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## Regulation of the LPA<sub>2</sub> Receptor Signaling through the Carboxyl-Terminal Tail-Mediated Protein-Protein Interactions

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### Abstract

While it is well known that lysophosphatidic acid (LPA) mediates diverse physiological and pathophysiological responses through the activation of G protein-coupled LPA receptors, the specificity and molecular mechanisms by which different LPA receptors mediate these biological responses remain largely unknown. Recent identification of several PDZ proteins and zinc finger proteins that interact with the carboxyl-terminal tail of the LPA<sub>2</sub> receptor provides a considerable progress towards the understanding of the mechanisms how the LPA<sub>2</sub> receptor specifically mediates LPA signaling pathways. These findings have led to the proposal that there are at least two distinct protein interaction motifs present in the carboxyl terminus of the LPA<sub>2</sub> receptor. Together, these data provide a new concept that the efficiency and specificity of the LPA<sub>2</sub> receptor-mediated signal transduction can be achieved through the cross-regulation between the classical G protein-activated signaling cascades and the interacting partner-mediated signaling pathways.

### Keywords

LPA; LPA<sub>2</sub> receptor; PDZ; LIM; zinc finger; protein-protein interactions

### 1. Introduction

LPA is a growth factor-like phospholipid, which mediates diverse biological responses such as cell proliferation, differentiation, migration, survival, angiogenesis, inflammation and platelet aggregation [1-5]. In the past decade, the identification of at least seven G protein-coupled LPA receptors, including LPA<sub>1</sub>/EDG2, LPA<sub>2</sub>/EDG4 and LPA<sub>3</sub>/EDG7 of the Endothelial Differentiation Gene (EDG) family and the structurally distinct LPA<sub>4</sub>/P2Y9/GPR23, LPA<sub>5</sub>/GPR92, GPR87, and P2Y5 of the P2Y purinoreceptor family has provided a big step toward the understanding of LPA actions [6-12]. These LPA-bound membrane receptors couple to G<sub>s</sub>, G<sub>i/o</sub>, G<sub>q</sub> and/or G<sub>12/13</sub> proteins to activate various signaling pathways. In addition, LPA can bind to the nuclear peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ) to regulate vascular remodeling [13,14]. Along with the rapid development of this field, more efforts have been made to determine the specificity and mechanisms by which different LPA receptors mediate LPA actions. Among all of the LPA receptors, the LPA<sub>2</sub> receptor is unique in the carboxyl-terminal tail, in which it contains two distinct protein-protein interaction domains to bind several PDZ proteins and zinc finger proteins, respectively [15-21]. These protein-protein interactions confer the specificity, efficiency and diversity by which the LPA<sub>2</sub> receptor mediates LPA-evoked signal transduction, which will be the focus of this review.

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## 2. Functional roles of the LPA<sub>2</sub> receptor

In response to LPA stimulation, the LPA<sub>2</sub> receptor couples to G<sub>i/o</sub>, G<sub>q</sub> and G<sub>12/13</sub> proteins to mediate cellular responses such as DNA synthesis, MAP kinase activation, AKT activation, inhibition of adenylyl cyclase, increase of inositol phosphates and intracellular calcium concentration, cell rounding, cell adhesion and cell migration [8]. These signaling events are partially impaired in mouse embryonic fibroblasts (MEFs) derived from the LPA<sub>2</sub><sup>-/-</sup> mice, and are dramatically reduced or completely abolished in MEFs derived from the LPA<sub>1</sub><sup>-/-</sup>, LPA<sub>2</sub><sup>-/-</sup> mice, suggesting a functional redundancy of LPA<sub>1</sub> and LPA<sub>2</sub> receptors in some of the signaling pathways. However, disruption of the LPA<sub>2</sub> receptor gene does not result in any developmental abnormality [22].

Although the LPA<sub>2</sub> receptor is not required for growth and development, increasing evidence has implicated LPA and the LPA<sub>2</sub> receptor as the key mediators in cancer progression. It has been reported that the expression of the LPA<sub>2</sub> receptor is up-regulated in ovarian cancers, invasive ductal carcinoma tissue of breast cancers, colorectal cancers and thyroid cancers [23-26]. Recently autotaxin/lysophospholipase D (ATX/lysoPLD) has been known to mediate tumor invasion, neovascularization and metastasis through the production of LPA in the cellular microenvironment [5,27]. And the platelet-derived LPA has been shown to act as a tumor cell mitogen and promoting factor of osteolysis during bone metastasis [28]. Although several actions of LPA can contribute to tumorigenesis, including cell migration, cell proliferation, cell survival and transcriptional activation of genes involved in these signaling events [5], it remains unclear whether the LPA<sub>2</sub> receptor mediates these actions through specific signaling pathways.

## 3. Regulation of LPA<sub>2</sub> receptor functions through the carboxyl-terminal tail-mediated protein-protein interactions

The classical paradigm of G protein-coupled receptor activation involves the coupling of ligand-bound receptors to heterotrimeric G proteins, which sequentially activates the downstream effector molecules and induces cellular responses. However, emerging evidence has revealed that other mechanisms can cross-regulate GPCR signaling pathways. For example, a number of GPCRs can interact with cellular proteins through the intracellular loops or carboxyl-terminal tail. These interacting partners participate in the regulation of receptor trafficking, dimerization, effector coupling, membrane expression and subcellular localization, etc., thereby determining the signal specificity and efficiency [29,30].

The LPA<sub>1</sub>, LPA<sub>2</sub> and LPA<sub>3</sub> receptors share a high homology in the sequences except for the carboxyl-terminal tail, suggesting that this region may specifically regulate the unique functions of each receptor. The structure of LPA<sub>2</sub>-CT (a.a. 296-351) contains a di-leucine motif and several putative palmitoylated cysteine residues in the proximal region, and a class I PDZ-binding motif at the distal end. It also contains several serine and threonine residues presumably can be phosphorylated by G protein-coupled receptor kinases (GRKs), which are important for β-arrestin binding and receptor internalization. The last four amino acids, DSTL, known as a class I PDZ-binding motif, mediates the interactions with NHERF2 (Na<sup>+</sup>/H<sup>+</sup> exchanger regulatory factor 2), PDZ-RhoGEF, LARG (Leukemia-associated RhoGEF) and MAGI-3 (membrane-associated guanylate kinase with an inverted domain structure-3) [16-18,20,21], whereas the proximal region is responsible for the association with several zinc finger proteins, including the LIM domain-containing TRIP6 (thyroid hormone receptor-interacting protein 6) and the proapoptotic Siva-1 protein [15,19] (Table 1). Except for PDZ-RhoGEF and LARG that can bind to both LPA<sub>1</sub> and LPA<sub>2</sub> receptors [18], other proteins selectively interact with the LPA<sub>2</sub> receptor but not other LPA receptors, suggesting that these interacting partners may play specific roles in regulating LPA<sub>2</sub> receptor functions.

### 3.1. PDZ-mediated protein-protein interactions

PDZ domains, derived from the initials of three proteins containing such domains including PSD-95, the Drosophila discs-large tumor suppressor protein DlgA and the tight junction protein ZO-1, are composed of ~90 amino acids that function as the protein interaction modules, which bind to the specific short peptide motif found in the carboxyl-terminus or internal region of a variety of target proteins [31-35]. Most of the PDZ domains are involved in the organization of multi-protein complexes, which allows the stable localization of interacting proteins to achieve the efficiency and fidelity of the signal transduction. The PDZ-binding motifs can be classified into three groups, which are composed of the four amino acids, X-S/T-X-L/V (class I), X- $\phi$ -X- $\phi$  (class II) and X-D-X-V (class III), respectively ( $\phi$ , hydrophobic amino acid; X, unspecified amino acid) [36]. These PDZ-binding motifs can be found in the carboxyl-terminus of several LPA receptors, such as LPA<sub>1</sub> (HSVV), LPA<sub>2</sub> (DSTL) and LPA<sub>5</sub> (DSAL).

Interestingly, the first reported LPA<sub>2</sub> cDNA clone was derived from an ovarian tumor library, which lacks the last four amino acids (DSTL), but is 31 amino acids longer than the reported human cDNA sequences [8]. This can be explained by one guanine base deletion at the 3' end of the coding sequences, which leads to a frame-shift mutation in the carboxyl-terminal region of the protein. However, whether these mutations lead to any de-regulation of LPA<sub>2</sub> in tumor development is not known.

**3.1.1. NHERF2 regulates LPA-mediated phospholipase C- $\beta$  signaling pathway and the activation of ERKs and AKT**—The Na<sup>+</sup>/H<sup>+</sup> exchanger regulatory factors NHERF1 (also known as EBP50) and NHERF2 (also known as E3KARP) possess two tandem PDZ domains and a carboxyl-terminal domain that binds to the actin-binding proteins ezrin, radixin, moesin and merlin [37,38]. The NHERF family members function as scaffold proteins by associating with a number of transporter, ion channels, transcription factors, different signaling molecules and growth factor receptors [38-40]. Emerging data also show that the two PDZ domains of NHERF1 and NHERF2 can interact with the carboxyl-terminal PDZ-binding motifs of a number of G protein-coupled receptors, and regulate receptor trafficking, localization and/or other functions [41]. However, NHERF1 and NHERF2 seem to have a distinct peptide-binding specificity. For example, PDZ1 of NHERF1 binds to the  $\beta_2$ -adrenergic receptor (DSLL), P2Y1 purinergic receptor (DTSL), and cystic fibrosis transmembrane regulator (CFTR)(DTRL) [42,43]. But NHERF1 does not bind to the LPA<sub>1</sub> receptor (HSVV) or LPA<sub>2</sub> receptor (DSTL) [17]. Likewise, PDZ1 of NHERF2 binds to the  $\beta_2$ -adrenergic receptor (DSLL) [42]; however, PDZ2 of NHERF2 specifically binds to the LPA<sub>2</sub> receptor (DSTL) but not LPA<sub>1</sub> receptor (HSVV) [17]. These observations suggest that although the last four amino acid residues of the target receptors are required for PDZ domain recognition, other adjacent residues are also involved in determining the specificity and/or affinity of the interaction.

Through the interaction with the LPA<sub>2</sub> receptor, NHERF2 potentiates LPA-induced ERK activation and COX-2 induction [17]. This effect can be partly attributed to the specific coupling of the LPA<sub>2</sub> receptor to phospholipase C- $\beta$ 3 (PLC- $\beta$ 3) by NHERF2, which regulates inositol phosphoate production, calcium mobilization and protein kinase C activation. Intriguingly, both PLC- $\beta$ 3 and the LPA<sub>2</sub> receptor bind to the same PDZ domain of NHERF2. Since NHERF proteins can form oligomers through PDZ domains [44,45], NHERF2 must be self-associated to form a multi-protein complex containing the LPA<sub>2</sub> receptor and PLC- $\beta$ 3. In addition, it has been reported that LPA-induced activation of ERKs and AKT is attenuated by the inhibition of NHERF2 expression in Caco-2 colon cancer cells that predominantly express the LPA<sub>2</sub> receptor but not LPA<sub>1</sub> or LPA<sub>3</sub> receptor [21].

**3.1.2. NHERF2 links the LPA<sub>2</sub> receptor with CFTR**—Cystic fibrosis transmembrane conductance regulator (CFTR) is a cAMP-regulated chloride channel, which plays a critical role in cholera toxin-induced secretory diarrhea [46]. Through the PDZ domain-mediated interactions, NHERF2 bridges the LPA<sub>2</sub> receptor and CFTR, and facilitates the macromolecular complex formation at the apical plasma membrane of intestinal epithelial cells [20]. When activated by LPA, the LPA<sub>2</sub> receptor couples to G<sub>i</sub> proteins to reduce cAMP levels, thereby inhibiting PKA-mediated activation of CFTR and cholera toxin-induced secretory diarrhea [20]. These studies demonstrate that the triple complex formation among the LPA<sub>2</sub> receptor, NHERF2 and CFTR plays a key role in LPA-mediated inhibition of CFTR activity.

**3.1.3. PDZ domain-containing Rho GEFs and MAGI-3 regulate LPA<sub>2</sub> functions in RhoA and ERK activation**—In line with the discovery of NHERF2 as an LPA<sub>2</sub> receptor-interacting protein, two reports have demonstrated that the PDZ-binding motif of LPA<sub>2</sub> receptor can also interact with the PDZ domains of two RhoA-specific guanine nucleotide exchange factors (GEFs) and the MAGI family members [16,18]. The activated LPA receptors couple to G<sub>12/13</sub> proteins, which can activate RhoA signaling through the recruitment of the RGS domain-containing Rho GEFs, including PDZ-RhoGEF, LARG and p115-RhoGEF [47-50]. Among these Rho GEFs, the PDZ domain-containing PDZ-RhoGEF and LARG are capable of binding to the LPA<sub>1</sub> and LPA<sub>2</sub> receptors, and regulate LPA-induced RhoA activation [18]. These findings suggest that the PDZ-mediated interaction may facilitate the recruitment of PDZ domain-containing RhoGEFs to the proximity of G<sub>12/13</sub> proteins, thereby enhancing their ability to catalyze the guanine nucleotide exchange of RhoA.

Using a PDZ proteomic array to screen the proteins that interact with the LPA<sub>2</sub> receptor, several PDZ domain-containing proteins have been identified, which include NHERF1 (PDZ1), NHERF2 (PDZ2), neurabin, MAGI-2 (PDZ5) and MAGI-3 (PDZ5) [16]. However, PDZ-RhoGEF and LARG were not found by this assay, suggesting a differential *in vitro* binding affinity among these PDZ-mediated interactions. MAGI proteins contain multiple PDZ domains, WW domains and a guanylate kinase-like domain, allowing them to function as scaffold proteins to enable multi-protein complex formation [51]. These proteins are present at the areas of cell-cell contact such as the synaptic junctions in neurons and the tight junctions in epithelial cells [51]. MAGI-3 can interact with PTEN, receptor tyrosine phosphatase- $\beta$ , transforming growth factor- $\alpha$  (TGF- $\alpha$ ), frizzled receptor and the  $\beta_1$ -adrenergic receptor [52-56]. It has been reported that MAGI-3 can interact with PTEN to negatively modulate AKT activity, and bind to the  $\beta_1$ -adrenergic receptor to impair G<sub>i</sub>-mediated AKT activation [52, 55]. However, overexpression of MAGI-3 or inhibition of MAGI-3 expression does not significantly affect LPA-induced AKT activation [16]. Instead, the interaction of MAGI-3 with the LPA<sub>2</sub> receptor regulates LPA-induced activation of RhoA and ERKs [16]. Overexpression of MAGI-3 enhances LPA-induced RhoA activation but has little effect on ERK activation, whereas knockdown of MAGI-3 inhibits LPA-induced RhoA and ERK activation. Together, these data demonstrate that the LPA<sub>2</sub> receptor functions can be differentially regulated through PDZ-mediated protein-protein interactions in different cellular contexts.

### 3.2. Zinc finger-mediated protein-protein interactions

Using the carboxyl-terminal tail of the LPA<sub>2</sub> receptor as bait in a yeast two-hybrid screen, two zinc finger-containing molecules have been identified, including TRIP6 and Siva-1 [15,19]. The structure of TRIP6 contains an N-terminal proline-rich domain, three carboxyl LIM domains and a PDZ-binding motif at the carboxyl-terminal end. The LIM domains (named by the initials of Lin-11, Isl-1, and Mec-3) contain two zinc finger motifs, which are critical for protein-protein interactions [57]. TRIP6 binds to the LPA<sub>2</sub> receptor mainly through LIM2-3 domains; however, LIM1 can facilitate this interaction [15]. The structure of Siva-1 protein contains an internal death domain homology region (DDHR) and two carboxyl zinc finger

motifs; however, lacking histidine residues [58]. The association with the LPA<sub>2</sub> receptor is mediated by the carboxyl-terminal zinc finger motif of Siva-1, which resembles the second half of TRIP6-LIM3 domain [19]. Indeed, the domain mapping shows that these two proteins bind to the same motif located in the proximal region of the carboxyl-terminal tail of the LPA<sub>2</sub> receptor (F.-T. Lin and G. Tigyi, unpublished). However, there are some differences in their interactions. TRIP6 associates with the membrane-bound LPA<sub>2</sub> receptor in an LPA-dependent manner, whereas Siva-1 binds to the LPA<sub>2</sub> receptor on the membrane or in the cytosol in the absence or presence of LPA [15,19]. The interaction of TRIP6 with the LPA<sub>2</sub> receptor is transient since LPA treatment for 15-20 min induces the translocation of TRIP6 to focal adhesion plaques where the LPA<sub>2</sub> receptor is not present. In contrast, Siva-1 seems to preferentially bind to the internalized LPA<sub>2</sub> receptor.

### **3.2.1. The LIM domain-containing TRIP6 regulates LPA<sub>2</sub> receptor-mediated cell migration**

TRIP6, also known as ZRP-1 (*Zyxin-related Protein 1*), is a focal adhesion molecule known to regulate cell motility and transcriptional control [15,59-62]. Together with LPP, zyxin, and more distantly with ajuba and LIMD1, these proteins form the zyxin family members [59]. Through the LIM domain-mediated protein-protein interactions, these molecules function as scaffold or adaptor proteins for the assembly of multi-protein complexes involved in actin rearrangement, cell adhesion and motility [63]. LPA stimulation promotes the recruitment of TRIP6 to the activated LPA<sub>2</sub> receptor, and induces the association of TRIP6 with the components of focal complexes, including paxillin, p130<sup>cas</sup>, FAK and c-Src [15]. Subsequently, TRIP6 is phosphorylated by c-Src at Tyr-55, a unique site only present in TRIP6 but not other zyxin family members [64]. This phosphorylation transforms the pY<sup>55</sup>-Q-A-P motif of TRIP6 into a docking site for Crk SH2 domain, thereby enhancing LPA-induced morphological changes and cell migration. In addition, knockdown of TRIP6 attenuates LPA-induced, c-Src-mediated ERK activation. In contrast to c-Src, the PTPL1/FAP-1 (Fas-associated phosphatase-1) tyrosine phosphatase binds to the carboxyl-terminal PDZ-binding motif and LIM3 of TRIP6 [65]. Dephosphorylation of TRIP6 by PTPL1/FAP-1 serves as a molecular switch by inhibiting TRIP6 binding to Crk, thereby negatively regulating TRIP6 function in LPA-induced adhesion turnover and cell migration [66]. Given the high sequence homology among the LIM domains of TRIP6, LPP and zyxin, LPP and zyxin are also found to bind to the LPA<sub>2</sub> receptor in an LPA-dependent manner.

### **3.2.2 The interaction of the LPA<sub>2</sub> receptor with Siva-1 promotes LPA-mediated down-regulation of Siva-1**

LPA is known as a promoting factor for cell survival [67]. Several pro-survival signaling pathways have been defined for this protective effect, including the activation of AKT/PKB, ERKs and NF-κB [68,69]. Among different LPA receptors, the LPA<sub>1</sub> and LPA<sub>2</sub> receptors have been shown to mediate these effects [70-72]. In contrast to the LPA<sub>2</sub> receptor, Siva-1 functions as a proapoptotic protein, which is transcriptionally activated by p53 and E2F1 during DNA damage response [73]. Although the mechanism how Siva-1 regulates apoptosis is not fully understood, it has been reported that Siva-1 promotes apoptosis through a caspase-3-dependent mitochondrial pathway [74], and can function as a negative regulator of NF-κB in T cell receptor-mediated activation-induced cell death [75]. In addition, Siva-1 binds to Bcl-xL and inhibits Bcl-xL-mediated protection against UV radiation-induced apoptosis [76].

Several G protein-coupled receptors have been shown to undergo ligand-stimulated ubiquitination and degradation through proteasomal or lysosomal pathways [77]. It has been noted that prolonged LPA stimulation not only down-regulates the LPA<sub>2</sub> receptor but also concomitantly targets the LPA<sub>2</sub> receptor-interacting Siva-1 for ubiquitination and proteasomal degradation [19]. As a result, the proapoptotic activity of Siva-1 during DNA damage response is attenuated by LPA. Thus, in addition to activating AKT/PKB and ERK pathways to promote



cell survival, LPA can directly inhibit the proapoptotic function of Siva-1 through the specific binding of Siva-1 to the LPA<sub>2</sub> receptor.

#### 4. Concluding remarks

As different LPA receptors may couple to the same G proteins to activate the downstream cascades, it appears that the LPA<sub>2</sub> receptor signaling can be achieved rapidly and specifically through these G protein-independent protein-protein interactions. It is of interest to note that these LPA<sub>2</sub> receptor-interacting PDZ proteins and LIM-containing proteins possess similar functions in the association with actin, and serve as scaffold or adaptor proteins for the assembly of multi-protein complexes. In particular, TRIP6 and the structurally related LPP contain a class I PDZ-binding motif at their carboxyl-termini, allowing them to interact with PDZ proteins. The PDZ-containing NHERF2, PDZ-RhoGEF, LARG and MAGI-3 mediate LPA-induced activation of RhoA, ERKs and/or AKT, and the LIM-containing TRIP6 regulates LPA-induced ERK activation, adhesion turnover and cell migration in a c-Src-dependent manner. Thus, it is likely that the LPA<sub>2</sub> receptor, PDZ proteins and LIM-containing proteins form a supramolecular complex in the microdomain of plasma membrane, and coordinately regulate LPA<sub>2</sub> receptor functions in actin remodeling, cell adhesion, migration and/or cell survival. However, several questions remain to be answered, such as the physiological relevance of these protein-protein interactions and the downstream signaling events initiated by these interacting proteins. As the number of the identified LPA receptors is increasing, the LPA signaling pathways are more complex than were previously imagined. To specifically target one particular LPA receptor subtype for therapeutic intervention, it is prerequisite to understand how different interacting proteins regulate the specific signal transduction of each LPA receptor. It is also anticipated that more interacting partners of a particular subtype of LPA receptor will be identified in the near future.

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**Table I**Proteins interacting with the carboxyl-terminal tail of the LPA<sub>2</sub> receptor

| <b>Binding motif</b>                              | <b>Interacting protein</b> | <b>Functional significance</b>   | <b>References</b> |
|---|----------------------------|--|-------------------|
| PDZ-binding motif<br>(distal end)                 | NHERF2                     | Activation of PLC-β3 signaling pathways  | [17]              |
|   |                            | Activation of AKT and ERKs   | [21]              |
|   |                            | Linking LPA <sub>2</sub> with CFTR to inhibit CFTR activity  | [20]              |
| Zinc finger-binding<br>motif<br>(proximal region) | PDZ-RhoGEF<br>LARG         | RhoA activation  | [18]              |
|   | MAGI-3                     | Activation of RhoA and ERKs  | [16]              |
|   | TRIP6                      | Regulation of cell migration through<br>c-Src/PTPL1-dependent phosphorylation<br>and dephosphorylation<br><br>ERK activation | [15,64,66]        |
|   | Siva-1                     | Interactions mediate down-regulation of<br>Siva-1 to promote cell survival   | [19]              |