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Cell surface receptors in lysophospholipid signaling

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Abstract

The lysophospholipids, lysophosphatidic acid (LPA) and sphingosine 1-phosphate (S1P), regulate various signaling pathways within cells by binding to multiple G protein-coupled receptors. Receptor-mediated LPA and S1P signaling induces diverse cellular responses including proliferation, adhesion, migration, morphogenesis, differentiation and survival. This review will focus on major components of lysophospholipid signaling: metabolism, identification and expression of LPA and S1P receptors, general signaling pathways and specific signaling mechanisms in mouse embryonic fibroblasts. Finally, in vivo effects of LP receptor gene deletion in mice will be discussed.

Keywords: G protein-coupled receptor; Lysophosphatidic acid; LPA; Sphingosine 1-phosphate; S1P

1. Introduction

Lysophospholipids (LPs) are not only metabolites in membrane phospholipid synthesis, but also omnipresent bioactive molecules influencing a broad variety of biological processes by binding to cognate G protein-coupled receptors (GPCRs). The best characterized representatives of signaling LPs are lysophosphatidic acid (LPA) and sphingosine 1-phosphate (S1P). Although their signaling role has been recognized for decades, the identification of high-affinity receptors for LPA and S1P in the last several years dramatically improved our comprehension of LP signaling. The widespread expression of cell surface LP receptors and coupling to several classes of G proteins allow regulation of various cellular processes with particular impact on neurogenesis, vascular development, wound healing, immunity, and cancer.

2. Metabolism of LPA and S1P

LPA is a simple lipid molecule made up of a glycerol backbone with a hydroxyl group, a phosphate group, and a long saturated or unsaturated fatty acid chain (Fig. 1). Several pathways for LPA synthesis and degradation have been implicated [1]. Extracellular LPA is likely to be generated by the phospholipases (PL) A_1 and A_2 mediating deacylation of phosphatidic acid and, additionally, by lysophospholipase D (lysoPLD) that hydrolyzes lysophosphatidylcholine to LPA [1]. Recently, lysoPLD from bovine and human serum was identified as autotaxin, an ectophosphodiesterase originally implicated in nucleotide signaling ([2,3] and see Aoki, this issue). Inside the cell, LPA is likely generated by PLs, and by acylation of glycerol 3-phosphate catalyzed by glycerophosphate acyltransferase, by reduction of acyl dihydroxy acetone phosphate or by phosphorylation of monoacylglycerol mediated by monoacylglycerol kinase [1]. Intracellular LPA serves primarily as a metabolite in the glycerolipid and phosphatidylinositol synthesis [1]. Whether intracellular LPA can also be released to mediate its bioactive effects on cell surface GPCRs is currently unknown. Degradation of extracellular LPA is mediated by integral membrane lipid phosphate phosphatases (LPPs). When localized in the plasma membrane, the catalytic site of LPPs protrudes into the extracellular space mediating ecto-phosphatase activity ([4] and see Pyne, this issue). So far, four enzymes, LPP-1, LPP-2, LPP-3 and plasticity-related gene-1 (PRG-1) have been described, which in mammals have been reported to mediate hydrolysis of LPA to monoacylglycerol [5,6]. Overexpression of LPP-1 in Rat2 fibroblasts induced dephosphorylation of exogenous LPA thereby attenuating LPA elicited cellular effects [4]. The physiological significance of LPP-1 activity was demonstrated by the finding that endogenous expression of LPP-1 at the cell surface of intact platelets increased after LPA exposure and actively dephosphorylated LPA [7]. Moreover, an action of LPPs "*in trans*" is assumed since overexpression of LPP-3 in ovarian cancer cells terminated LPA signaling in parental cells [8]. PRG-1, specifically

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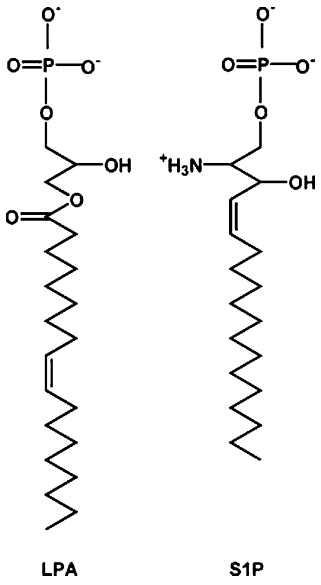

Fig. 1. Chemical structure of the signaling lysophospholipids LPA and S1P. LPA: (1-oleoyl) lysophosphatidic acid; S1P: sphinosine 1-phosphate.

expressed in neurons, attenuates LPA-induced axon collapse mediated by its ecto-phosphatase activity [6]. These findings indicate a pivotal role of LPPs as negative regulators of LPA signaling by reducing extracellular LPA concentrations.

S1P is composed of a sphingoid backbone and a phosphate group (Fig. 1). Intracellularly, sphingosine kinase (SPHK) phosphorylates sphingosine to S1P, whereas specific S1P phosphatases hydrolyze S1P to sphingosine [9]. An alternative degradation of S1P is mediated by S1P lyase, an enzyme that cleaves S1P to phosphoethanolamine and hexadecanal [9]. S1P generating and degrading enzymes are highly conserved throughout evolution. Homologous proteins for SPHK and S1P lyase were found in plant, yeast, worm, and mammals, whereas S1P phosphatases have been found in yeast and mammals [9,10]. So far, two sphingosine kinases, SPHK1 and SPHK2, have been identified in mammals [11,12]. The existence of additional sphingosine kinases is very likely since in some tissues, e.g., spleen, small intestine and lung, sphingosine kinase activity was reported despite lacking expression of SPHK1 and SPHK2 [13]. Two S1P-specific phosphohydrolases, SPP1 and SPP2 have been cloned in human [14,15]. Both enzymes are located in the endoplasmic reticulum indicating dephosphorylation of intracellular S1P. The importance of a tight regulation of intracellular S1P levels became evident by the disruption of S1P lyase in different organisms resulting in severe developmental defects manifested in (i) *Dictyostelium discoideum* by aberrant morphogenesis, a higher viability during stationary phase, reduced spore differentiation, and defective slug migration, (ii) *Caenorhabditis elegans* by severe intestinal damage, egg laying defects and semi-lethality, and (iii) *Drosophila melanogaster* by pattern abnormalities in flight muscles, egg laying defects, increased apoptosis during embryonic stages, and semi-lethality ([16–18] and see Oskouian and Saba, this issue). These severe phenotypes support the hypothesis that S1P may act as a second messenger molecule in addition to its well-characterized function as an extracellular ligand for several cell surface receptors [9]. However, the intracellular concentration of S1P may also influence extracellular S1P levels since the bulk of extracellular S1P seems to be secreted by so far unknown mechanisms [19]. It remains to be seen, whether S1P is also efficiently generated extracellularly, e.g., by fractions of constitutively secreted SPHK [20] or by autotaxin that was also shown to hydrolyze sphingosylphosphorylcholine to S1P, even though the catalytic efficiency was 4.5-fold lower compared to the hydrolysis of lysophosphatidylcholine to LPA [21].

Highest concentrations of both, LPA and S1P, were found in serum after platelet activation. S1P is abundantly stored within platelets that have high levels of active SPHK but lack S1P lyase and is rapidly secreted after platelet induction [22]. The level of S1P in human serum is estimated to be 0.5–0.8 μM [23]. In contrast to S1P, increased LPA concentrations in serum arise extracellularly by de novo generation catalyzed by secreted PLA_1, PLA_2 and lysoPLD [19]. In humans, serum LPA levels increase within 1–24 h of blood clotting from approximately 1 μM to 5–6 μM [24]. Beside platelets, several other cell types generate extracellular LPA as shown for neural cells, adipocytes, fibroblasts and ovarian cancer cells [1,25,26]. Similarly, high S1P levels were found to be produced by ovarian cancer cells and peripheral blood cells including erythrocytes, neutrophils and mononuclear cells [22]. The widespread expression of LPA and S1P generating enzymes and the generation of extracellular LPA and S1P by a wide variety of cell types under normal or pathological conditions indicate that LPs are omnipresent bioactive molecules (see Sengupta et al., and Hla, this issue). However, the most crucial factors for the signaling capacity of LPs are the expression and distribution of cell surface LP receptors and their coupling to downstream signaling pathways.

3. Identification of LPA and S1P receptors

The first LP receptor was identified in 1996, during a search for genes with predominant expression in the ventricular zone (VZ) of the cerebral cortex. This led to the identification of ventricular zone gene 1 (VZG-1), that was shown to encode a high-affinity GPCR for LPA [27,28]. Subsequently, sequence similarities allowed rapid identification of further cognate LPA and S1P receptors [29,30]. In mammals, four high-affinity cell surface receptors for LPA have been described so far. Three of them, originally named EDG-2/VZG-1/rec1.3, $EDG\text{-}4_{(non\text{-}mutant)}$ and EDG-7 are closely related GPCRs [27,31–34]. Because of the inconsistency of the LP receptor nomenclature, EDG-2/VZG-1/rec1.3, $EDG\text{-}4_{(non\text{-}mutant)}$ and EDG-7 genes have been renamed LPA_1, LPA_2 and LPA_3 following the guidelines of IUPHAR [35]. Recently, a fourth LPA receptor,

LPA_4/GPR23/P2Y9, was cloned in human [36]. With 20–24% amino acid identity to LPA_1, LPA_2 and LPA_3, LPA_4 is evolutionarily distant from the other LPA receptors. Instead, LPA_4 is more closely related to nucleotide receptors of the P2Y GPCR family [36], and it is notable that the aforementioned metabolic enzyme autotaxin also shares ligand relationships with nucleotides.

Five cognate GPCRs for S1P have been renamed by the IUPHAR nomenclature as $S1P_1$, $S1P_2$, $S1P_3$, $S1P_4$, and $S1P_5$ [30,37] (formerly EDG-1, EDG-5/AGR16/H218, EDG-3, EDG-6 and EDG-8/NRG-1, respectively [35]). $S1P_1$ was originally isolated as an immediate early gene with a potential role in endothelial cell differentiation [38]. In 1998, two groups reported independently the identification of S1P as a high-affinity ligand for $S1P_1$ [39,40]. Similarly, $S1P_2$ and $S1P_3$ were identified as orphan GPCRs before S1P was reported as high-affinity ligand for these receptors [30]. $S1P_4$ was isolated from in vitro differentiated human and murine dendritic cells and subsequently was shown to be a high-affinity receptor for S1P [41–43], although its preferred ligand appears to be phytosphingosine 1-phosphate [44]. $S1P_5$ was originally cloned from rat pheochromocytoma 12 (PC12) cells and designated nerve growth factor-regulated gene-1 (NRG-1) since nerve growth factor repressed expression of this gene in PC12 cells [45]. Shortly after the first report on $S1P_5$, another lab isolated $S1P_5$ from rat brain and identified S1P as high-affinity ligand for this receptor [46]. It has to be highlighted that LP signaling is a rapidly growing field and future studies will presumably clarify the identities of new LP receptors.

4. Expression of LPA and S1P receptors

LP receptors have been investigated based on mRNA expression of the respective receptors in different tissues of rodents and human (Table 1). In adult mice, LPA_1 is widely expressed with high mRNA levels in testis, brain, lung, heart, spleen and intestine, and moderate levels in kidney, thymus, stomach and muscle [27,29,47]. No LPA_1 expression was detected in liver of adult mice [27,29,47]. LPA_1 is similarly expressed in adult human organs showing high mRNA expression in brain, heart, colon, small intestine, placenta, prostate, ovary, pancreas, testis and spleen, and lower expression levels in skeletal muscle and kidney [32]. Hardly any LPA_1 mRNA was detected in human lung and thymus, whereas LPA_1 expression was completely absent in liver and peripheral blood leukocytes [32]. Expression of LPA_1 is characterized in detail within the mouse nervous system showing a tight spatio-temporal regulation during development [29,30]. At embryonic stages, LPA_1 is predominantly expressed in the VZ during cortical neurogenesis. Shortly before birth, however, LPA_1 expression in the cortex declines simultaneously with the end of the cortical neuroblast proliferation phase [27,48]. After birth, LPA_1 expression reappears in brain where it is closely associated with developing white matter tracts and coincides with the process of myelination showing highest expression between postnatal days 18 and 21 [48]. In situ hybridization analysis revealed oligodendrocytes, the myelinating glia cells in the central nervous system as LPA_1 expressing cells [48]. Subsequently, LPA_1 expression was also demonstrated in sciatic nerve and Schwann cells, the myelinating cells in the peripheral nervous system [49]. LPA_2 and LPA_3 show a more restricted expression pattern than LPA_1. LPA_2 is most abundantly expressed in testis and kidney from adult mice [29,50]. Low expression levels were found in brain, heart, lung, spleen, thymus, and stomach, whereas hardly any or no LPA_2 transcripts were detectable in liver, muscle and small intestine [29,50]. Unlike adult brain, embryonic mouse brain showed high levels of LPA_2 mRNA [29,50]. In humans, LPA_2 was strongly detectable in testis and leukocytes [32]. Moderate LPA_2 levels were reported in pancreas, thymus, spleen and prostate [32]. In human adult brain, heart, lung, liver, kidney,

Table 1
Expression of LP receptors in rodents

Tissue	LPA_1	LPA_2	LPA_3	$S1P_1$	$S1P_2$	$S1P_3$	$S1P_4$	$S1P_5$	References
Embryonic brain	++	++	(+)	+++	++	+	–	(+)	[27,50,51,29,58,56]
Adult brain	+++	(+)	(+)	+++	+	+	–	+++	[27,47,50,51,29,54,53,52,55,56,45,46]
Heart	++	+	++	+++	+++	+++	–	–	[29,47,50,51,54,53,52,55,45,46]
Lung	+++	+	++	+++	+++	+++	+++	(+)	[27,47,50,51,29,54,53,41,52,58,55]
Liver	–	(+)	–	++	+	–	–	–	[27,47,50,51,29,54,53,52,58,55,45,46]
Kidney	+	+++	+++	+	+	+++	–	–	[27,47,50,51,29,54,53,52,58,55,45,46]
Spleen	+	+	+	+++	+	+++	+++	++	[27,47,50,51,29,54,53,41,52,55,45,46]
Thymus	+	+	+	+	++	++	+++	–	[29,50,51,53,41,52]
Stomach	+	+	+	(+)	++	(+)	–	n.a.	[29,50,51,53,41,55]
Intestine	++	–	+	(+)	+	(+)	–	–	[29,50,51,53,41,55,46]
Testis	+++	+++	+++	(+)	+	++	–	–	[47,50,51,29,53,52,55,45,46]
Uterus	n.a.	n.a.	n.a.	+	++	+	–	–	[52]
Muscle	+	–	–	+	(+)	+	n.a.	–	[47,29,53,55,45]
Skin	n.a.	n.a.	n.a.	(+)	+	+	–	+++	[52]

Expression of LPA and S1P receptors in different tissues are shown. +++, strong expression; ++, moderate expression; +, weak expression; (+), very weak expression or inconsistent data; –, no expression; n.a., not analyzed.

muscle, ovary, placenta, intestine and colon LPA_2 is apparently not expressed [32]. Strong LPA_3 expression was found in kidney, testis, lung, and to a lesser extent in small intestine, heart, spleen, thymus, and stomach from adult mice [29,51]. Within the mouse brain, LPA_3 transcripts show highest expression around birth, whereas expression of LPA_3 is very low during embryonic development and in adult brain [29]. Abundant LPA_3 expression was also found in rat testis and kidney [34]. Human LPA_3 is detectable at highest levels in heart, prostate, pancreas and testis and at moderate levels in lung and ovary [33,34]. Finally, LPA_3 expression was also reported in human brain with particularly strong expression in the amygdala, frontal cortex and hippocampus [34]. Human LPA_4 seems to be very weakly expressed in every tissue examined with the exception of ovary, where LPA_4 expression is strongly upregulated [36]. So far, the expression pattern of LPA_4 in rodents has not been examined.

Generally, $S1P_1$, $S1P_2$ and $S1P_3$ receptors are widely expressed in human and rodents, whereas $S1P_4$ and $S1P_5$ expression is restricted to single tissues (Table 1). $S1P_1$ was found to be highly expressed in spleen, brain, heart, lung, adipose tissue, liver and moderately in thymus, kidney, muscle and uterus of adult mice [52–54]. Hardly any expression was found in skin, stomach, intestine and testis [52,53]. $S1P_2$ is expressed at high levels in adult mouse heart, lung, thymus, adipose tissue, spleen, uterus, kidney, brain, and at lower levels in liver, skin, muscle, stomach, intestine, and testis [52,53,55,56]. In rats, transcripts were found in heart, lung, stomach, intestine, and adrenal gland [55]. $S1P_3$ mRNA was detectable in adult mouse spleen, heart, lung, kidney, thymus, brain, adipose tissue, testis, uterus, muscle, and skin but was absent in liver, stomach and intestine [52,53]. In human, $S1P_3$ mRNA was detectable in heart, placenta, kidney, liver, pancreas, muscle, lung and brain [57]. All three receptors, $S1P_1$, $S1P_2$ and $S1P_3$, were also reported to be expressed during embryonic stages in the rodent brain [56,58]. In contrast to the ubiquitous expression of $S1P_1$, $S1P_2$ and $S1P_3$, human and mouse $S1P_4$ expression was found to be confined to lung, lymphoid and hematopoietic tissues including thymus and spleen [41,52]. Similarly, $S1P_5$ showed a restricted expression pattern with strong $S1P_5$ levels in adult human brain and spleen as well as in adult rodent brain, spleen and skin [45,46,52]. Within the adult rat brain, $S1P_5$ seems to be strongly localized to white matter tracts as shown by in situ hybridization analysis [46].

5. General aspects of LP signaling

Essentially, all cells in mammals respond in one way or another to LPA and S1P. The most common cellular responses are proliferation, cell survival, cell motility and differentiation. These effects are mediated by coupling of LP receptors to G proteins that regulate the activity of intracellular messenger molecules (Fig. 2). LP receptors couple to members of three major G protein families, the G_i (G_{i1}, G_{i2}, G_{i3}, G_{o1}, G_{o2}, G_z, G_t, G_{gus}), G_q (G_q, G_{11}, G_{14}, $G_{15/16}$), and G_{12} (G_{12}, G_{13}) family. LPA_1, LPA_2, $S1P_2$ and $S1P_3$ are known to interact with all three G protein families [30,37,59]. LPA_3 interacts with G_i and G_q, but not with G_{12} proteins. An exception may be LPA_4, that appears to couple with the fourth subclass, the G_s (G_s, G_{olf}) family, although

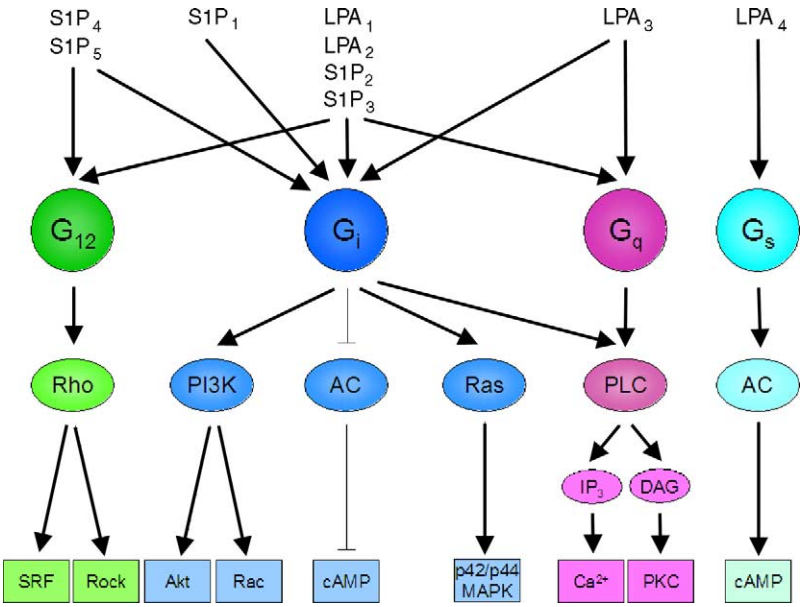

Fig. 2. General signaling pathways regulated by LPA and S1P. Interaction of LPA and S1P receptors with G protein families and subsequent downstream effects on second messenger and effector molecules are indicated. AC, adenylyl cyclase; cAMP, cyclic adenosine monophosphate; DAG, diacylglycerol; p42/p44 MAPK, p42/p44 mitogen-activated protein kinase; IP_3, inositol 1,4,5-triphosphate; PI3K, phosphoinositide 3-kinase; PKC, protein kinase C; PLC, phospholipase C; Rock, Rho-associated kinase; SRF, serum response factor.

coupling with other G proteins can not be excluded [36]. $S1P_1$ interacts exclusively with G_i, whereas $S1P_4$ and $S1P_5$ have been shown to couple to both, G_i and G_{12} [37,59,60]. General downstream effects of most LP receptors include activation of phospholipase C (PLC) and Ca^{2+} mobilization [30,37,59]. Activation of mitogen-activated protein kinase (MAPK) is another common effect observed after activation of $LPA_{1–3}$ and $S1P_{1–4}$. An exception hereof is the $S1P_5$-mediated inhibition of MAPK. Rho activation has been observed for LPA_1, LPA_2, $S1P_1$, $S1P_2$, $S1P_3$, and $S1P_4$ receptors [30,37,59]. Activation of PLC, MAPK and Rho via LPA and S1P receptors result in cell proliferation, cell survival and changes in cell morphology such as cell rounding [30,37,59]. Adenylyl cyclase (AC) is differentially regulated by LP receptors. LPA_1, LPA_2, $S1P_1$, and $S1P_5$ inhibit AC activity whereas $S1P_2$ and $S1P_4$ activate AC. For LPA_3 and $S1P_3$ both activation and inhibition of AC have been reported [30,37,59]. LPA_4 was shown to activate AC resulting in cAMP accumulation and to induce Ca^{2+} mobilization [36]. Phosphoinositide 3-kinase (PI3K) and its substrate Akt are activated by LPA_1 thereby enhancing cell survival [49]. Phosphorylation of Akt was also reported following activation of LPA_2 and $S1P_3$ [61,62]. $S1P_1$ and $S1P_3$ were found to enhance cell migration via activation of the small GTPase Rac [63]. The opposite effect, inhibition of Rac activity and prevention of cell migration, was attributed to activation of the $S1P_2$ receptor [63].

In addition to the well-characterized cell surface receptor-mediated responses of LPs, actions of intracellular LPA and S1P as second messengers have been proposed by several studies [5,9]. For S1P, direct intracellular signaling functions have been suggested that result in Ca^{2+} mobilization, activation of MAPK, DNA synthesis, and suppression of apoptosis [64]. However, most of these studies could not formally exclude the release of intracellular S1P followed by activation of known and especially unknown cell surface S1P receptors. As importantly, intracellular targets for S1P have not been identified so far. Therefore, unequivocal proof for an intracellular signaling role of S1P are still lacking. For LPA, an intracellular target has been reported recently [65]. LPA was shown to bind to the nuclear hormone receptor peroxisome proliferator-activated receptor γ (PPARγ). The physiological significance of LPA binding to PPARγ is challenged by the observation that other ligands, such as eicosanoids and anionic fatty acids, also interact with PPARγ [66]. Furthermore, analysis of PPARγ expression and gene deletions in mouse revealed restricted expression and functions in adipocyte tissue [67,68], whereas LPA-mediated effects were observed in many other tissues. These findings require additional studies to clarify the physiological significance of LPA interaction with PPARγ.

PLC activation, Ca^{2+} mobilization, MAPK regulation, AC inhibition/activation and small GTPases activation are frequently observed in different cell types in response to LPA and S1P. However, LP-mediated signaling can vary from one cell type to another depending on the composition and expression levels of the receptors and downstream signaling proteins. Furthermore, accumulating data suggest crosstalk between LP and growth factor signaling pathways such as S1P and platelet-derived growth factor (PDGF) crosstalk in chemotaxis, p42/p44 MAPK or Akt activation [62,69–71]. These factors require detailed examination of LP signaling in individual cell types.

6. LP signaling in mouse embryonic fibroblasts

Probably one of the best characterized primary cell type in terms of LP signaling are mouse embryonic fibroblasts (MEFs). In particular the analysis of MEFs derived from different LP receptor-null mice allowed clarification of the signaling pathways elicited by different LPA and S1P receptors (Fig. 3). MEFs express LPA_1, LPA_2, $S1P_1$, $S1P_2$ and $S1P_3$ receptors [52,61,72,73]. Stimulation with LPA in wild-type MEFs induce activation of PLC resulting in inositol 1,4,5-triphosphate (IP_3) and diacylglycerol (DAG) production, and subsequently in Ca^{2+} mobilization and protein kinase C (PKC) activation [61]. Analysis of $LPA_1^{(-/-)}$ and $LPA_2^{(-/-)}$ MEFs revealed an involvement of both LPA receptors in PLC activation and Ca^{2+} mobilization, whereby LPA_2 had a slightly greater effect than LPA_1 [61]. S1P-induced PLC activation and concomitant mobilization of Ca^{2+} is largely attributable to $S1P_3$ involving a pertussis toxin (PTX)-insensitive pathway, whereas $S1P_2$ has no

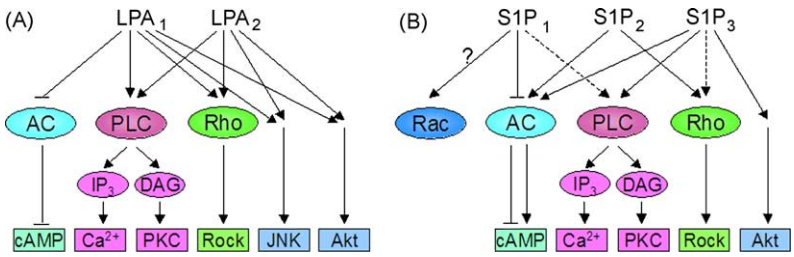

Fig. 3. LP signaling in mouse embryonic fibroblasts (MEFs). (A) LPA_1- and LPA_2-mediated effects on signaling molecules. (B) Intracellular signaling effects of S1P through $S1P_{1–3}$ receptors. Whether $S1P_1$ mediates activation of Rac in MEFs is presently controversial. Weak activation of signaling molecules by distinct receptors are indicated by dashed arrows. AC, adenylyl cyclase; cAMP, cyclic adenosine monophosphate; DAG, diacylglycerol; IP_3, inositol 1,4,5-triphosphate; JNK, c-Jun N-terminal kinase; PKC, protein kinase C; PLC, phospholipase C; Rock, Rho-associated kinase.

effect on PLC activation and intracellular Ca^{2+} levels [52,73]. A slight PTX-sensitive activation of PLC was still detectable in LPA_3-null cells. This remaining activation of PLC is presumably mediated by $S1P_1$ coupled to G_i. LPA-induced AC inhibition in wild-type MEFs can be attributed solely to LPA_1 activation, since inhibition of AC was comparable in $LPA_2^{(-/-)}$ and wild-type cells [61]. In $LPA_1^{(-/-)}$ MEFs, however, AC inhibition was no longer detectable. The analysis of $S1P_2^{(-/-)}$, $S1P_3^{(-/-)}$ and $S1P_2^{(-/-)}$ $S1P_3^{(-/-)}$ double-null cells demonstrated the presence of two opposing S1P receptor-mediated effects on AC and basal cAMP levels in MEFs [73]. $S1P_1$ mediates inhibition of AC through coupling to G_i, whereas $S1P_2$ and $S1P_3$ induce AC activation in a PTX-insensitive manner. Unexpectedly, LPA did not induce activation of p42/p44 MAPK as observed in most other cell type, whereas p42/p44 MAPK activation in MEFs was observed in response to S1P [61,62]. Instead, activation of c-Jun N-terminal kinase (JNK) and Akt was observed in MEFs following LPA treatment [61]. Analysis of $LPA_1^{(-/-)}$ and $LPA_2^{(-/-)}$ cells showed redundant functions of LPA_1 and LPA_2 on activation of these kinases that were abolished in $LPA_1^{(-/-)}$ $LPA_2^{(-/-)}$ double-null MEFs [61]. S1P-mediated Akt activation was reported to be mediated through $S1P_3$ [62]. Stress fiber formation normally mediated through $G_{12/13}$/Rho/ROCK-induced actin polymerization was still observed in $LPA_1^{(-/-)}$ and $LPA_2^{(-/-)}$ single but not in $LPA_1^{(-/-)}$ $LPA_2^{(-/-)}$ double-null MEFs [61]. Rho activation induced by S1P is mainly mediated by $S1P_2$ [73]. For $S1P_3$ only marginal effects on Rho activation are reported [73]. Finally, S1P increased migration of MEFs by activation of $S1P_1$ receptors [72]. Whether or not $S1P_1$-induced cell migration in MEFs involves activation of Rac is contradictory, since Rac activation by S1P could not be confirmed in other studies [52,73].

In summary, analysis of wild-type and receptor-deficient MEFs outline complex LPA and S1P signaling pathways with partially redundant but also unique actions of single LP receptors.

7. Phenotypes of LP receptor-null mice

A significant step towards unraveling the physiological relevance of LP signaling is the analysis of consequences arising from single or combined genetic deletions of LP receptors in mice. So far, $LPA_1^{(-/-)}$, $LPA_2^{(-/-)}$, $LPA_1^{(-/-)}$ $LPA_2^{(-/-)}$, $S1P_1^{(-/-)}$, $S1P_2^{(-/-)}$, $S1P_3^{(-/-)}$, and $S1P_2^{(-/-)}$ $S1P_3^{(-/-)}$ mice have been described [52,61,72–76]. In the following section, macro- and microscopic phenotypes of these mice will be outlined.

$LPA_1^{(-/-)}$ mice revealed semi-lethality with death of 50% of the LPA_1-null mice within the first 3 weeks of age [74]. Semi-lethality of $LPA_1^{(-/-)}$ pups is attributable to an impaired suckling behavior. Nearly all $LPA_1^{(-/-)}$ pups had no or only little milk in their stomach at postnatal days 0 and 3. Consistently, surviving $LPA_1^{(-/-)}$ mice revealed a decreased postnatal growth rate leading to a 30% reduction in body weight compared to control littermates [74]. Furthermore, juvenile and adult $LPA_1^{(-/-)}$ mice displayed craniofacial deformities including shorter snouts and more widely spaced eyes relative to control siblings. A small percentage of $LPA_1^{(-/-)}$ embryos and neonatal pups displayed frontal hematomas. In newborns, these hematomas were not lethal per se and dissipated after several days [74]. At the microscopic level, no abnormalities were found within the olfactory epithelia, olfactory bulb or cortex of $LPA_1^{(-/-)}$ mice, that might explain the impaired suckling behavior by defects related to olfactant detection or processing. Whereas, so far, no abnormalities at the cellular level were found in the central nervous system of LPA_1-null mice, alterations were found associated with Schwann cells (SCs), the major LPA_1 expressing cell type in the postnatal peripheral nervous system. $LPA_1^{(-/-)}$ mice displayed increased apoptosis of SCs in the sciatic nerve confirming the previously observed survival effect of LPA on cultured primary SCs [49,74]. The signaling mechanism underlying cell survival involves coupling of LPA_1 receptors to G_i proteins, that activates PI3K presumably via the βγ-subunit of G_i. Subsequently, PI3K phosphorylates the serine/threonine kinase Akt [49]. Phosphorylated Akt likely inhibits apoptosis through phosphorylation of Bad, a proapoptotic member of the Bcl-2 family, since the peptide SC survival factor, neuregulin, was reported to prevent SC death through activation of PI3K/Akt/Bad [77]. Another study suggested a slightly different mechanism for LPA-mediated cell survival. Li et al. identified PI3K-dependent activation of p42/p44 MAPK in parallel with a weak activation of Akt. Based on the observation that PD98059, an inhibitor of the p42/p44 MAPK-activating MAPK kinase, MEK, largely inhibited LPA dependent cell survival, they suggested that LPA mediates cell survival through a G_i/PI3K/MEK/p42/p44 MAPK pathway [78]. The increase of apoptotic SCs in sciatic nerve from 10% in wild-type to 18% in LPA_1-null mice, however, did not affect movements or locomotion of $LPA_1^{(-/-)}$ mice.

In contrast to $LPA_1^{(-/-)}$ mice, $LPA_2^{(-/-)}$ mice did not reveal obvious phenotypic abnormalities, and the generation of $LPA_1^{(-/-)}$ $LPA_2^{(-/-)}$ double-null mice resulted in no additional macro- or microscopic effects beside an increased incidence of neonatal frontal hematomas compared to $LPA_1^{(-/-)}$ mice [61]. General histology, cell number, proliferation and thickness of cerebral cortices in $LPA_1^{(-/-)}$ $LPA_2^{(-/-)}$ double-null mice were comparable to controls. These findings are striking in view of the LPA-mediated reduction of cell death and increased terminal mitosis of cortical neuroblasts observed in an ex vivo culture system for intact cerebral cortices [79]. LPA-effects in cortical ex vivo cultures finally become manifested in increased cell numbers, increased thickness of the cortices and in cortical folding [79]. Although, these effects were shown to depend on LPA_1 and LPA_2 receptor functions, $LPA_1^{(-/-)}$ $LPA_2^{(-/-)}$ double-null mice did not revealed corresponding

phenotypes. These apparently contradictory findings suggest the existence of compensatory signaling mechanisms in the double-null mice. A strong candidate for compensation is undoubtedly S1P signaling, since S1P and LPA often display redundant functions and LPA_1 and $S1P_1$ receptors were reported to be similarly expressed within the developing cortex [58].

$S1P_1$-null mice displayed the most severe phenotype characterized by strong and widespread embryonic hemorrhage leading to intrauterine death between embryonic day (E) 12.5 and E14.5 [72]. Whereas vasculogenesis and angiogenesis were not affected in $S1P_1{}^{(-/-)}$ embryos, recruitment of vascular smooth muscle cells (VSMCs) to blood vessel walls was severely impaired resulting in defective ensheathment of vessels by VSMCs and incomplete vessel maturation. Defects in vascular development in $S1P_1{}^{(-/-)}$ mice were somehow predictable due to several studies showing S1P-mediated signaling mechanisms in VSMCs and vascular endothelial cells [80]. In particular, $S1P_1$ and $S1P_3$ receptors have been implicated in processes relevant for vascular maturation and angiogenesis such as cell migration and formation of adherence junctions [80]. $S1P_2{}^{(-/-)}$ mice have been generated independently by two groups [73,75]. Both reports identified no anatomical or physiological defects at birth. The average litter size was slightly reduced in $S1P_2{}^{(-/-)}$ females, although $S1P_2$-null pups were born with the expected Mendelian frequency [73]. One report described the occurrence of spontaneous and sporadic seizures between 3 and 7 weeks of age [75]. Subsequently, whole-cell patch-clamp recordings revealed hyperexcitability of $S1P_2{}^{(-/-)}$ neocortical pyramidal neurons indicating a possible function of $S1P_2$ in neuronal excitability [75]. This potential $S1P_2$ function may be dependent on the C57BL/6 (albino) genetic background, since $S1P_2{}^{(-/-)}$ mice generated in a different background (C57BL/6N) did not reveal comparable effects [73]. $S1P_3{}^{(-/-)}$ mice appeared healthy with no apparent morphological defects [52]. Similar to $S1P_2{}^{(-/-)}$ mice, $S1P_3$-null mice were born at the expected Mendelian frequencies, but again, the average litter size was slightly reduced in $S1P_3{}^{(-/-)}$ mice intercrosses compared to $S1P_3{}^{(+/-)} \times S1P_3{}^{(+/+)}$ crosses. In $S1P_2{}^{(-/-)}$ $S1P_3{}^{(-/-)}$ crosses, the average litter size was severely reduced. However, the reason for this is currently not clear [73]. In addition to the reduction of the litter sizes, $S1P_2{}^{(-/-)}$ $S1P_3{}^{(-/-)}$ double-null mice often did not survive through infancy, although no obvious phenotype was observed [73].

8. Concluding remarks

The identification of high-affinity surface receptors for LPA and S1P has allowed rapid progress in understanding LP signaling mechanisms, clearly demarcating receptor-dependent from receptor-independent functions. A particularly valuable tool in this regard for studying the significance of LP receptor-mediated signaling has been single or combined targeted deletions of LP receptors in mouse. Analyses of LP receptor-null mice have revealed important insights into single cell signaling, allowing attribution of distinct signaling pathways to specific receptors as shown in MEFs, that can allow signaling studies in primary cells. Similar correlations between receptors and LP-mediated effects by pharmacological studies have not been reported so far, due to the lack of subtype-specific agonists and antagonists for LP receptors, although this situation is changing rapidly, and the combination of pharmacological tools with genetic nulls represents new and fertile territory. Further analysis of both receptor-null animals, and related enzyme-deficient animals will also provide new insights in the near future. The growing list of genetic and chemical tools will have greatest impact on understanding in vivo actions of LP signaling, and will further clarify LP receptor-mediated physiological as well as pathophysiological functions.

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