protease inhibitor cocktail). After incubation on ice for 30 min, the lysates were centrifuged (15,000 × *g*, 15 min). Supernatants were collected and protein concentrations were determined by Bradford assay (Bio-Rad, USA). Equal amounts of protein were boiled and separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) transferred to polyvinylidene difluoride membranes (Millipore, USA), and blocked with 5% non-fat milk. Membranes were incubated in primary antibody overnight at 4°C. Membranes were then washed in TBST (10 mM Tris, 140 mM NaCl, 0.1% Tween 20, pH 7.6), incubated with appropriate secondary antibody, and washed again in TBST. Bands were visualized by chemiluminescence and exposed to X-ray film.

DNA constructs

pCAGIG-LPA_1 expression vector design was based on the pCAGIG vector. The pCAGIG vector, which contains the IRES-EGFP cDNA under the control of the CMV enhancer and chick β-actin promoter, was a gift from Dr. C. Cepko (Matsuda and Cepko, 2004). The full coding sequence for murine LPA_1 was obtained by reverse transcription PCR. Total RNA was prepared from the cerebellum of adult C57BL/6N mice using TRIzol reagent (Invitrogen). Total RNA (2 μg) was converted to cDNA using AMV reverse transcriptase (Promega, USA). The coding regions of mouse LPA_1 were PCR amplified from cDNA with the following 5′-*Eco*RI-mLPA$_1$ and 3′-*Not*I-mLPA$_1$ primer sets: 5′-CCGGAATTCATGGCAGCTGCCTCTACTT-3′, and 5′-GAGAGCGGCCGCTACACGGTCACCCCAGA-3′. The PCR product was subcloned into the pCAGIG vector at *Eco*RI and *Not*I sites. pCAGIG-LPA_1 construct was confirmed by automated sequencing.

In utero electroporation

Timed-pregnant C57BL/6N females were anesthetized at stage embryonic day 13.5 (E13.5) with isoflurane (4% during induction, 2.5% during surgery), and the uterine horns were exposed by way of a laparotomy. The 1 μl of expression vector (4 μg/μl for pCAGIG and pCAGIG-LPA_1 constructs) in phosphate buffered saline (PBS) containing 0.05% fast green (Sigma-Aldrich, USA) was injected into the lateral ventricle of the embryo using a glass capillary with a length of 90 mm and a diameter of 1 mm (GD-1; Narishige, Japan). Electroporation was performed with a Tweezertrodes (diameter, 5 mm; BTX, USA) with 5 pulses of 45 V for 50 millisecond duration and 950 millisecond interval using a square-wave pulse generator (ECM 830; BTX). The uterine horns were then returned into the abdominal cavity, the wall and skin were sutured, and embryos were allowed to continue their normal development.

Immunohistochemistry

Electroporated embryonic brains were fixed with 4% paraformaldehyde (PFA) at 4°C for 2 h. Brains were cryoprotected in 30% sucrose /1× PBS at 4°C overnight and embedded in Tissue-Tek OCT (Sakura Finetek, USA). Cryosections (10 μm) were collected on MAS-coated glass slides (Matsunami Glass, Japan). Sections on glass slides were treated with heat in citrate buffer (10 mM, pH 6.0) at 95°C for 5 min. Samples were blocked in PBST containing 10% normal goat serum. The sections were incubated with primary antibodies against GFP (Abcam) (1:1000) and LPA_1 (Abcam) (1:1000) overnight at 4°C and then incubated in Alexa 488 and Alexa 568 conjugated secondary antibodies (Invitrogen) (1:1000) for one hour at room temperature. After washing, the specimens were mounted onto cover slips using Vectashield (Vector Laboratories, USA). The fluorescent images were acquired with a laser scanning confocal microscope (LSM510; Zeiss). For identify distribution of the GFP positive cells, images were converted gray values and normalized to background staining. Images were divide 10 equal bins spanning the cortical thickness and measured by ImageJ program (NIH).

In situ hybridization

Cryosections (18 μm) were collected on MAS-coated glass slides (Matsunami Glass). mRNA for LPA_1 was detected by in situ hybridization using digoxygenin (DIG)-labeled antisense riboprobes. The sections were treated with proteinase K (1

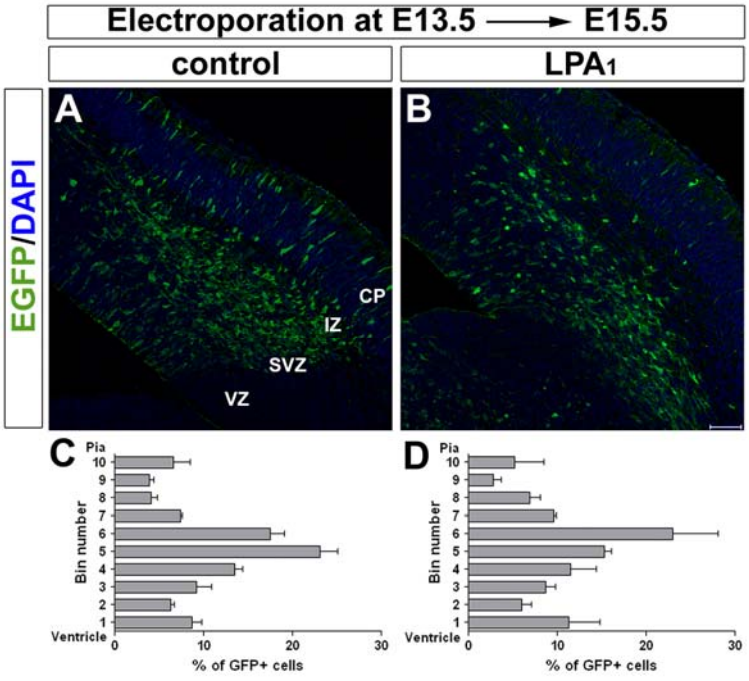

Fig. 1. *In vivo* overexpression of LPA_1 protein by *in utero* electroporation. Confocal images of coronal sections of cortices of embryonic (E13.5) mice transfected by *in utero* electroporation with control (A, C; expressing EGFP; pCAGIG) or LPA_1 (B, D; expressing EGFP and LPA_1; pCAGIG-LPA_1) constructs that were harvested 48 h post electroporation and immunostained for GFP. Distribution of GFP positive cells in 10 bins spanning cortical thickness are shown as histograms (C, D). DAPI staining was used as a nuclear marker. CP, cortical plate; IZ, intermediate zone; SVZ, subventricular zone; VZ, ventricular zone. Scale bar, 100 μm.

μg/ml, 5-30 min, room temperature) and hybridized with 0.3 μg/ml riboprobe in a hybridization buffer (50% formamide, 20 mM Tris-HCl at pH 7.5, 600 mM NaCl, 1 mM EDTA, 10% dextran sulfate, 200 μg/ml yeast tRNA, 1× Denhardt's solution, 0.25% SDS) at 65°C overnight. The sections were washed three times with 1× SSC containing 50% formamide at 65°C, followed by maleic acid buffer (0.1 M, pH 7.5) containing 0.1% Tween 20 and 0.15 M NaCl. DIG-labeled probe was visualized by overnight incubation of the sections with anti-DIG antibody conjugated to alkaline phosphatase (1:2,000; Roche, USA) and NBT/BCIP reaction. The open reading frame of *Lpar1* antisense riboprobe was synthesized using a digoxygenin-labeled riboprobe with T7 RNA polymerase and a DIG-RNA labeling mix according to the manufacturer (Roche). The *Lpar1* riboprobe was a gift from Dr. Jerold Chun (The Scripps Research Institute).

RESULTS

Characterization of the spatiotemporal-specific LPA_1-overexpressing mouse cerebral cortex

To evaluate the importance of LPA_1 in neuronal migration in the developing cerebral cortex, we examined the overexpression of LPA_1 in the VZ by *in utero* electroporation. First, we made a vector containing LPA_1 and tested it in B103 rat neuroblastoma cells. pGAGIG-LPA_1 encoding [LPA_1/enhanced green fluorescent protein (EGFP)] - transfected cells displayed a flattened and more migratory morphology compared with pCAGIG (encoding EGFP)-transfected cells (data not shown). We injected a construct encoding EGFP or LPA_1/EGFP into the lateral ventricle of E13.5 mouse embryos and transferred it into neuronal progenitor cells in the VZ by *in utero* electroporation (Saito, 2006). After allowing normal *in vivo* embryonic development, immunohistochemistry for GFP was performed on coronal brain sections obtained from E15.5 mice. The expression of EGFP was

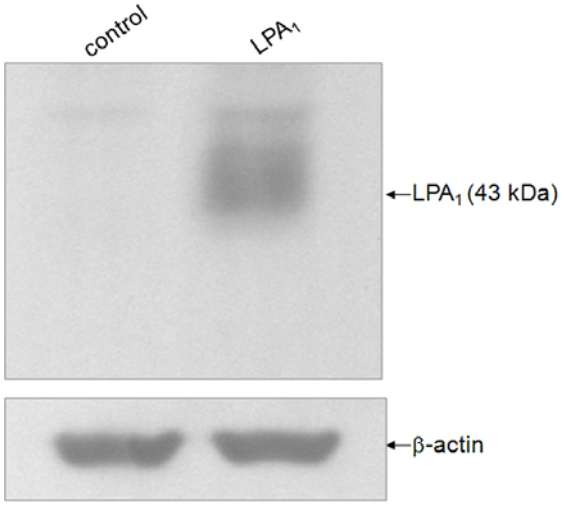

Fig. 2. Western blot analysis of LPA_1 proteins in TR neocortical neuroblast cells. TR cells were transiently transfected with control (expressing EGFP) or LPA_1 (expressing EGFP and LPA_1) constructs. Twenty-four hours post-transfection, the cells were lysed, probed with an anti-LPA_1 antibody and a β-actin antibody, and analyzed by Western blots.

detectable in many neurons of mice transfected with EGFP (Fig. 1A) or with LPA_1 and EGFP (Fig. 1B). In EGFP-transfected control (Figs. 1A and 1C), transfected cells were mainly located in the subventricular zone (SVZ) and intermediate zone (IZ), and some populations entered the cortical plate (CP). These neuronal migration patterns correlated with previously reported results (Langevin et al., 2007). With LPA_1/EGFP-transfection

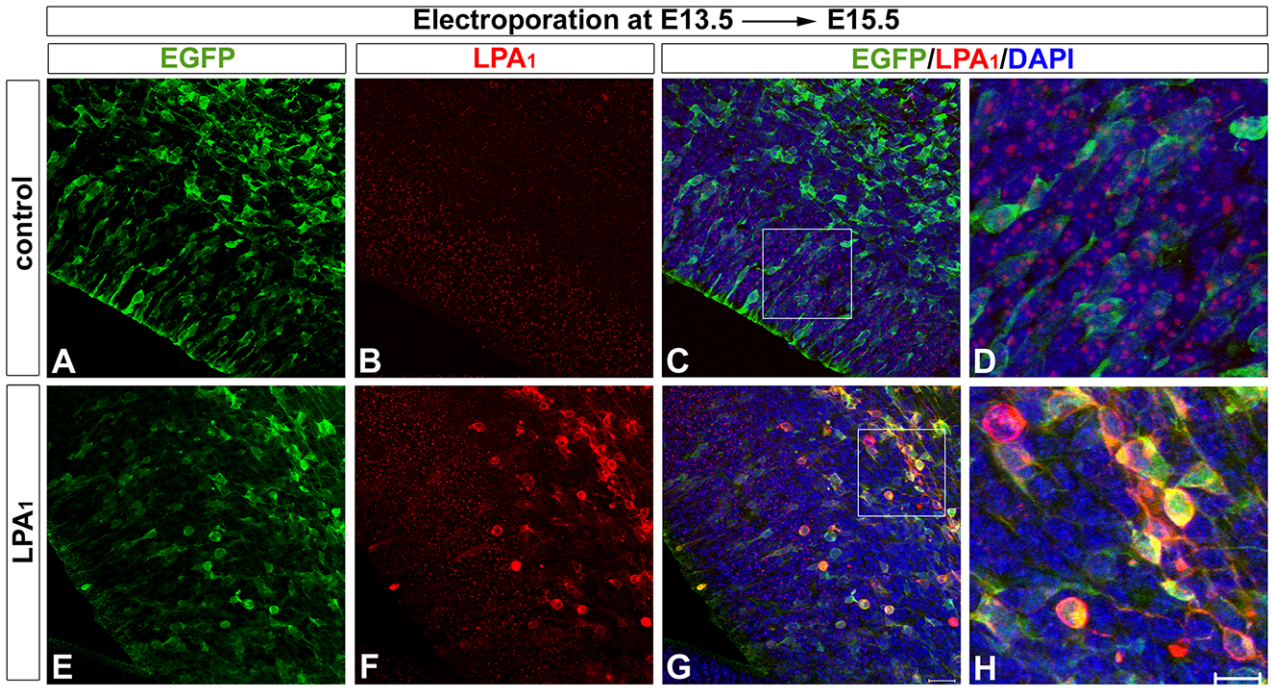

Fig. 3. Immunohistochemical localization of LPA_1 in the *in utero* electroporated cortices of embryonic mice. Embryonic mice cortices that were *in utero* electroporated at E13.5 with control (A-C; expressing EGFP) or LPA_1 (E-G; expressing EGFP and LPA_1) constructs and harvested at E15.5 were immunostained with anti-GFP antibody (A, E) or anti-LPA_1 antibody (B, F). (C) Merge of (A) and (B). (G) Merge of (E) and (F). DAPI staining was used as a nuclear marker. Samples were analyzed by confocal microscopy in the following way: 10 z-stacks of each sample (1 μm apart) were taken and used to generate a two-dimensional image with Zeiss LSM software (Zeiss). Projections of these 2-D images are shown. Scale bar, 20 μm. (D, H) Higher magnification view of the square region in (C) and (G). Scale bar, 10 μm.

(Figs. 1B and 1D), LPA_1 did not affect neuronal migration or neuronal morphology in developing mouse cerebral cortex. Although LPA1 overexpression appeared to slightly delay neuronal migration in CP, it was not statistically significant. (Figs. 1C and 1D). These results indicate that LPA_1 overexpression in cerebral cortex did not affect radial migration of neuronal progenitor cells.

Subcellular localization of LPA_1 in LPA_1-overexpressing mice

To delineate the subcellular localization of LPA_1, we examined its protein distribution in EGFP- and LPA_1/EGFP-transfected mice cerebral cortex. First, we tested the antibody specificity to LPA_1. Western blot analysis of EGFP and LPA_1/EGFP overexpressing TR neocortical neuroblast cells, which revealed a single band at the expected size for LPA_1 (Fig. 2), demonstrated the specificity of the antibody used to detect LPA_1. We next characterized the subcellular localization of LPA_1 using immunohistochemistry (Fig. 3). At E15.5, 2 days after *in utero* electroporation, we performed GFP and LPA_1 double immunostaining in EGFP- and LPA_1/EGFP-transfected mice cerebral cortex. In EGFP-transfected controls, LPA_1 did not co-localize with the GFP signal (Figs. 3A-3C). Interestingly, endogenous LPA_1-positive signal, which was observed in the nuclear/perinuclear area of neuronal cells located in the VZ, formed a spot-like pattern (Fig. 3D). In LPA_1/EGFP-overexpressing cortices, LPA_1 expression co-localized with GFP (Figs. 3E-3G). As shown in Fig. 3H, LPA_1 was mainly located in plasma membrane in LPA_1-overexpressing neuronal cells. Additionally, endogenous LPA_1-positive signals were the same as those seen in EGFP transfected controls (Fig. 3G). Thus, these data show that the endogenous subcellular localization of LPA_1, which exhibited a spot-like pattern, was located in the nuclear/perinuclear area, whereas the LPA_1 in LPA_1-overexpressing cells was located in the plasma membrane.

LPA_1 is mainly expressed in ventricular zone of mouse embryonic cerebral cortex

Due to the fact that our Western blotting and *in utero* electroporation experiment revealed that the LPA_1 antibody was specific for detecting LPA_1, we further identified the LPA_1 protein expression pattern in the cerebral cortex of (E13.5 to E17.5) embryonic mice (Figs. 5 and 6). First, we identified that the *Lpar1* transcript was mainly expressed in the VZ of cerebral cortex using in situ hybridization, as previously described (Hecht et al., 1996) (Fig. 4). At E13.5, *Lpar1* antisense riboprobes revealed that a high level of expression of *Lpar1* was detected strictly in the VZ of cerebral cortex (Fig. 4A). At E15.5, the level of *Lpar1* mRNA expression, which were exhibited in the VZ were slightly diminished (Fig. 4C). At E17.5, *Lpar1* mRNA in VZ was barely detectable (Fig. 4E). Immunohistochemical analysis confirmed that LPA_1 was located in the cerebral cortex at E13.5 (Fig. 5). In particular, LPA_1 levels were highly enriched within the VZ (Figs. 5A and 5B) and the lens (Figs. 5A and 5C). To examine the expression pattern of LPA_1 during cortical development, we performed immunostaining on sagittal brain sections obtained

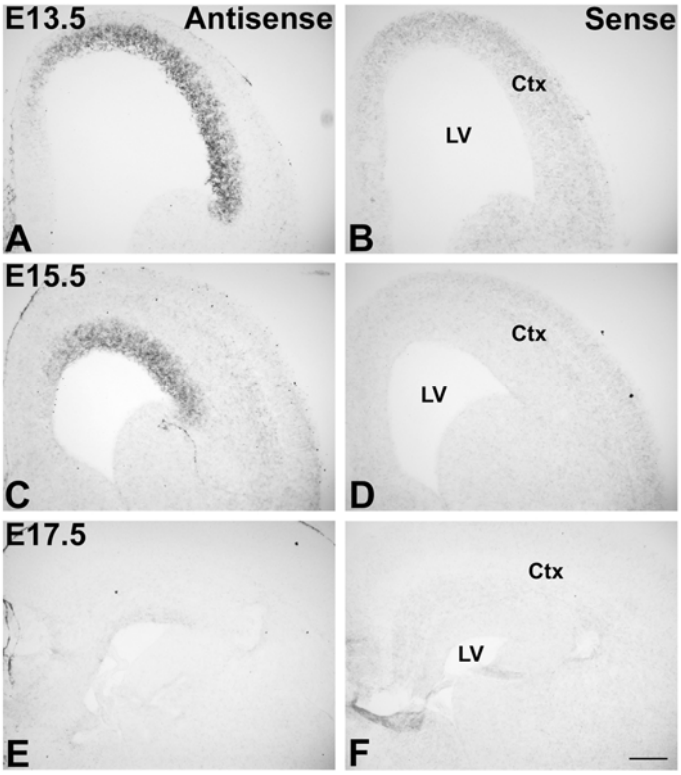

Fig. 4. Expression pattern of *Lpar1* in mouse embryo. *In situ* hybridization performed with DIG-labeled riboprobes in the coronal slices showed that *Lpar1* transcripts were expressed throughout the ventricular zone at E13.5 (A), E15.5 (C) and E17.5 (E). *In situ* hybridization with sense probe (B, D, and F) is negative controls. Scale bar, 200 μm.

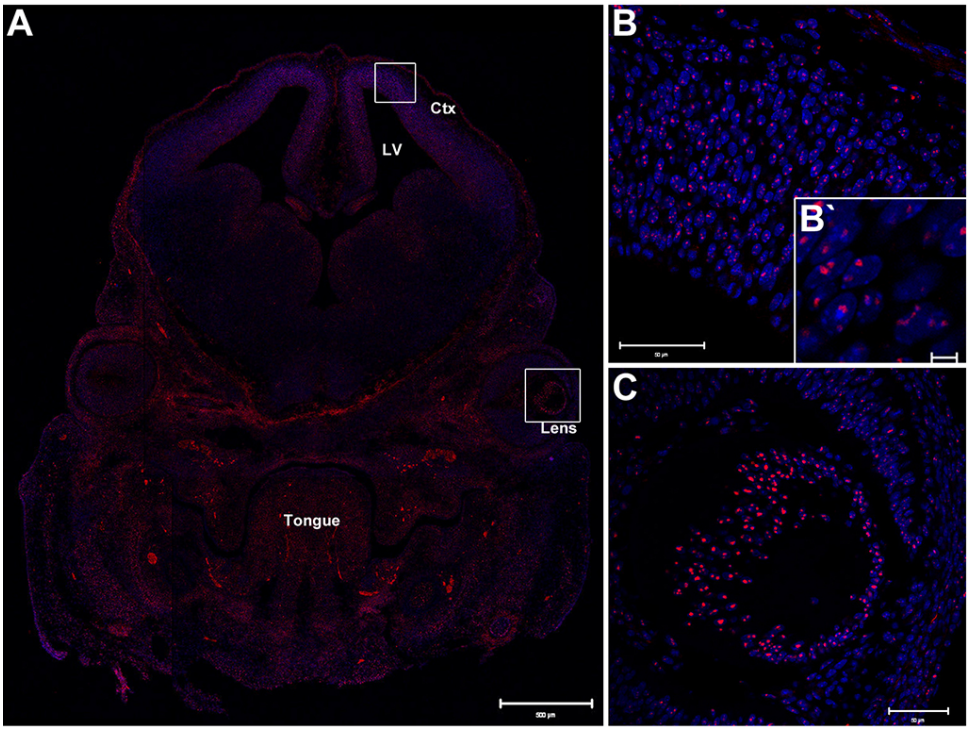

Fig. 5. Expression pattern of LPA_1 in mouse embryo 13.5 cortices. (A) The distribution of LPA_1 was examined by immunostaining with an anti-LPA_1 antibody (red) in fixed coronal sections of E13.5 embryonic brain. LPA_1 protein was expressed in the cells located predominately in the VZ and the lens. (B) Higher magnification view of the square in upper region of A. (B′) Higher magnification view of (B). (C) Higher magnification view of the square in lower region of A. DAPI (blue) staining was used as a nuclear marker. Samples were analyzed by confocal microscopy. (A) Scale bar, 500 μm. (B, C) Scale bar, 50 μm. (B′) Scale bar, 5 μm. Ctx, Cortex; LV, lateral ventricle.

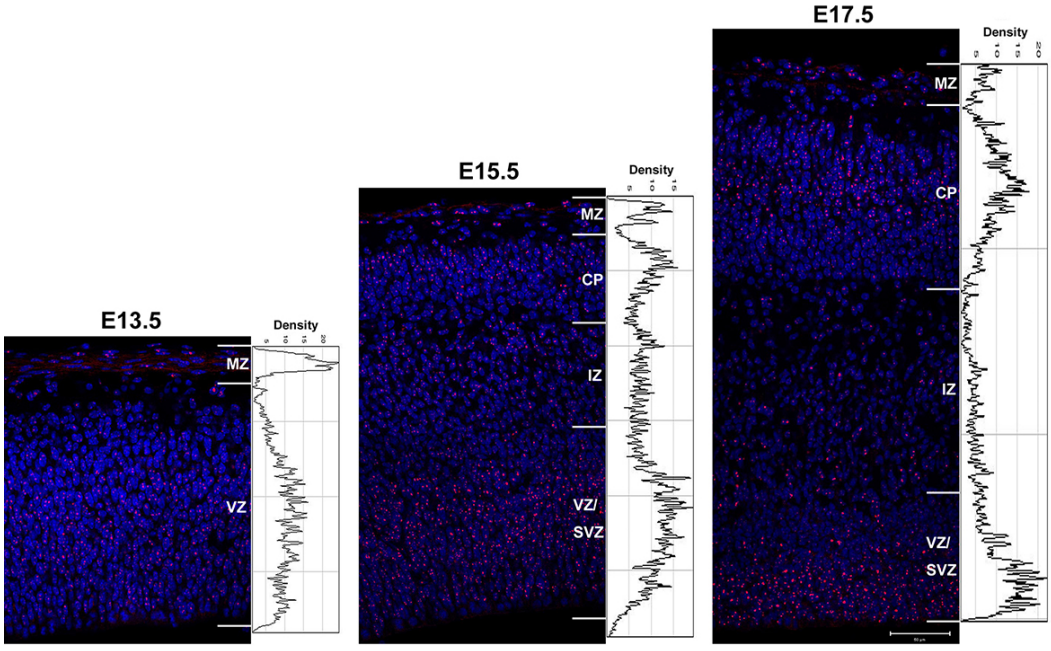

Fig. 6. Expression pattern of LPA_1 during cortical development. Sagittal sections of the cortex from E13.5-E17.5 were immunostained to anti-LPA1 antibody (red). DAPI (blue) staining was used as a nuclear marker. Densities of each red signal were measured by dynamic profiler plugin in ImageJ (NIH) and shown in right panel. Samples were analyzed by confocal microscopy. Scale bar, 50 μm. MZ, marginal zone.

from E13.5 to E17.5 mice (Fig. 6). At E13.5, immunostaining of LPA_1 revealed that a high level of LPA_1 was detected in the VZ of cerebral cortex. At E15.5, LPA_1 levels were high in the VZ and the CP. At E17.5, the levels of LPA_1 were highly enriched within the VZ and upper layer of the CP. Taken together, these data demonstrate that LPA_1 was expressed in the VZ and upper layer of the CP in the cerebral cortex of developing mice.

DISCUSSION

The aim of the present study was to characterize functional consequence of LPA_1 in the developing cerebral cortex. Our results provide an additional understanding of the role of LPA_1 in the developing cerebral cortex and of the technical application of *in utero* electroporation for the validation of antibodies.

We used *in utero* electroporation to uncover the role of LPA_1 in neuronal migration from the VZ to the CP. The overexpression of LPA_1 in newly postmitotic cells of the VZ did not show any obvious abnormality in the neuronal migration to the CP (Fig. 1). LPA_1, which is endogenously expressed in the VZ (Hecht et al., 1996), has an important role in the maintenance of neuroprogenitor pools in the VZ (Kingsbury et al., 2003). The LPA receptor-mediated signaling is regulated by their ligand, LPA. Thus, the levels of LPA may be low in the SVZ and IZ. Thus, the ectopic expression of LPA_1 did not change neuronal migration in the cerebral cortex.

An examination of the subcellular localization of LPA_1 was performed by immunohistochemistry (Fig. 3). In LPA_1-overexpressing cerebral cortical cells, LPA_1, which colocalized with EGFP fluorescence, was predominantly expressed in the plasma membrane (Fig. 3H). These results were similar to those previously reported in a study of LPA_1 overexpression in a cell-based assay (Avendano-Vazquez et al., 2005; Murph et al., 2003; Urs et al., 2005).

In EGFP expressing control cerebral cortex, LPA_1 and EGFP signal did not colocalize, and the endogenous LPA_1-positive signal was located in the perinuclear area and looked like an LPA_1 oligomer (Fig. 3D). G protein-coupled receptor (GPCR) oligomerization has recently been widely accepted, and the number of documented oligomeric GPCR combinations is extensive (Filizola, 2010; Gurevich and Gurevich, 2008; Maggio et al., 2005; Milligan, 2009; Vidi et al., 2011). Active GPCRs are specifically phosphorylated by G-protein-coupled receptor kinases (GRKs), initiating arrestin recruitment. Receptor/arrestin complexes than recruit two components of the internalization machinery (clathrin and AP-2) and a variety of other proteins, initiating the second round of signaling (DeWire et al., 2007; Moore et al., 2007). Thus, GPCR oligomerization may be regulated by arrestin-mediated signaling (Gurevich and Gurevich, 2008). Arrestin is important in the LPA induced signaling cascade, and LPA_1 internalization is regulated by arrestin mediated pathway (DeWire et al., 2007; Gesty-Palmer et al., 2005; Sun and Lin, 2008; Sun and Yang, 2010; Urs et al., 2005; 2008). Another report showed that LPA_1 can dimerize (Zaslavsky et al., 2006). Our observation of ventricular zone cells that were immunopositive to LPA1 antibody with puncta morphology is intriguing. We speculate that these intracellular puncta might have been formed after LPA1 are bound to ligands (i.e., lysophosphatitic acids), followed by internalization of this receptor-ligand complex into the cells. Taken together, these results suggest that activated LPA_1 is internalized and oligomerized by arrestin scaffolding.

In a previous *in situ* hybridization study, the *Lpar1* expression pattern was mainly located in the VZ of the cerebral cortex of developing mouse (Hecht et al., 1996). We performed *in situ* hybridization of *Lpar1* in the cerebral cortex and found the

same expression pattern of the *Lpar1* transcript (Fig. 4). In Figs. 5 and 6, the protein expression pattern of LPA_1 is mainly located in the VZ and the CP. During cortical development, postmitotic neurons migrate toward the CP from the VZ and pass the IZ. Within the CP, radially migrating cells become arranged in an inside-out pattern in which the earlier generated neurons occupy deeper layers, and those generated later become located in more superficial layers (Aboitiz et al., 2001). These previous reports indicated that the transcripts of *Lpar1* are located in the VZ, and LPA_1-expressing cells migrate toward the CP from VZ. Thus, LPA_1 protein is located in the VZ and CP of the cerebral cortex of mice. This observation requires further studies with functional consequence of LPA_1 expressed in VZ and CP.

Nowadays, *in utero* electroporation is a widely used and well established experimental method. It has a several advantage like region-specific, cell type-specific, inducible, and multiple gene targeting. Moreover, it can apply to region dependent behaviors and functional outcome test (De Vry et al., 2010; Taniguchi et al., 2012). Besides the above advantages of the *in utero* electroporation, it can easily introduce specific genes *in vivo* condition. Despite many targeted GPCR antibody is available but can't easily find validated antibody, for this reason *in utero* electroporation is a powerful tools for quick and easily find good antibody from hardly discern GPCR.

In summary, our results provide information on the subcellular localization and tissue distribution of LPA_1 and the technical advantages of the use of *in utero* electroporation for the validation of antibodies.

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