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JB Review

Non-Edg family LPA receptors: the cutting edge of LPA research

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Keisuke Yanagida[1] and Satoshi Ishii[2,*]

[1]Department of Biochemistry and Molecular Biology, Faculty of Medicine, The University of Tokyo, Tokyo 113-0033; and [2]Department of Immunology, Graduate School of Medicine, Akita University, Akita 010-8543, Japan

*Satoshi Ishii, Department of Immunology, Graduate School of Medicine, Akita University, Akita 010-8543, Japan. Tel: +81 18 884 6089, Fax: +81 18 884 6444, email: satishii@med.akita-u.ac.jp

Lysophosphatidic acid (LPA) is a bioactive lipid mediator with diverse physiological and pathological actions on many types of cells. Originally, LPA was thought to elicit its biological functions through three subtypes of endothelial differentiation gene (Edg) family G protein-coupled receptors (LPA1, LPA2 and LPA3) until our group identified a fourth subtype, LPA4. The discovery of this receptor, which is structurally distinct from the Edg family LPA receptors, led to the identification of two additional LPA receptors, LPA5 and LPA6, homologous to LPA4. These 'non-Edg family' LPA receptors now provide a new framework for understanding the diverse functions of LPA, including vascular development, platelet activation and hair growth. In this review, we summarize the identification, intracellular signalling and biological functions of this novel cluster of LPA receptors.

Keywords: Edg family LPA receptors/G protein-coupled receptor/lysophosphatidic acid/ non-Edg family LPA receptors/orphan receptor.

Abbreviations: acyl-LPA, ester-linked LPA; alkyl-LPA, alkyl ether-linked LPA; ATX, autotaxin; CHO, Chinese hamster ovary; DRG, dorsal root ganglion; Edg, endothelial differentiation gene; FPP, farnesyl pyrophosphate; GPCR, G protein-coupled receptor; hMSC, human mesenchymal stem cell; HUVEC, human umbilical vein endothelial cell; *K*d, dissociation constant; LPA, lysophosphatidic acid; MEF, mouse embryonic fibroblast; mPA-PLA1α, membrane bound phosphatidic acid-selective phospholipase A1α; PTX, pertussis toxin; ROCK, Rho-associated kinase.

Lysophosphatidic acid (LPA) is a simplest phospholipid consisting of a phosphate group, a glycerol moiety and a fatty acid. In spite of its simple structure, LPA exerts various biological functions and is involved in processes such as cell growth, differentiation, survival, motility and cytoskeletal changes (*1*). LPA is not a single molecular entity, and in fact, there are many LPA species (Fig. 1) (*2*). Either a saturated or unsaturated fatty acid is esterified at the *sn*-1 or *sn*-2 position of the glycerol backbone. In addition, the *sn*-1 alkyl or alkenyl ether-linked LPA species also exist. The biological activities of LPA depend on the length and the degree of unsaturation of the carbon chain attached to the glycerol backbone, as well as the type of linkage and its position.

In 1960, Vogt (*3*) found that LPA induced the contraction of isolated rabbit duodenum preparations. To our knowledge, this is the first report of the biological activity of LPA. Subsequently, Tokumura *et al.* (*4*) reported vasopressor activities of several molecular species of LPA along with the relationship between their structure and activity. These pioneering works led to the disclosure of numerous bioactivities of LPA. However, because of experimental difficulties in its handling or its detergent-like property, questions remained about whether LPA serves as an agonist for LPA-specific receptors. Then, in 1989, Moolenaar and colleagues showed the GTP-dependency of LPA-induced phosphoinositide hydrolysis and the pertussis toxin (PTX) sensitivity of adenylyl cyclase inhibition (*5*). Subsequently, van der Bend *et al.* (*6*) reported strong evidence for the existence of a specific LPA-binding protein of 38–40 kDa using radiolabelled diazirine-LPA, a photoaffinity probe. These studies clearly proved the existence of a specific G protein-coupled receptor (GPCR) for LPA.

Classic LPA Receptors (Edg family LPA Receptors)

In 1990, Hla and colleagues found that one of the immediate early response genes cloned from human umbilical vein endothelial cells (HUVECs) encodes a rhodopsin family GPCR (*7*). They named it endothelial differentiation gene 1 (Edg1). Subsequently, several GPCRs that share substantial homology to Edg1 in their predicted amino acid sequences were cloned. These 'Edg family' GPCRs were considered to constitute a family of 'orphan GPCRs' whose ligands were yet to be identified. In 1996, Chun and colleagues demonstrated that overexpression of Edg2 in cultured neuronal cells resulted in enhanced morphological changes and adenylyl cyclase inhibition in response to LPA (*8*), providing the first evidence that a member of the Edg family GPCRs represented a functional receptor for LPA. This was quickly followed by the report by Hla and colleagues that Edg1 is a receptor for the related lysophospholipid, sphingosine-1-phosphate (*9*). Before 2000, reports from a number of research groups established that there are eight

Edg receptor genes in the human genome, three of which encode LPA receptors and share 50–57% amino acid identity with each other: LPA1/Edg2 (*8*), LPA2/Edg4 (*10*), and LPA3/Edg7 (*11*, *12*).

Edg family LPA receptors mediate intracellular Ca^{2+} mobilization, adenylyl cyclase inhibition and mitogen-activated protein kinase activation through PTX-sensitive and -insensitive G proteins. LPA1 and LPA2 couple with Gi, Gq and G12/13 proteins. Meanwhile, LPA3 couples with Gi and Gq proteins but not with G12/13 proteins. Knockout mice for these receptors have already been established. By analysing these mice, researchers have revealed important roles of Edg family LPA receptors in the nervous system (*13*–*21*), cancer progression (*22*), cardiovascular function (*23*), reproduction (*24*) and other processes (Table I). Details are discussed in a number of excellent reviews (*1*, *25*, *26*).

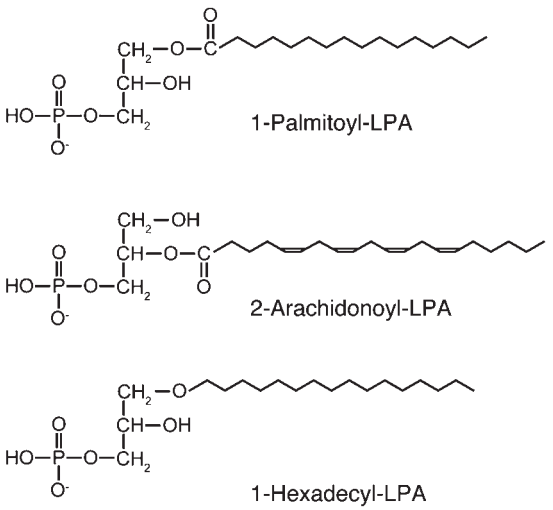

Fig. 1 Chemical structures of the molecular species of LPA. LPA contains an acyl- or ether-linked fatty acyl chain of various lengths and unsaturation levels. The acyl chain is esterified either at the *sn*-1 or *sn*-2 position of the glycerol backbone (1-acyl-LPA and 2-acyl-LPA, respectively), and the ether-linked LPAs carry an alkyl- or an alkenyl-linkage at the *sn*-1 position (alkyl-LPA and alkenyl-LPA, respectively). 1-Palmitoyl-LPA, 2-arachidonoyl-LPA and hexadecyl-LPA are shown as representative molecular species for 1-acyl-LPA, 2-acyl-LPA, and alkyl-LPA, respectively.

Non-Edg Family LPA Receptors

Even after the identification of Edg family LPA receptors, there were several reports implying the existence of an additional LPA receptor(s). First, LPA-induced human platelet aggregation showed distinct ligand specificity, which was not accounted for by Edg family LPA receptor-mediated responses (*27*–*29*). Human platelets aggregated more potently in response to alkyl ether-linked LPA (alkyl-LPA) than to the corresponding ester-linked LPA (acyl-LPA). However, all Edg family LPA receptors prefer acyl-LPA to alkyl-LPA. Secondly, rat hepatoma RH7777 cells that do not express any of Edg family LPA receptors showed a mitogenic response to LPA and its analogs (*30*). Of note, the stereoselectivity of the response was similar to that of human platelets. Thirdly, in a study of fibroblasts from LPA1/LPA2 double-knockout mice, some LPA-induced intracellular responses, including inositol phosphate production, adenylyl cyclase inhibition and stress fiber formation, were still observed but at reduced levels (*31*).

Table I. Six subtypes of LPA receptors.

Receptor	Aliases	Coupled G proteins	Phenotypes of knockout mice
LPA1	Edg2, vzg-1	Gi, Gq, G12/13	Craniofacial dysmorphism (*13*) Decreased post-natal growth rate (*13*) Increased apoptosis of Schwann cells in the sciatic nerve (*13*) Impaired suckling behaviour and partial neonatal lethality (*13*) Abnormal behaviours (*15*, *17*, *20*, *21*) Increased adiposity (*77*) Abnormal cortical/neuronal development (*18*, *19*) Resistance to neuropathic pain (*16*) Attenuated renal fibrosis (*78*) Attenuated pulmonary fibrosis (*79*)
LPA2	Edg4	Gi, Gq, G12/13	Increased radiation sensitivity of intestinal crypt (*80*) Attenuated injury-induced neointimal hyperplasia (LPA1/2-double knockout mice) (*23*) Attenuated tumourigenesis in the colon (*22*) Reduced allergic airway inflammation (*81*)
LPA3	Edg7	Gi, Gq	Delayed implantation (*24*) Altered embryo spacing (*24*)
LPA4	p2y9, GPR23	Gs, Gq, G12/13, (Gi)[a]	Increased bone density (*39*) Partial embryonic lethality with blood and lymphatic vessel malformation (*44*)
LPA5	GPR92, GPR93	Gq, G12/13	N/A[b]
LPA6	p2y5	G12/13, (Gs), (Gi)	N/A

[a]Preliminary data that require further confirmation are given in parentheses.
[b]N/A, not applicable.

Importantly, LPA3 mRNA was not detected in these double-knockout fibroblasts.

In 2003, we successfully identified the first non-Edg family LPA receptor, LPA4, in our attempt to de-orphanize the receptor p2y9/GPR23, which is structurally distant from the Edg family LPA receptors (*32*). As described below, our finding has led to identification of two additional LPA receptors, LPA5 and LPA6. These three receptors share 35–55% amino acid identity with each other and constitute a new family of LPA receptors, i.e., non-Edg family LPA receptors. From here, we extend our previous review (*33*) with the recent important findings on non-Edg family LPA receptors and their biological functions.

LPA4

LPA4 (p2y9/GPR23) was cloned as an orphan GPCR and is closely related to the purinergic P2Y receptors (*34*, *35*) whereas, it shares only 20–24% amino acid identity with Edg family LPA receptors. As p2y9/GPR23 shows some homology with platelet-activating factor receptors and cysteinyl-leukotriene receptors (Fig. 2), we assumed that the orphan receptor might be activated by some lipid ligands. We screened 198 lipids with Chinese hamster ovary (CHO) cells stably transfected with p2y9/GPR23 and found that LPA induced both intracellular Ca^{2+} mobilization and cAMP formation through p2y9/GPR23 (*32*). Radioligand binding assays with membrane fractions of RH7777 cells transiently transfected with p2y9/GPR23, demonstrated specific binding of LPA to p2y9/GPR23. Scatchard analysis of the binding data gave a dissociation constant (K_d) of 44.8 nM, which is approximately equivalent to the K_d values of LPA with LPA1 and LPA2. Later, Lee *et al*. (*36*) confirmed p2y9/GPR23 as a fourth LPA receptor using mouse p2y9/GPR23 cDNA, and it is now generally accepted as LPA4 (*37*).

Intracellular signalling of LPA4

As described above, LPA increased intracellular levels of Ca^{2+} and cAMP in CHO cells stably expressing LPA4 (CHO-LPA4 cells) (*32*). These observations suggest that LPA4 couples to both Gs and Gq proteins. Since PTX-treatment enhanced the LPA-induced increase of intracellular cAMP, its coupling to Gi protein also seemed possible. Lee *et al*. (*36*) confirmed that LPA4 couples to Gs, Gi, and Gq proteins using rat neuroblastoma B103 cells. They demonstrated that LPA-induced Ca^{2+} mobilization in B103-LPA4 cells was mediated by both Gq and Gi proteins. The increase of Gs-dependent intracellular cAMP levels from exposure to LPA was also shown in these cells.

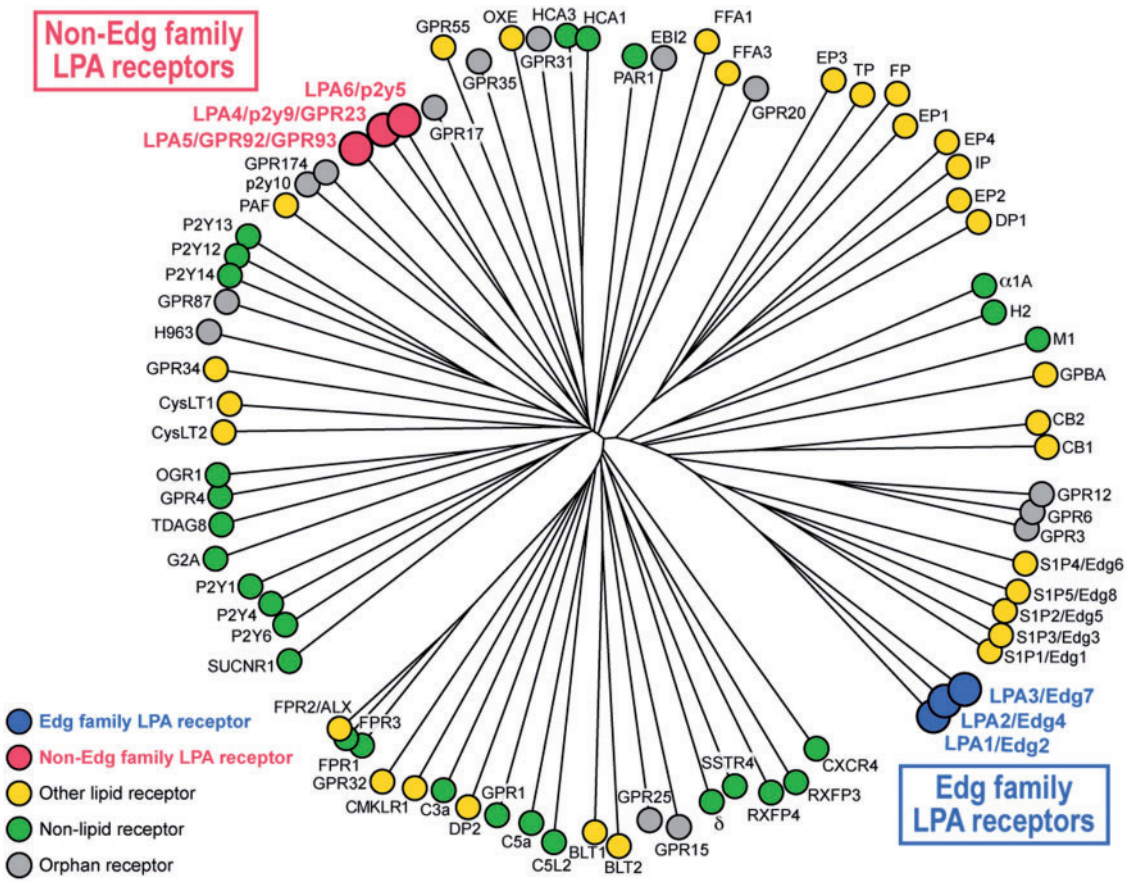

Fig. 2 A representative phylogenetic tree of human GPCRs. Amino acid sequences of selected human GPCRs were obtained from GenBank and SwissProt and aligned using the program, ClustalW, distributed by DNA Data Bank of Japan (DDBJ) (http://clustalw.ddbj.nig.ac.jp/top-j.html). Phylogenetic tree was generated using the FigTree v1.3.1 program. Note that non-Edg family LPA receptors are located rather distantly from Edg family LPA receptors.

However, contrary to this study, LPA did not increase intracellular cAMP in B103-LPA4 cells from our laboratory (*38*). In addition, we found that LPA-induced Ca^{2+} mobilization in B103-LPA4 cells completely depends on Gq protein in an experiment with YM254890, a Gq-inhibitor. Although the reasons for the apparent discrepancy are still unknown, coupling efficiency of LPA4 to Gs or Gi protein may depend on the culture conditions or the receptor expression levels. The cell-type specificity of the coupling to Gs was observed for endogenously expressed LPA4 as well. In human mesenchymal stem cells (hMSCs) after osteogenic induction (*39*) and human fibrosarcoma HT1080 cells (*40*), the LPA–LPA4 interaction evoked Gs-dependent cellular functions. However, no increase in intracellular cAMP was observed in mouse embryonic fibroblasts (MEFs) (*31*), which express functional LPA4 receptors (*41*).

Importantly, LPA4 couples to G12/13 proteins and activates Rho. We found that LPA4 mediates Rho-associated kinase (ROCK)-dependent neurite retraction and cell aggregation in B103-LPA4 cells (*38*). Consistently, Lee *et al.* (*36*) showed G12/13-dependent cytoskeletal changes of RH7777-LPA4 cells and B103-LPA4 cells. In the presence of serum, B103-LPA4 cells formed cell aggregates through activation of the G12/13-Rho-ROCK signalling axis (*38*). In sharp contrast with B103-LPA4 cells, B103-LPA1 cells exhibited a flattened morphology, which was likely evoked by the LPA1-Gi-Rac signalling axis (*38*, *42*). It is interesting that inactivation of Gi protein in B103-LPA1 cells by PTX treatment led to formation of the aggregated morphology, as observed in B103-LPA4 cells (*38*). Given that Rho and Rac activities are mutually inhibitory (*43*), LPA1 and LPA4 may suppress the intracellular signalling to each other through Rac and Rho, respectively. The LPA4-G12/13-Rho pathway was also observed in MEFs (*41*). In MEFs from LPA4 knockout embryos, LPA-induced Rho activation was severely attenuated. Instead, they showed extremely enhanced LPA1-dependent Rac activation and chemotaxis.

Biological functions of LPA4

To date, three independent research groups, including ours, have generated LPA4 knockout mice. Lee *et al.* (*41*) observed no obvious abnormality in their LPA4 knockout mice with a mixed 129/Sv and C57BL/6 genetic background. In another study, Liu *et al.* (*39*) showed increased trabecular bone density in LPA4 knockout mice. This study further demonstrated that the mRNA expression of LPA4 is extremely high in the hMSCs after osteogenic induction, and in the mouse tibia. Using siRNA against LPA4, LPA4 was shown to mediate the increase of intracellular cAMP level in hMSCs, which seems responsible for the inhibition of osteogenic differentiation.

We generated LPA4 knockout mice with a pure C57BL/6 genetic background (*44*). In contrast to the previous two reports, our LPA4 knockout mice displayed reduced litter size compared with wild-type mice. The apparent discrepancy between previous reports and our study might be caused by strain differences and/or different experimental conditions. Subsequent analysis revealed that ~30% of LPA4 knockout embryos did not survive gestation and displayed haemorrhages and/or oedema in many organs, including the heart, skin and lung. In LPA4 knockout embryos with bleeding, the blood vessels were dilated. Furthermore, the recruitments of smooth muscle cells and pericytes were impaired. The defects in blood vessel formation in LPA4 knockout mice were also observed in Matrigel plug assays using adult LPA4 knockout mice, which showed no obvious abnormalities. In the embryos with oedema, lymphatic vessels were also dilated. These data indicate an essential role of LPA4 in normal blood and lymphatic vessel development. Of note, the LPA producing enzyme autotaxin (ATX) has also been shown to have a key role in vascular development (*45*). All ATX knockout embryos died with severe vascular defects in the embryos (*46–49*). Interestingly, the phenotypes of G13 knockout mice were remarkably similar to those of ATX-knockout mice, which included impaired vascular development (*50–52*). These studies suggested that LPA produced by ATX has a critical role in vascular development through a G13-coupling LPA receptor(s). However, the responsible LPA receptor(s) had been unknown because none of the single or multiple knockout mice for LPA1, LPA2 or LPA3 showed such vascular anomalies (*45*). Currently, LPA4 seems to be one of the 'missing links' connecting ATX and G13. However, the phenotype observed in LPA4 knockout mice was partially penetrant, suggesting that other G13-coupling LPA receptor(s) is involved in the vascular development. Since five out of six LPA receptors couple to G12/13 proteins, multiple LPA receptors might have cooperative and redundant roles in this developmental process. LPA4 mRNA is abundant not only in embryos but also in ovary, uterus and placenta in mice (*33*). Whether LPA4 has some roles in female reproduction must await further analysis of LPA4 knockout mice.

LPA5 (GPR92/93)

In 2005, two independent research groups identified a fifth LPA receptor, LPA5 (GPR92/93). While Lee *et al.* (*53*) searched for an additional LPA receptor that affects actin cytoskeleton from collections of GPCR cDNAs, they observed a positive response in cells expressing GPR92/93, which was an orphan GPCR closely related to LPA4 with ~35% amino acid identity. Independently, Kotarsky *et al.* (*54*) focused on serum as a source of an endogenous ligand for GPR92/93 that shows high expression in leukocyte cell lines. Using a reporter gene assay, they isolated a fraction from bovine serum that activated GPR92/93 and found that LPA bound to albumin is an agonist. Both reports showed specific bindings of radiolabelled LPA to membrane fractions from GPR92/93-overexpressing cells. The K_d value for GPR92/93 shown in the latter report was 6.4 nM, which is much lower than the K_d value (88.6 nM) which we obtained with RH7777 cell membranes transiently expressing GPR92/93 (*55*).

Non-LPA agonists for LPA5

Although GPR92/93 was reported as a fifth LPA receptor, LPA5, there are several studies showing the existence of several non-LPA agonists for this receptor. Choi *et al*. (*56*) reported that peptone (protein hydrolysates) activated LPA5 in a dose-dependent manner with an EC_{50} of 10.6 mg/ml. They also showed the synergistic effect of peptone and LPA on the activation of LPA5. Oh *et al*. (*57*) reported that farnesyl pyrophosphate (FPP) at nM concentrations and *N*-arachidonoylglycine at μM concentrations showed potent agonist activities toward LPA5. In their study, FPP was a more potent agonist towards LPA5 than myristoyl-LPA. However, oleoyl-LPA showed higher activity than FPP in the report by Williams *et al*. (*58*). We also confirmed that oleoyl-LPA was a more potent activator of LPA5 than FPP (K.Y. and S.I., our unpublished data). Further analysis with knockout mice will be necessary to determine whether these non-LPA agonists for LPA5 actually function *in vivo*.

Intracellular signalling of LPA5

LPA5 couples to Gq and G12/13 proteins (*53*). In B103-LPA5 cells, LPA increased intracellular Ca^{2+} and cAMP levels and induced Rho-dependent neurite retraction. Experiments with minigenes revealed the involvement of Gq protein in Ca^{2+} mobilization and of G12/13 proteins in neurite retraction. LPA5-dependent increase of intracellular cAMP was observed not only in B103-LPA5 cells but also in LPA5-overexpressing HeLa cells (*54*). However, a Gs minigene did not affect the LPA-induced cAMP production in B103-LPA5 cells, indicating that LPA5 mediates the activation of adenylyl cyclase in a Gs-independent manner (*53*).

Biological functions of LPA5

Knockout mice with the *Lpar5* gene deletion have not yet been reported. However, LPA5 mRNA is highly expressed in small intestine and stomach in mice (*53*, *54*, *56*). Consistently, LPA5 expressed in intestinal epithelial cells has been suggested to play an important role in LPA-induced fluid absorption in mice (*59*). In mouse small intestine, LPA5 is expressed not only in epithelial cells, but also in intraepithelial lymphocytes, which mainly consist of $CD8^+$ cells (*54*). As LPA5 is also expressed in other immune cells and the spleen (*53*), some immunological functions of LPA5 are assumed. Very recently, Lundequist and Boyce (*60*) reported that human mast cells express LPA5 mRNA at a high level. LPA5-mediated LPA-induced release of macrophage inflammation protein-1β from these cells.

Mouse dorsal root ganglion (DRG) was also shown to be rich in LPA5 mRNA (*53*, *57*). Oh *et al*. (*57*) demonstrated the existence of a functional LPA5 receptor in rat cultured DRG neurons. They observed LPA5-dependent Ca^{2+} mobilization in DRG neurons with small diameters but not in those with large diameters. The study reported that there were many DRG neurons double-positive for the presence of LPA5 and the nociceptor transient receptor potential vanilloid type 1. Thus, LPA5 may play a significant role in nociceptive transmission and pain perception.

LPA directly induces human platelet aggregation. As discussed earlier, the LPA responses of platelets are inconsistent with the pharmacological properties of the classic Edg family LPA receptors, suggesting the existence of a 'platelet-type' LPA receptor(s) with a preference for alkyl-LPA over acyl-LPA (*29*). Notably, gene expression profiling of human platelets for GPCRs showed the highest expression of LPA5 among LPA receptors identified (*61*). In recent studies with LPA5-selective agonists, LPA5 was suggested to be the 'platelet-type' LPA receptor (*58*, *62*). More recently, Khandoga *et al*. (*63*) showed that LPA-induced cell shape changes were abolished by siRNA against LPA5 in two human megakaryocytic cell lines (Dami and Meg-01). The rank order of activation by LPA species in these cells was similar to that of human platelets, supporting the idea that LPA5 corresponds to the 'platelet-type' LPA receptor. Importantly, LPA was identified as a main platelet-activating lipid of mildly oxidized low-density lipoprotein and human atherosclerotic lesions (*64*). Thus, LPA5 may be a novel target for anti-thrombotic therapy for patients with myocardial infarction and stroke.

LPA6 (p2y5)

LPA6 (p2y5) was originally an orphan receptor encoded in an intron of the retinoblastoma gene (*65*). The fact that p2y5 shares the highest sequence homology with p2y9/GPR23/LPA4 among all GPCRs strongly suggested that LPA is a ligand for p2y5. However, we could not detect LPA-induced Ca^{2+} mobilization or cAMP level changes in p2y5-overexpressing cells initially at the time of the identification of LPA4 (*32*). However, we noticed that B103-p2y5 cells formed aggregates when cultured with serum (*55*). As was described above, we previously showed that B103-LPA4 cells formed aggregates through the activation of Rho in serum-containing medium, which is abundant in LPA (*38*). Thus, the similar cell morphology between B103-p2y5 cells and B103-LPA4 cells implied activation of the p2y5-Rho signalling axis by LPA. By analysing RH7777-p2y5 and B103-p2y5 cells, we obtained consistent data that p2y5 actually responded to LPA with activation of the G12/13-Rho signalling pathway (discussed below) (*55*). Furthermore, membrane fractions of RH7777 cells stably expressing p2y5 displayed specific binding to radiolabelled LPA (*55*).

During our analysis of p2y5 as a novel LPA receptor, two independent research groups reported that several mutations in the p2y5 gene underlie autosomal recessive hypotrichosis/woolly hair (*66*, *67*). It is noteworthy that membrane-bound phosphatidic acid-selective phospholipase A1α (mPA-PLA1α or LIPH), a 2-acyl-LPA-producing enzyme (*68*), is encoded by a causative gene for autosomal recessive hypotrichosis/woolly hair (*69*), which has clinical features quite similar to those caused by the p2y5 mutations. Based on this similarity, Pasternack *et al*. (*66*) showed LPA-dependent cAMP level changes and specific

binding of radiolabelled LPA in p2y5-overexpressing cells and proposed that p2y5 is a LPA receptor. Lee *et al.* (*70*) independently confirmed that LPA is a ligand for p2y5 by biochemical analysis. They also showed the specific binding of radiolabelled LPA to membrane fractions from p2y5-overexpressing cells. p2y5 was recently designated as LPA6 by the International Union of Basic and Clinical Pharmacology Committee for LPA receptor nomenclature (*37*).

Intracellular signalling of LPA6

Pasternack *et al.* (*66*) showed that LPA induced the increase of intracellular cAMP levels in CHO-LPA6 cells. However, in our research, no significant change in intracellular cAMP levels was detected by LPA in CHO, B103 and RH7777 cells overexpressing LPA6 (*55*). An independent study by Lee and colleagues (*66*) also showed no cAMP increase in CHO-LPA6 cells. Instead, they showed LPA-dependent inhibition of forskolin-triggered elevation of cAMP in CHO-LPA6 cells. The reason for these discrepancies is unknown. Since CHO cells have intrinsic responses to LPA, the confirmation with other cell lines would be necessary to elucidate the efficacy of its coupling to Gs and Gi proteins.

While no LPA6-mediated changes of intracellular cAMP or Ca^{2+} levels were detected, we found that LPA rapidly induces neurite retraction in B103-LPA6 cells and membrane blebbing in RH7777-LPA6 cells (*55*). These morphological changes were Y27632-sensitive, indicating involvement of ROCK in the LPA6 signalling. When B103-LPA6 cells were transfected with Gs/13 protein, a chimeric α subunit of Gs and G13 proteins that enables G13-coupling GPCRs to activate adenylyl cyclases (*71*), LPA increased the cAMP level in a dose-dependent manner. The coupling of LPA6 to G13 protein was further confirmed by the examination of [^{35}S]GTPγS incorporation into Gα13 protein by immunoprecipitation with anti-Gα13 protein antibody. Consistent with our results, Lee *et al.* (*70*) also demonstrated that LPA6 couples to G12/13 proteins.

Biological functions of LPA6

Consistent with the involvement in human hair growth, LPA6 mRNA expression is localized in Huxley's layer of the hair follicle inner root sheath and the epidermis in humans (*67*). As mRNA of mPA-PLA1α shows similar expression distribution to that of LPA6 (*72*), mPA-PLA1α-derived 2-acyl-LPA is likely to act on LPA6 in an autocrine/paracrine manner (*73*). Indeed, we revealed that LPA6 shows ligand preference for 2-acyl-LPA over 1-acyl-LPA in heterologous expression experiments (*55*). Further studies are needed for understanding the precise mechanisms by which LPA regulates human hair growth, including responsible G proteins that function downstream of LPA6. It is interesting that the guanine nucleotide-binding protein subunit alpha-12 gene (*GNA12*) that encodes G12 protein was recently reported to be a strong candidate for the autosomal recessive transmission of hereditary hypotrichosis in a Pakistani family, although direct sequencing analysis of coding sequence failed to detect mutations (*74*).

It has been reported that HUVECs show Rho-dependent actin reorganization in response to LPA (*75*, *76*). We detected high expression of LPA6 mRNA in HUVECs (*55*). Following siRNA-mediated knockdown of LPA6, remarkable inhibition of the LPA-induced HUVEC contraction was observed. Thus, endogenously expressed LPA6 in HUVECs is likely to play a major role in the Rho-mediated morphological changes induced by LPA, raising a possibility that LPA6 regulates vascular permeability as another of its physiological roles. LPA6 is also expressed in cells and tissues other than hair follicles and vascular epithelium (K.Y. and S.I., our unpublished data). Although no other defects including vascular dysfunction have been reported in hypotrichosis/woolly hair patients with the *LPAR6* gene mutations, this LPA receptor may play important, yet overlooked, roles in some physiological and/or pathological situations.

Conclusions

In this review, we have discussed the recent studies on the identification, intracellular signalling and biological functions of non-Edg family LPA receptors, LPA4, LPA5 and LPA6. The discovery of these novel LPA receptors now provides a new framework for understanding pleiotropic functions of LPA. Recent studies have revealed physiological and pathological roles of these receptors, including vascular development, platelet activation and hair growth. However, much less is known about these receptors compared with the Edg family LPA receptors. Further studies, including those with knockout mice, will be necessary for further understanding of this novel cluster of LPA receptors. In addition, the development of pharmacological tools, including specific antagonists and agonists for these receptors, will not only help to reveal the physiological and pathological roles of the receptors but also lead to development of novel therapeutic strategies for various diseases.

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Conflict of interest

None declared.

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